Platelet Sodium-Hydrogen Antiport in Obese and Diabetic Black Women

Zoltan Zentay, Alluru Reddi, Maya Raguwanshi, Jeffrey P. Gardner, Jwa Hwa Cho, Norman Lasker, Amita Dasmahapatra, and Abraham Aviv

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. (Hypertension 1992;20:549–554)

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.1–4 NIDDM and essential hypertension also share a tendency for accelerated atherosclerotic and thromboembolic complications,5–8 which may arise from serum lipid abnormalities and platelet hyperactivity9–12 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,13–17 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/m². 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%) 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 1,000 g for 10 minutes, and cells were washed three times (by centrifugation at 1,000 g 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., St. Louis, Mo.). Changes in the cytosolic pH (pHᵢ) were followed up to 80 seconds. Basal pHᵢ and cellular buffering power were measured in nonacidified platelets. The latter measurement used the ammonium chloride method.¹⁸ Measurements of the pHᵢ 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. Illustration of the analytical approach to obtain the parameters of the Na-H antiport. Initial rates of recovery from different degrees of acidification are plotted as a function of initial cellular proton concentration ([H⁺]ᵢ), from which the recovery proceeded. The x-axis intercept is the [H⁺]ᵢ, at which the activity of the Na-H antiport converges to zero. When expressed in pH units, it represents the apparent cytosolic pH (pHᵢ) set point for activation of the Na-H antiport. A shift to the left in the x-axis intercept denotes an alkaline shift in the Xᵢ. An increase in the slope indicates an increase in the Zᵢ. Open circles indicate platelets acidified with sodium propionate without thrombin in the medium; closed circles indicate platelets acidified with sodium propionate in the presence of 0.1 National Institutes of Health units of thrombin per milliliter. B([H⁺]ᵢ), is the cellular H⁺ concentration of untreated platelets that is equivalent to the basal pHᵢ. The Xᵢ and Zᵢ values for thrombin-nontreated platelets are 0.1421 μM H⁺ (pH 6.847) and 0.0832 pH units/sec x μM H⁺. The Xᵢ and Zᵢ values for thrombin-treated platelets are 0.0877 μM H⁺ (pH=7.057) and 0.2529 pH units/sec x μM H⁺, respectively.

The apparent set point (Xᵢ), expressed in cytosolic H⁺ concentration ([H⁺]ᵢ), and the activity index (Zᵢ) of the Na-H antiport were obtained by linear regressions describing the relation between the initial rate of pHᵢ recovery (y axis) as a function of the initial [H⁺]ᵢ, from which the recovery proceeded (x axis). This approach is illustrated in Figure 1. The x-axis intercept of the linear regression is the Xᵢ, whereas the slope is the Zᵢ for the Na-H antiport within the tested range of [H⁺]ᵢ. An alkaline shift in the Xᵢ is expressed by a shift to the left in the x-axis intercept, whereas an increase in the Zᵢ is described by an increment in the slope of the regression line. A usual response to thrombin and other agonists that activate the Na-H antiport is an alkaline shift in the Xᵢ, increased Zᵢ, or both (for further details, see References 20 and 21). The ΔZᵢ expresses the increase in the activity index of the Na-H antiport after thrombin treatment, and it represents the extent of Na-H antiport stimulation by thrombin within the range of [H⁺]ᵢ tested.

Statistical evaluation used one-way analysis of variance (followed, if necessary, by the Duncan multiple range test) and correlation analyses. All computations were performed with an IBM-compatible personal computer (SAS, REG, and GLM programs, SAS Institute, Cary, N.C.). Because of technical problems, not all platelet
TABLE 1. Characteristics of Subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age (years)</th>
<th>BMI* (kg/m²)</th>
<th>SBP* (mm Hg)</th>
<th>DBP* (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norm</td>
<td>44.1±2.4</td>
<td>25.0±0.6</td>
<td>111.8±4.2</td>
<td>71.9±2.7</td>
</tr>
<tr>
<td>Obese</td>
<td>45.3±2.6</td>
<td>34.4±1.1</td>
<td>130.4±4.6</td>
<td>82.8±3.1</td>
</tr>
<tr>
<td>NIDDM</td>
<td>46.0±1.8</td>
<td>33.9±1.0</td>
<td>124.4±3.1</td>
<td>80.2±2.5</td>
</tr>
</tbody>
</table>

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; NIDDM, diabetic obese group; Obese, nondiabetic obese group; Norm, nondiabetic nonobese group.

Obese 188.0±11.1
NIDDM 191.4±14.2

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, under 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 Zj 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 ΔZj and blood pressure may therefore reflect increased platelet sensitivity to thrombin and a higher thrombin-evoked Ca2+ response associated with a higher blood pressure. Our recent findings (unpublished data, Zentay et al) indicates that the Zj was positively correlated with the thrombin-evoked rise in the Ca2+.

In a recent study Livne et al have shown that resting platelets of presumably white hypertensive men exhibited an alkaline shift in the Xj 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 Xj. 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. Effect of HDL on platelets may relate to its capacity to release cholesterol from the plasma membrane since increased serum cholesterol or platelet cholesterol concentration...
We recently proposed that an alkaline shift in the $X_i$ may be the lasting imprint of elevated $Ca$ and platelet hyperactivity. A number of investigations have shown a positive correlation between platelet $Ca$ and blood pressure. A rise in platelet $Ca$ 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 nondiabetic nonobese, nondiabetic obese, and diabetic obese black women, parameters of the Na-H antiport in aspirin-treated platelets were significantly correlated with blood pressure as well as serum HDL and the TC/HDL ratio.

**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 range (a negative x-axis intercept in Figure 1). Insets do not include this subject since data are expressed in pH units. ⋄, Diabetic obese group (n=12); □, nondiabetic obese group (n=12); and ○, nondiabetic nonobese group (n=11). For the entire sample population the correlations for the $X_i$ vs. HDL were $r=0.5679$ and $p=0.0004$; for the nondiabetic population, $r=0.6044$ and $p=0.0023$. Correlations of the $X_i$ vs. TC/HDL ratio for the entire population were $r=-0.70613$ and $p=0.0001$, and for the nondiabetic population, $r=-0.6150$ and $p=0.0018$.

References
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