Na^+/Li^+ and Na^+/H^+ Countertransport Activity in Hypertensive Non–Insulin-Dependent Diabetic Patients: Role of Insulin Resistance and Antihypertensive Treatment

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We measured erythrocyte Na^+/Li^+ and Na^+/H^+ countertransport (CT) activity (millimoles per liter per cell per hour) in 10 healthy control subjects (age, 38 ± 4 years; body mass index, 25 ± 1 kg/m^2) and in 25 hypertensive patients with non–insulin-dependent diabetes mellitus ([NIDDM] age, 49 ± 3 years; body mass index, 29 ± 1 kg/m^2; fasting plasma glucose, 157 ± 12 mg/dL) 4 weeks after discontinuation of previous antihypertensive treatment. Na^+/Li^+ CT was significantly increased in hypertensive NIDDM patients compared with controls (0.56 ± 0.04 *v* 0.30 ± 0.03, $P < .01$), whereas Na^+/H^+ CT was similar to control levels (21 ± 1 *v* 20 ± 2). A positive correlation was found between Na^+/Li^+ CT and the severity of insulin resistance ($r = .69$, $P < .01$), mean arterial pressure ([MAP] $r = .64$, $P < .01$), plasma triglyceride concentration ($r = .46$, $P < .05$), and plasma total cholesterol ($r = .41$, $P < .05$). An inverse correlation was found between Na^+/Li^+ CT activity and plasma insulin concentration ($r = -.47$, $P < .05$). No relationship was observed between Na^+/Li^+ CT activity and either creatinine clearance or proteinuria. Stepwise multiple regression analysis for all metabolic variables and blood pressure showed that only the severity of insulin resistance was positively correlated with increased Na^+/Li^+ CT activity. Na^+/H^+ and Na^+/Li^+ CT activity were not altered by 3 hours of euglycemic physiologic hyperinsulinemia (84 ± 3 μU/mL). Hypertensive NIDDM subjects were treated for 3 months with captopril, nifedipine, or doxazosin. After captopril, a reduction of Na^+/H^+ CT was observed (22 ± 4 *v* 13 ± 2, $P < .05$); Na^+/Li^+ CT decreased after doxazosin (0.56 ± 0.06 *v* 0.45 ± 0.05, $P < .05$) and nifedipine (0.52 ± 0.06 *v* 0.42 ± 0.05, $P < .05$). In conclusion, in hypertensive NIDDM subjects, (1) Na^+/Li^+ CT is increased and is correlated with the level of insulin resistance and the MAP; (2) acute physiologic hyperinsulinemia does not affect Na^+/Li^+ or Na^+/H^+ CT activity; and (3) Na^+/H^+ CT activity is reduced by captopril, and Na^+/Li^+ CT is decreased by doxazosin and nifedipine.

THE Na^+/H^+ countertransport (CT) exchanger is a ubiquitous electroneutral system involved in the regulation of intracellular pH, cell growth, cell volume regulation, and transepithelial Na transport.[1,2] Recent studies have implicated increased Na^+/H^+ CT activity in the pathogenesis of essential hypertension in man.[3-5]

Because the Na^+/H^+ CT exchanger also can exchange Li^+ for H^+ and Li^+ for Na^+, the Na^+/Li^+ CT pump may represent a functional mode of the pH regulation system (Na^+/H^+ CT) or of a closely related antiporter isoform.[6] The similarities and differences between Na^+/Li^+ and Na^+/H^+ CT have been examined extensively.[6] The maximal transport activity of the Na^+ exchanger is about 25 to 30 mmol/L · cell · h. At intracellular sites, Na^+ can be replaced by Li^+. However, Na^+ cannot occupy the intracellular regulatory site. As a result, the rate of Na^+/Li^+ exchange for external Na^+ is approximately 100 times slower than for H^+ efflux in exchange for Na^+ influx. A recent finding by Busch et al[7] suggests that Na^+/Li^+ CT reflects Na^+/H^+ CT activity at pH 7.4. These investigators found that expression of the human Na^+/H^+ CT exchanger in *Xenopus* oocytes enhances Na^+/H^+ CT activity and establishes Na^+/Li^+ CT activity.[7]

Several studies have demonstrated that red blood cell (RBC) Na^+/Li^+ CT activity is increased in hypertensive patients and their first-degree relatives,[8] and this abnormality has been suggested as a preclinical marker of hypertension.[9] Previous reports also have shown that increased RBC Na^+/Li^+ CT activity is associated with insulin resistance in patients with essential hypertension[10,11] and in patients with IDDM.[12] In IDDM individuals, increased RBC Na^+/Li^+ CT activity also has been correlated with glomerular hyperfiltration[13] and has been suggested to represent a genetic marker for the development of diabetic nephropathy.[14,15]

In patients with NIDDM, the findings have been more conflicting. Some investigators have reported increased RBC Na^+/Li^+ CT activity in type II diabetic patients with hypertension[16,17] and with diabetic nephropathy,[16-18] whereas others have failed to demonstrate abnormal RBC Na^+/Li^+ CT activity in either normotensive or hypertensive NIDDM subjects.[19,20] No data about the relationship between insulin sensitivity and RBC Na^+/Li^+ CT activity are available in NIDDM patients. This is surprising, since elevated blood pressure occurs in 40% to 50% of type II diabetic patients[21] and both essential hypertension[22] and diabetes[23,24] have been shown to be associated with insulin resistance. The effect of the major classes of antihypertensive drugs on these ion countertransport systems has yet to be investigated.

The present study was undertaken to evaluate the following in hypertensive NIDDM patients: (1) the maximal activity of RBC Na^+/H^+ and Na^+/Li^+ CT pumps; (2) the relationship between Na^+/H^+ and Na^+/Li^+ CT and the characteristic metabolic abnormalities found in patients with NIDDM; and (3)

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the effect of antihypertensive drug treatment on Na^+/H^+ and Na^+/Li^+ CT activity.

SUBJECTS AND METHODS

Subjects

The study population consisted of 10 healthy control subjects and 25 hypertensive non–insulin-dependent diabetic (NIDDM) patients (Table 1). The normal subjects were aged 33 to 45 years. There were six men and four women, and all were within 90% to 120% of their ideal body weight based on the midpoint for medium-frame individuals from the 1959 Metropolitan Life Insurance Tables. Their weight and height were 76 ± 2 kg and 171 ± 3 cm, respectively. No subject had any history of renal, metabolic, or cardiovascular disease, and routine screening tests, including blood chemistry and urinalysis, were normal.

NIDDM patients were aged 35 to 65 years and were within 100% to 140% of ideal body weight. Although their mean age and ideal body weight were slightly greater than in controls, there are no data to suggest that such small differences have any effect on either Na^+/Li^+ or Na^+/H^+ CT activity.[8-10] Their fasting plasma glucose was between 120 and 220 mg/dL. To be included in the study, NIDDM subjects were required to have a blood pressure greater than 140/90 mm Hg without antihypertensive medication for a period of at least 4 weeks. NIDDM subjects had no evidence of renal (as defined by serum creatinine <1.5 mg/dL), endocrine (excluding diabetes), or other major organ system disease as determined by a complete medical history, physical examination, and routine laboratory tests. Although serum creatinine was less than 1.5 mg/dL in all diabetic subjects and the glomerular filtration rate was normal in absolute terms (Table 1), this does not exclude the possibility that some individuals had early, mild diabetic renal disease. A more detailed description of the characteristics of the diabetic patient population is shown in Table 1. Other than sulfonylurea drugs, no subject was taking any other medication known to affect glucose or lipid metabolism before entry into the study. Patients who had previously taken insulin were excluded from the study. For patients who were taking antihypertensive drugs, the medication was discontinued gradually over a period of 2 weeks, and they remained untreated with any blood pressure medications for an additional 2 weeks. Body weight was stable for at least 3 months before entry to the study in all participants. Subjects participated in light to moderate physical activity, and none were enrolled in a physical training program or performed any heavy exercise, ie, weight-lifting, running, etc., for at least 3 months before participation in the study.

Table 1. Characteristics of the Hypertensive NIDDM Subjects

Characteristic	Value
No. of subjects	25
Sex (male/female)	18/7
Race (Caucasian/Mexican-American)	10/15
Age (yr)	49 ± 3
Ideal body weight (%)	112 ± 3
FPG (mg/dL)	157 ± 12
FPI (mU/mL)	19 ± 2
MAP (mm Hg)	114 ± 2
Total cholesterol (mg/dL)	193 ± 9
HDL cholesterol (mg/dL)	40 ± 3
Triglycerides (mg/dL)	205 ± 26
Creatinine clearance (mL/min)	96 ± 7
Proteinuria (mg/d)	260 ± 48
Duration of diabetes (yr)	7 ± 2
Duration of hypertension (yr)	4 ± 2
Antihypertensive treatment (n)	20
Sulfonylurea treatment (n)	21
Diet only (n)	4

Data with "±" are the mean ± SEM.

Abbreviations: FPG, fasting plasma glucose; FPI, fasting plasma insulin.

The purpose, nature, and potential risks of the study were explained to all subjects, and their written voluntary consent was obtained before study participation. The experimental protocol was reviewed and approved by the Institutional Review Board of The University of Texas Health Science Center at San Antonio.

Study Design

In the morning on the day of the insulin clamp, a fasting blood sample was drawn for the basal measurement of RBC Na^+/H^+ and Na^+/Li^+ CT activity. In diabetic subjects, these measurements were performed 4 weeks after discontinuation of all antihypertensive medications. The following measurements also were performed in the diabetic and control subjects: fasting plasma glucose and insulin, hemoglobin A_{Ic}, serum total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, 24-hour endogenous creatinine clearance, and 24-hour urinary protein excretion.

Insulin sensitivity was assessed in all subjects with the euglycemic insulin clamp,[25] which was performed in combination with 3-^{3}H-glucose infusion and indirect calorimetry. At 8 AM, after an overnight fast, a 20-gauge Teflon catheter was inserted retrogradely into a wrist vein for blood sampling, and the hand was placed into a heated (60°C) box to achieve arterialization of the venous blood. An antecubital vein in the contralateral arm was cannulated with an 18-gauge catheter, and this line was used for infusion of insulin, cold glucose, and 3-^{3}H-glucose. Catheters were kept patent with a slow infusion of 0.9% saline. A primed constant infusion of 3-^{3}H-glucose (Dupont, Boston, MA) was started and continued for the entire length (360 minutes) of the study. The prime infusion of 3-^{3}H-glucose was calculated as follows: 20 μCi × (fasting plasma glucose ÷ 100). The continuous titrated glucose infusion rate was 0.20 μCi/min. At the end of the basal tracer equilibration period (180 minutes), the euglycemic insulin clamp was performed. The rate of insulin (Humulin-R; Eli Lilly, Indianapolis, IN) infusion was 40 $mU/m^2 \cdot min$ during the subsequent 180 minutes. In NIDDM patients, after the insulin infusion was started, no glucose was infused until the plasma glucose decreased to 100 mg/dL, at which level it was clamped by measuring at 5-minute intervals and periodically adjusting a 20% dextrose infusion.[25] During the last 50 minutes of the basal period and throughout the 180-minute insulin clamp study, blood samples were drawn every 5 to 15 minutes for determination of plasma 3-^{3}H-glucose specific activity and plasma insulin concentration. During the last 60 minutes of the basal period and the last 60 minutes of the insulin clamp, continuous indirect calorimetry was performed as previously described.[26] A computerized, continuous open-circuit system (Deltatrac; Sensormedics, Anaheim, CA) was used to measure gas exchange. Timed urine collections were obtained during the basal and insulin clamp periods for determination of urinary nitrogen. At the end of the insulin clamp, a blood sample was drawn for measurement of erythrocyte Na^+/H^+ and Na^+/Li^+ countertransport activity in all subjects. The insulin clamp results have been reported previously.[27]

Following completion of the insulin clamp studies, NIDDM patients were assigned to one of three groups: captopril (n = 7, Capoten 25 to 50 mg twice daily; Bristol-Myers Squibb, Princeton, NJ), nifedipine (n = 9, Procardia XL 30 to 60 mg once daily; Pfizer Labs, New New York, NY), or doxazosin (n = 9, Cardura 1 to 8 mg once daily or 8 mg twice daily; Pfizer Labs). Captopril and nifedipine were administered in double-blind fashion. Every third patient was assigned to the doxazosin group. The dosage of each medication was adjusted until diastolic blood pressure (DBP) was reduced to less than 85 mm Hg. No limiting side effects occurred in any individual. Patients returned to the Clinical Research Center at least twice weekly during the first 4 weeks of treatment and one to two times per week thereafter. Blood pressure was measured at each visit after a period of 15 minutes in the supine

position, using an automatic recording sphygmomanometer (Dynamap; Critikon, Tampa, FL). The mean of three blood pressure measurements taken at 1-minute intervals was recorded. Blood pressure medication was titrated to the maximum dose over the first 4 weeks. Once the desired blood pressure was attained, patients were maintained on a constant dosage for an additional 8 weeks. The mean dosages for the last 8 weeks of treatment were as follows: captopril 55 ± 5 mg/d, nifedipine 50 ± 4 mg/d, and doxazosin 8 ± 2 mg/d.

At the end of the 12-week treatment period, fasting blood samples were drawn for measurement of RBC Na^+/H^+ and Na^+/Li^+ CT activity. The following measurements also were repeated: fasting plasma glucose and insulin, hemoglobin A_{1c}, serum total cholesterol, HDL cholesterol, triglycerides, 24-hour creatinine clearance, and 24-hour urinary protein excretion.

Measurement of Erythrocyte Na^+/H^+ and Na^+/Li^+ CT Activity

A fasting blood sample was drawn into heparinized tubes for measurement of RBC Na^+/H^+ and Na^+/Li^+ CT activity. The blood was centrifuged at 2,000 × *g* for 4 minutes at 4°C. The plasma and buffy coat were removed by aspiration, and the RBCs were suspended at 20% hematocrit in cold preserving solution containing 10 mmol/L NaCl, 140 mmol/L KCl, 2.5 mmol/L KPO_4 buffer, pH 7.4, and 1 mmol/L $MgCl_2$, with osmolarity of 300 mOsm. The RBC samples were shipped overnight to Boston in Styrofoam buckets containing crushed ice. The next morning, RBCs were processed as follows. The plasma and the buffy coat were removed by aspiration, and the RBCs were washed four times with ice-cold washing solution containing 150 mmol/L choline chloride, 1 mmol/L $MgCl_2$, and 10 mmol/L Tris-MOPS, pH 7.4 (4°C). A 50% cell suspension was made using the ice-cold washing solution, and the hematocrit was measured after centrifuging capillary tubes for 10 minutes in a microhematocrit centrifuge. Aliquots of this suspension were diluted (1:50) with 0.02% Acationox detergent (Sherwood, Davis, & Geck, St Louis MO) in double-distilled water for hemoglobin determination by optical density at 540 nm, and Na^+ and K^+ concentrations were determined by atomic absorption spectrophotometry.

Erythrocyte Na^+/H^+ CT activity was measured by determining the initial rate of net Na^+ influx after an outward H^+ gradient was imposed.[11] This was performed by measuring Na^+ influx after incubation of erythrocytes in media with pH 8.0 and pH 6.0 to block the Na^+/H^+ exchanger. The difference in the rate of Na^+ influx determined at pH 8.0 and pH 6.0 provides a measure of the Na^+ influx that is driven by an outward H^+ gradient. A detailed description of the methodology has been published previously.[11]

Na^+/Li^+ exchange was measured as external sodium-stimulated Li^+ efflux from preloaded erythrocytes as previously reported.[11] The nystatin loading procedure was used to load RBCs to 7 mmol Li^+/L cells, to enhance the precision of measurement of Li^+ concentration in the flux media. A detailed description of the methodology has been published previously.[11]

Metabolic Measurements

Plasma glucose concentration was measured with a Beckman glucose oxidase analyzer (Beckman Instruments, Fullerton, CA). For determination of tritiated glucose specific activity, the plasma was deproteinized according to the Somogyi procedure and centrifuged for 10 minutes at 4,000 rpm. The clear supernatant was evaporated to dryness at 55°C, resuspended in 1 mL distilled water, mixed with 5 mL Scintiverse II (Fisher Scientific, Pittsburgh, PA), and counted in a Beckman LS 6000 IC scintillation counter. Hemoglobin A_{1c} levels were measured by affinity chromatography with a commercial kit (Isolab; Biochemical Methodology, Akron, OH). Plasma insulin was determined by radioimmunoassay (Coat-A-Count; Diagnostic Products, Los Angeles, CA). Plasma total cholesterol and triglyceride levels were measured enzymatically on a Hitachi 704 Autoanalyzer (Boehringer-Mannheim Biochemicals, Indianapolis, IN) [28] HDL cholesterol level was measured enzymatically on the Hitachi 704 Autoanalyzer after precipitation of chylomicrons, very–low-density lipoprotein, and low-density lipoprotein (LDL) cholesterol by phosphotungstic acid.[28] LDL cholesterol content was calculated from the Friedewald equation. Urinary nitrogen level was measured by the method of Kjeldahl.[29] Urinary protein content was measured on 24-hour urinary samples using a modification of the Coomassie blue method.[30] Interassay and intraassay coefficients of variation for urinary protein concentration were less than 10%.

Calculations and Statistical Analysis

Mean arterial pressure (MAP) was calculated from systolic (SBP) and diastolic (DBP) blood pressure readings as follows: (SBP − DBP)/3 + DBP. During the last 40 minutes of the basal period, a steady-state plateau of 3-^{3}H-glucose specific activity (dpm per milligram) was achieved in all subjects, and hepatic glucose production (HGP) was determined by dividing the continuous 3-^{3}H-glucose infusion rate (dpm per minute) by the steady-state plasma tritiated glucose specific activity (dpm per milligram). After administration of insulin and glucose, non–steady-state conditions exist, and the rates of glucose appearance and disappearance were calculated from the isotopic data using Steele's equation.[31] The rate of HGP (milligrams per square meter per minute) was calculated by subtracting the rate of exogenous glucose infusion from the rate of total glucose appearance determined by the isotope-tracer technique. Total-body glucose disposal (milligrams per square meter per minute) was calculated as the sum of the exogenous glucose infusion plus the rate of residual HGP. This value agreed closely with the rate of disappearance determined from the kinetics of tritiated glucose. Glucose oxidation and lipid oxidation (milligrams per square meter per minute) were calculated from the nonprotein respiratory quotient as previously described.[26] Nonoxidative glucose disposal, which provides an index of glycogen formation,[32] was calculated by subtracting the rate of glucose oxidation from the rate of whole-body glucose uptake.

All values are expressed as the mean ± SEM. Comparisons between the normal group and the three diabetic groups were performed using ANOVA. In NIDDM patients, comparison of data between pretreatment and posttreatment periods within a study group (intragroup) was performed using Student's *t* test for paired data. Correlation coefficients were determined using standard equations. In NIDDM subjects, a stepwise multiple regression analysis was performed between RBC Na^+/Li^+ taken as the dependent variable and the metabolic/hemodynamic parameters taken as independent variables. Because of skewed distribution, the metabolic/hemodynamic parameters (MAP, insulin-mediated glucose disposal, basal HGP, glucose oxidation, nonoxidative glucose disposal, body mass index, fasting plasma insulin, fasting plasma glucose, triglycerides, total cholesterol, LDL cholesterol, and HDL cholesterol) were analyzed with logarithmic transformation. All statistical analyses were performed with StatView software (Abacus Concepts, Berkeley, CA).

RESULTS

Arterial Blood Pressure and Glucose Metabolism

At the end of the 4-week washout period of previous antihypertensive medications, arterial blood pressure was 152 ± 4/93 ± 1 mm Hg (captopril), 154 ± 3/94 ± 1 (nifedipine), and 157 ± 3/95 ± 2 (doxazosin). Following 12 weeks of antihypertensive treatment, both SBP and DBP decreased significantly in all groups: 140 ± 3/85 ± 2 (captopril), 141 ± 2/85 ± 1 (nifedipine), and 142 ± 4/82 ± 3 (doxazosin) (all $P < .001$ *v* pretreatment). The decrements in SBP and DBP were similar in all three groups.

Before the start of antihypertensive therapy, fasting plasma

glucose was 146 ± 16, 161 ± 9, and 149 ± 10 mg/dL in the captopril, nifedipine, and doxazosin groups, respectively, and did not change significantly following treatment in any of the three groups (151 ± 17, 171 ± 6, and 148 ± 12 mg/dL, respectively, for captopril, nifedipine, and doxazosin). Pretreatment hemoglobin A_{1c} levels were similar in the captopril (6.3% ± 0.9%), nifedipine (6.8% ± 0.8%), and doxazosin (6.5% ± 1.0%) groups and did not change significantly with hypertensive treatment (6.3% ± 1.0%, 6.6% ± 0.9%, and 6.2% ± 1.0% for the captopril, nifedipine, and doxazosin groups, respectively).

During the euglycemic insulin clamp performed before the start of antihypertensive treatment in NIDDM patients, fasting plasma insulin increased from 19 ± 2 μU/mL to 84 ± 3, while plasma glucose was maintained at 101 ± 4 mg/dL. In all studies, the coefficient of variation for plasma glucose was less than 5%. Basdal HGP, which equals tissue glucose disposal in the postabsorptive state, was 74 ± 4 mg/m^2 · min. During the 40-mU/m^2 · min euglycemic insulin clamp, total-body glucose disposal, glucose oxidation, and nonoxidative glucose disposal were 147 ± 13, 80 ± 5, and 72 ± 6 mg/m^2 · min, respectively. Basal HGP was suppressed to 4 ± 2 mg/m^2 · min during insulin infusion.

In normal subjects, the basal plasma insulin level was 5 ± 1 μU/mL and increased to 78 ± 5 μU/mL during the last 60 minutes of the insulin clamp study, while the basal plasma glucose concentration (85 ± 4 mg/dL) was maintained at 88 ± 3 mg/dL. In all studies, the coefficient of variation for plasma glucose was less than 5%. During the 40-mU/m^2 · min euglycemic insulin clamp, total-body glucose disposal, glucose oxidation, and nonoxidative glucose disposal were 264 ± 18 ($P < .01$ *v* NIDDM), 106 ± 11 ($P < .01$), and 158 ± 14 ($P < .01$) mg/m^2 · min, respectively. During insulin infusion, basal HGP (65 ± 5 mg/m^2 · min) declined to 1 ± 2 mg/m^2 · min. In NIDDM subjects, total-body glucose disposal was positively correlated with the fasting plasma insulin concentration ($r = .43$, $P < .05$).

Erythrocyte Na^+/H^+ and Na^+/Li^+ CT Activity

At the end of the 4-week run-in period and following a 12-hour overnight fast, the maximal velocity of RBC Na^+/H^+ CT (20.8 ± 2 mmol/L · cell · h), the K_m for intracellular H^+ (pH_i 6.34 ± 0.2), and the Hill coefficient (n_{app} 2.24 ± 0.4) in NIDDM patients were similar to the values (20.4 ± 2 mmol/L · cell · h, pH_i 6.41 ± 0.1, and n_{app} 2.43 ± 0.3, respectively) obtained in normal subjects. In contrast, RBC Na^+/Li^+ CT in NIDDM patients (0.56 ± 0.04 mmol/L · cell · h) was significantly increased compared with that in normal subjects (0.30 ± 0.03 mmol/L · cell · h, $P < .01$) (Fig 1).

In 25 hypertensive NIDDM patients, a significant correlation was found between RBC Na^+/Li^+ CT activity at 150 mmol/L Na^+ and MAP ($r = .64$, $P < .01$; Fig 2). The correlation between MAP and Na^+/Li^+ CT activity was maintained if the data from normal subjects were included in the analysis ($r = .47$, $P < .05$). In addition, both SBP ($r = .47$, $P < .05$) and DBP ($r = .44$, $P < .05$) were significantly correlated with Na^+/Li^+ CT activity. Positive correlations also were noted between RBC Na^+/Li^+ CT activity and HGP ($r = .46$, $P < .05$), plasma triglyceride concentration ($r = .46$, $P < .05$; Fig 2), and

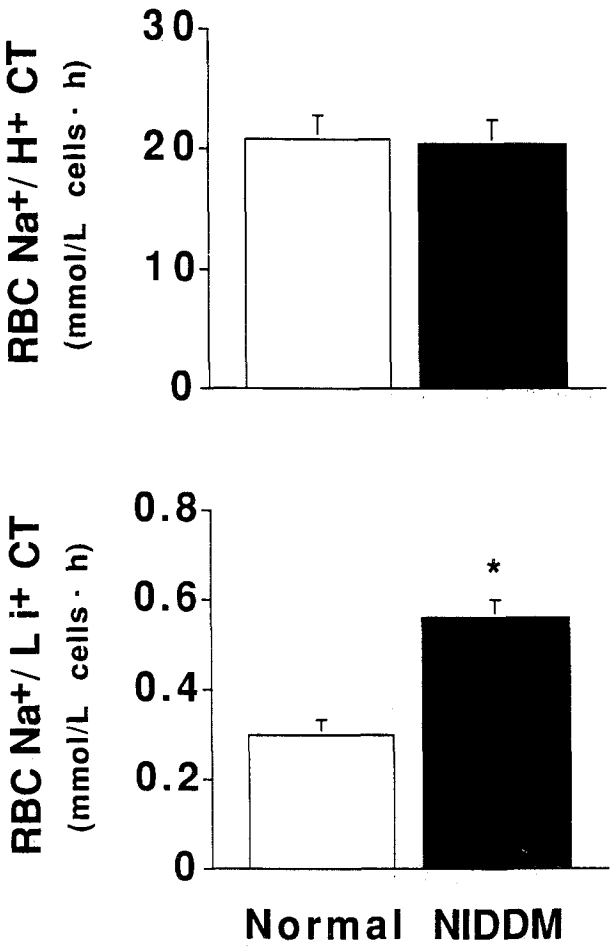

Fig 1. Maximal rate of RBC Na^+/H^+ and Na^+/Li^+ CT activity in normal subjects and in hypertensive NIDDM patients. Values are the mean ± SEM. *$P < .05$ *v* normal.

plasma total cholesterol concentration ($r = .41$, $P < .05$). Plasma triglyceride concentration was correlated positively with MAP ($r = .606$, $P < .01$) and inversely with insulin-mediated glucose disposal ($r = -.53$, $P < .05$). Doxazosin treatment significantly reduced plasma triglyceride concentrations (from 218 ± 18 to 178 ± 14, $P < .05$), but had no effect on plasma cholesterol levels (198 ± 6 *v* 194 ± 8). Captopril and nifedipine had no effect on any lipid parameters.

In diabetic patients, a significant inverse correlation was found between RBC Na^+/Li^+ CT activity and both the rate of insulin-mediated total-body glucose disposal ($r = -.69$, $P < .01$) and the fasting plasma insulin concentration ($r = -.47$, $P < .05$; Fig 2). When stepwise multiple regression analysis was performed between RBC Na^+/Li^+ CT activity as the dependent variable and the metabolic/hemodynamic variables (including whole-body insulin-mediated glucose disposal, nonoxidative glucose disposal, glucose oxidation, basal HGP, fasting plasma glucose, fasting plasma insulin, hemoglobin A_{1c}, body mass index, plasma triglycerides, plasma total cholesterol, plasma LDL cholesterol, plasma HDL cholesterol, SBP, and DBP), the strongest correlation was with insulin-mediated total-body glucose disposal ($r = .41$, $P < .01$).

In diabetic patients at the end of the 3-hour euglycemic

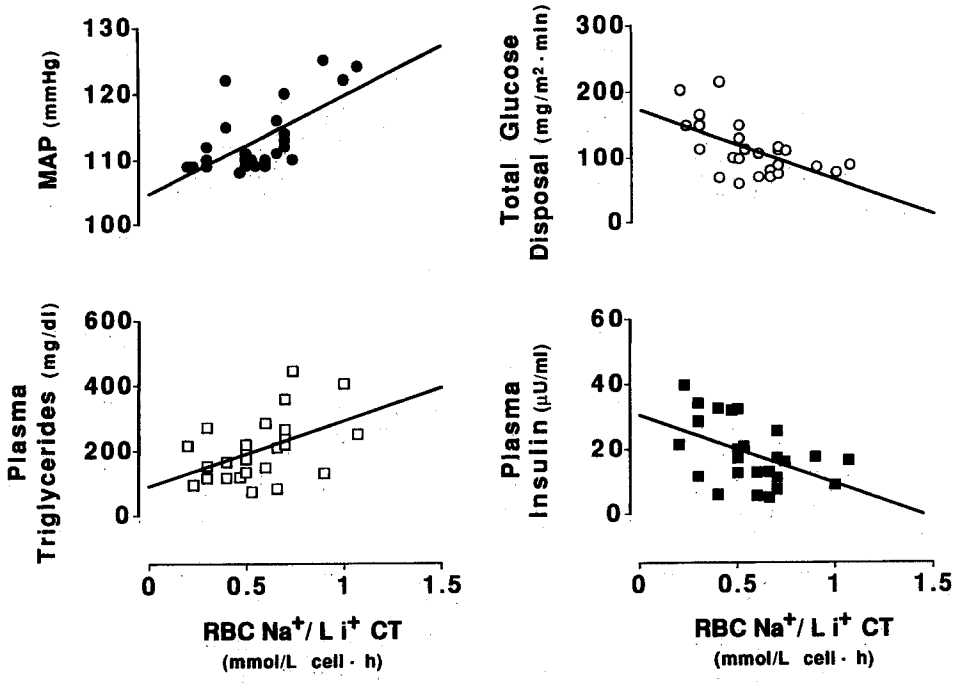

Fig 2. Correlation between the maximal rate of RBC Na^+/Li^+ CT activity and MAP ($r = .64$, $P < .01$), insulin-mediated total-body glucose disposal ($r = -.59$, $P < .01$), plasma triglyceride concentration ($r = .47$, $P < .05$), and fasting plasma insulin concentration ($r = -.47$, $P < .05$) in hypertensive NIDDM patients.

insulin clamp, no changes were observed in RBC Na^+/H^+ CT activity (22.6 ± 3 mmol/L · cell · h), the K_m for intracellular H^+ (pH_i 6.25 ± 0.1), the Hill coefficient (n_{app} 2.31 ± 0.4), or RBC Na^+/Li^+ CT activity (0.55 ± 0.2 mmol/L · cell · h) in comparison to baseline. Similarly, in normal subjects, euglycemic hyperinsulinemia failed to alter RBC Na^+/H^+ CT activity (25.8 ± 3 mmol/L · cell · h), the K_m for intracellular H^+ (pH_i 6.43 ± 0.1), the Hill coefficient (n_{app} 2.91 ± 0.5), or RBC Na^+/Li^+ CT activity (0.26 ± 0.05 mmol/L · cell · h) in comparison to basal values.

In diabetic subjects after 12 weeks of antihypertensive treatment, RBC Na^+/H^+ CT activity decreased significantly in the captopril group (from 21.5 ± 4 to 12.7 ± 2 mmol/L · cell · h, $P < .05$) without a change in either the K_m for intracellular H^+ (pH_i 6.28 ± 0.1 *v* 6.20 ± 0.1) or the Hill coefficient (n_{app} 2.44 ± 0.3 *v* 2.60 ± 0.3). In contrast, no changes were observed after nifedipine treatment for RBC Na^+/H^+ CT activity (22.9 ± 5 *v* 23.1 ± 4 mmol/L · cell · h), the K_m for intracellular H^+ (pH_i 6.44 ± 0.1 *v* 6.35 ± 0.1), or the Hill coefficient (n_{app} 2.17 ± 0.2 *v* 2.08 ± 0.4). Doxazosin treatment also failed to alter RBC Na^+/H^+ CT activity (18.1 ± 4 *v* 18.0 ± 4 mmol/L · cell · h), the K_m for intracellular H^+ (pH_i 6.27 ± 0.1 *v* 6.36 ± 0.1), and the Hill coefficient (n_{app} 2.11 ± 0.4 *v* 2.93 ± 0.6) (Fig 3). A significant decrease in RBC Na^+/Li^+ CT activity was obtained after treatment with nifedipine (0.52 ± 0.06 *v* 0.42 ± 0.05 mmol/L · cell · h, $P < .05$) and

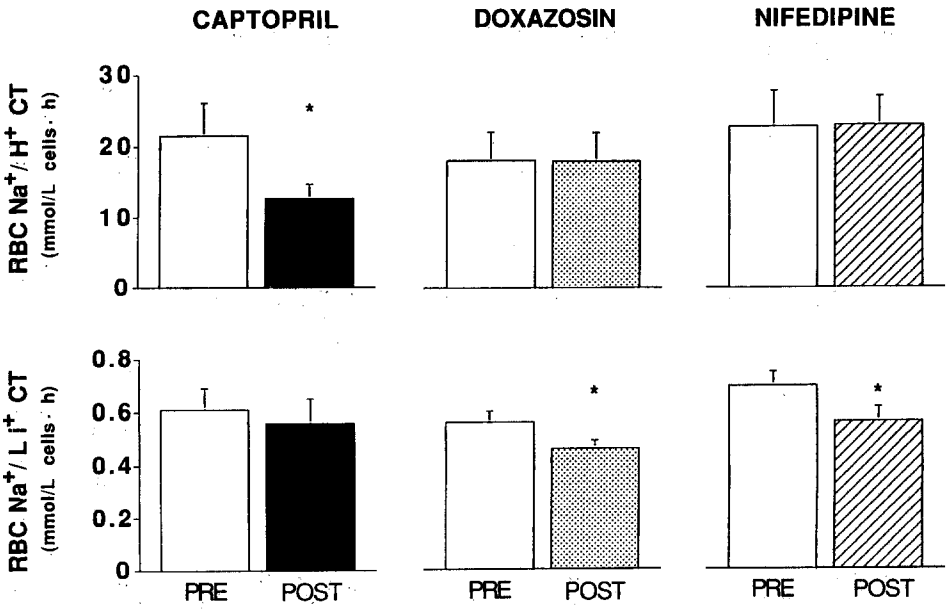

Fig 3. Maximal rate of RBC Na^+/Li^+ and Na^+/H^+ CT activity in hypertensive NIDDM patients before (PRE) and after (POST) 3 months of treatment with captopril, doxazosin, and nifedipine. *$P < .05$ *v* baseline.

doxazosin (0.56 ± 0.06 *v* 0.45 ± 0.05 mmol/L · cell · h, $P < .05$), whereas captopril treatment did not significantly affect this parameter (0.61 ± 0.08 *v* 0.56 ± 0.09 mmol/L · cell · h; Fig 3).

DISCUSSION

The results of the present study demonstrate that RBC Na^+/Li^+ CT activity is increased by about 85% in hypertensive NIDDM patients compared with normal subjects, whereas RBC Na^+/H^+ CT activity is unchanged. Our findings also demonstrate for the first time in type II diabetics that there is an association between elevated RBC Na^+/Li^+ CT activity and insulin resistance, blood pressure, and plasma triglyceride concentration. These results suggest that enhanced Na^+/Li^+ CT activity represents an additional feature of the metabolic and hemodynamic abnormalities that characterize insulin-resistant patients with NIDDM.[23,24] The data also provide evidence that acute physiological hyperinsulinemia does not affect either Na^+/Li^+ or Na^+/H^+ CT activity. Finally, antihypertensive treatment with captopril significantly decreased RBC Na^+/H^+ CT activity, whereas a reduction in RBC Na^+/Li^+ CT activity was observed after doxazosin and nifedipine administration.

The association between insulin resistance and increased Na^+/Li^+ CT activity deserves further comment. A large proportion of nondiabetic hypertensive patients are characterized by insulin resistance[22] and increased Na^+/Li^+ CT activity.[10,11] Conversely, in nondiabetic hypertensive patients with normal insulin sensitivity, Na^+/Li^+ CT activity is unchanged.[10] In normotensive IDDM individuals, increased Na^+/Li^+ CT activity is associated with reduced insulin sensitivity and hypertriglyceridemia.[12,33] In the present study, plasma triglyceride concentration was significantly correlated with Na^+/Li^+ CT activity, blood pressure, and insulin resistance. Wu et al[34] recently demonstrated a genetic linkage between SBP and a locus near the lipoprotein lipase gene in Asian hypertensive families. Whether a similar association also is present for Na^+/Li^+ CT activity is presently unknown. Taken collectively, previously published results[10-12,22,33,34] and results of the present study suggest that increased Na^+/Li^+ CT activity, plasma triglyceride levels, blood pressure regulation, and insulin resistance are closely related.

In this study, we observed that increased RBC Na^+/Li^+ CT activity in hypertensive NIDDM patients was inversely correlated with total glucose disposal. Similar findings have been reported by Doria et al[10] in essential hypertension patients. In our NIDDM subjects, the most insulin-resistant individuals had the lowest fasting plasma insulin levels, most likely reflecting the progressive time-related decline in β-cell secretory capacity that occurs during the natural history of NIDDM.[35,36] Since the most insulin-resistant NIDDM patients have the lowest fasting insulin levels (Fig 2), it is obvious that insulin resistance, and not hyperinsulinemia, is primarily related to the increased Na^+/Li^+ CT activity. Consistent with this concept, acute physiologic hyperinsulinemia, while maintaining euglycemia, had no effect on RBC Na^+/Li^+ and Na^+/H^+ CT activity. This observation is consistent with the failure of insulin infusion to alter either RBC Na^+/Li^+ or Na^+/H^+ CT activity in patients with borderline hypertension.[11] A stepwise multiple regression analysis between Na^+/Li^+ CT as the dependent variable and all metabolic/hemodynamic parameters showed that the severity of insulin resistance correlated best with the increased Na^+/Li^+ CT activity. These observations suggest that abnormalities in Na^+/Li^+ CT activity might result from the long-term effect of the insulin-resistant state.

Previous studies in NIDDM patients have yielded conflicting results about RBC Na^+/Li^+ CT activity. Trevisan et al[19] failed to observe an increase in erythrocyte Na^+/Li^+ CT activity, whereas other investigators have reported increased RBC activity in hypertensive NIDDM patients[16,17] and in NIDDM patients with clinical nephropathy.[16-18]

To our knowledge, Na^+/H^+ CT activity in erythrocytes of NIDDM patients has not been examined previously. In the present study, we failed to demonstrate significant changes in Na^+/H^+ CT activity or an association between Na^+/H^+ CT activity and any metabolic parameter or blood pressure in our diabetic patients. Elevated Na^+/H^+ CT has been associated with essential hypertension.[5,11] However, in those studies, elevated Na^+/Li^+ CT activity was not strongly associated with increased Na^+/H^+ CT activity.[5] The different changes in Na^+/H^+ and Na^+/Li^+ CT activity may reflect two different modes of action of the Na^+ antiporter. In particular, Na^+/Li^+ CT activity has been suggested to reflect the low activity of the Na^+ transporter at pH 7.4, whereas Na^+/H^+ activity reflects its maximal transport activity.

The ability of captopril treatment to reduce erythrocyte Na^+/H^+ CT activity by approximately 40% in hypertensive NIDDM patients is novel and may be of importance in understanding the well-established protective effect of angiotensin-converting enzyme (ACE) inhibitors in diabetic nephropathy.[37,38] Our observation that ACE inhibitors reduce Na^+/H^+ exchange in type II hypertensive individuals is consistent with previous findings in nondiabetic hypertensive subjects.[39] The mechanism(s) responsible for this effect remains unclear, but may be mediated by the decline in plasma angiotensin II levels following captopril administration. In vitro, angiotensin II directly stimulates Na^+/Li^+ activity, as well as cell hypertrophy and proliferation.[40-43] However, erythrocytes are not known to possess angiotensin II receptors,[44] and direct effects of angiotensin II on erythrocytes are not known. Since there is no evidence that ACE inhibitors bind directly to erythrocytes, an as yet unknown indirect mechanism is suggested in the action of captopril on Na^+/H^+ CT activity. Treatment with captopril had no effect on Na^+/Li^+ activity. This observation is consistent with a report by Niutta et al.[45] In contrast, both doxazosin and nifedipine significantly reduced the activity of the Na^+/Li^+ CT system. The differential responses between nifedipine and doxazosin versus the ACE inhibitors cannot be ascribed to the effect of the agents on blood pressure, which was similarly reduced in all three groups.

In summary, the present results demonstrate that RBC Na^+/Li^+ CT activity is significantly increased in hypertensive NIDDM patients and is strongly associated with insulin resistance. In contrast, RBC Na^+/H^+ CT activity is not increased in type II diabetic subjects. Despite a similar reduction in MAP, antihypertensive treatment showed a differential inhibitory effect on Na^+/H^+ and Na^+/Li^+ CT activity. ACE inhibition significantly reduced RBC Na^+/H^+ CT, whereas calcium-

channel antagonists and α_1-adrenergic blockers reduced RBC Na^+/Li^+ CT.

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