Cellular Sodium Membrane Transport and Cardiovascular Risk Factors in Non–Insulin-Dependent Diabetes Mellitus

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Associations have been described between cardiovascular risk factors and abnormalities of both sodium-lithium countertransport (SLC) and sodium-hydrogen ion exchange in subjects with insulin-dependent diabetes mellitus. The data in subjects with non–insulin-dependent diabetes mellitus (NIDDM) are few and more conflicting. This investigation examines erythrocyte SLC rates and platelet sodium-hydrogen ion-exchange kinetics and their relationship to cardiovascular risk factors in 45 nondiabetic and 35 NIDDM white men. The two groups did not differ significantly in erythrocyte SLC or platelet buffering capacity, sodium-hydrogen ion-exchange maximal rate (Vmax), or K_m for extracellular sodium. Within the whole group, controlling for the presence of diabetes, SLC correlated weakly with triglyceride concentration ($r = .23$, $P = .05$), but not with urinary albumin excretion rate (AER), systolic or diastolic blood pressure, body mass index (BMI), or concentrations of glucose, insulin, or total or high-density lipoprotein (HDL) cholesterol. Platelet sodium-hydrogen exchange was not significantly related to any cardiovascular risk factor studied. In conclusion, (1) SLC activity was not increased in NIDDM subjects; (2) SLC rates correlated weakly with serum triglyceride concentrations; (3) platelet sodium-hydrogen exchange Vmax and K_m for extracellular sodium and platelet buffering capacity did not differ between diabetic and nondiabetic groups; and (4) there was no significant relationship between platelet Na^+/H^+-exchange kinetics and any of the cardiovascular risk factors studied.

REAVEN[1] POSTULATED that the clustering of cardiovascular risk factors, including hypertension, dyslipidemia, and non–insulin-dependent diabetes mellitus (NIDDM), could be explained by assigning a primary role to insulin resistance. However, in addition to the insulin-resistant phenotype, some subjects at high risk for vascular disease exhibit a range of abnormalities of cell membrane sodium transport.

Elevated rates of erythrocyte sodium-lithium countertransport (SLC) have been described in subjects with essential hypertension,[2-5] and subsequent reports suggested correlations with body mass index (BMI),[6-8] fasting insulin,[9,10] and plasma lipids.[9,11,12] Increases in SLC have been shown in insulin-dependent diabetic subjects with nephropathy,[13-15] and in such subjects SLC rates correlate closely with insulin resistance.[16] Increased rates of Na^+-H^+ exchange in various cell types have also been shown to associate with essential hypertension.[17,18]

There are few data on erythrocyte SLC,[19-21] and even fewer on Na^+-H^+ exchange, in NIDDM. The present investigation was undertaken (1) to examine whether activities of the two ion-transport systems, erythrocyte SLC and platelet Na^+-H^+ exchange, differ between NIDDM and control subjects, and (2) to define their relationships to a range of cardiovascular risk factors.

SUBJECTS AND METHODS

Subjects

We studied 35 white men with NIDDM recruited consecutively from the diabetic clinic at Whittington Hospital. Forty-five white nondiabetic men aged 40 to 75 years registered with a North London group practice were studied as controls. These subjects were recalled as part of a population-screening study for microalbuminuria,[22] and represented all white men invited for recall. The selection criteria for recall matched each microalbuminuric subject with two normoalbuminuric subjects. For this reason, there was a high prevalence of microalbuminuria in the nondiabetic group. Nine diabetics but no control subjects were taking antihypertensive medication, which was discontinued 48 hours before study. Subjects reported to the Clinical Investigation Unit after an overnight fast. Each individual underwent a timed urine collection for measurement of albumin excretion rate (AER). Height and weight were measured without shoes and in light clothing, and BMI was calculated as weight in kilograms divided by height in meters squared. Blood pressure was measured after 15 minutes' rest using a mercury sphygmomanometer. Fasting blood samples were taken for determination of plasma glucose, insulin, and lipid concentrations, erythrocyte SLC activity, platelet buffering capacity, and platelet Na^+-H^+–exchange transport kinetics.

Assays

Urinary AER was determined using an in-house enzyme-linked immunosorbent assay similar to that described by Chesham et al.[23] Plasma glucose was determined by a glucose oxidase method (Beckman Analyzer; Beckman Instruments, Brea, CA) and insulin by an in-house two-site immunoenzymometric assay,[24] with monoclonal antibodies supplied by Serono Diagnostics (Woking, Surrey, UK). Intraassay and interassay coefficients of variation for the latter technique are 5.9% and 11.8%, respectively.

Erythrocyte SLC

SLC activity was determined as reported previously[25] according to the published method of Canessa et al[2] as later modified by Mangili et al.[26] Using this technique, passive leaking of lithium in sodium-free medium represents 30% to 35% of efflux in sodium-rich medium, comparing favorably with results obtained by others.[2] With careful control of osmolality, pH, and temperature, no hemolysis occurs in this assay. We have documented a within-assay coefficient of variation of 9.5% and a between-assay plus biological variation of 12.4% in eight subjects studied on five occasions over 9 to 21 months.[25]

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Platelet Sodium-Hydrogen Ion Exchange

Platelets were isolated from platelet-rich plasma by gel filtration through Sepharose CL 2B (Pharmacia, Milton Keynes, UK), and measurement of Na^+-H^+–exchange kinetics was made using the technique of Halkin et al.[27] In brief, cells were loaded with the pH-dependent fluorescent dye, biscarboxyethylcarboxyfluorescein-acetoxymethyl ester, and changes in intracellular pH were measured by dual-excitation fluorimetry on a Perkin Elmer LS-3B fluorometer (Beaconsfield, UK). Changes in the excitation to emission ratio using wavelengths of 440 nm, 500 nm (excitation), and 530 nm (emission) were recorded. For the assay, platelets were acidified by incubating the cells in and then removing 30 mmol · L^{-1} NH_4Cl to activate the antiporter, and six concentrations of NaCl (5 to 140 mmol · L^{-1}) were added to separate aliquots of the cell suspension, with the initial rate of alkalinization being calculated as the tangent of the initial slope of the fluorescence ratio. By assessing this rate at six different extracellular sodium concentrations, the maximal rate of Na^+-H^+ ion exchange (Vmax) and K_m for extracellular sodium were determined using a Lineweaver-Burke plot. A standard curve constructed by equilibrating intracellular and extracellular pH using 8 μg · mL^{-1} nigericin allowed changes in the fluorescence ratio to be converted to changes in intracellular pH. The correlation coefficient of the straight line produced exceeded .98 in every case. Buffering capacity was calculated for each individual as the pH change induced by the addition of 8 mmol · L^{-1} NH_4Cl.[28] Mean interassay coefficients of variation for these variables have been previously assessed and found to be 12.5% (K_m), 18.2% (Vmax), and 11.8% (buffering capacity). However, these values are likely to be overestimates, since they are based on measurements made in a single individual on separate days, and thus represent not only assay variation but also biological variation in transport activity.

Statistical Analysis

Statistical analysis was performed on the entire group of subjects. Comparisons were made using Student's *t* test for normally distributed data and the Mann-Whitney *U* test for skewed data. ANOVA with logarithmic transformation of skewed variables was used to permit adjustment for covariates in such analyses. Linear or multiple regression with logarithmic transformation of skewed data was also used, with a dummy variable to allow for the presence of diabetes. *P* values less than .05 were taken as significant. This number of subjects was sufficient to demonstrate significant relationships for correlation coefficients greater than .23 overall and approximately .3 in diabetic and nondiabetic subgroups separately.

RESULTS

The diabetic cohort was significantly younger and had a greater BMI than the nondiabetic group (Table 1). Mean systolic but not diastolic blood pressure was also higher in the diabetic group. Although plasma glucose was, by definition, increased in the diabetic group, this was not reflected in the fasting insulin concentration, although the range of values was diverse. There was no difference in total cholesterol, but high-density lipoprotein (HDL) cholesterol was significantly lower and triglycerides were higher in diabetic subjects. There was no difference in erythrocyte SLC, platelet buffering capacity, or K_m and Vmax for Na^+-H^+ exchange.

Figure 1 shows a breakdown of the study population according to blood pressure status and AER. In total, seven nondiabetic and nine diabetic subjects were hypertensive (blood pressure ≥ 160/95 mm Hg), and 12 nondiabetic and seven diabetic individuals were microalbuminuric (AER > 20 μg · min^{-1}). In 28 normotensive normoalbuminuric nondiabetic subjects, SLC was 0.395 ± 0.185 mmol · L^{-1} · h^{-1}. There was no significant difference in SLC, Na^+-H^+ exchange Vmax, K_m, or platelet buffering capacity

Table 1. Characteristics of the Two Study Groups

Characteristic	Nondiabetic (n = 45)	NIDDM (n = 35)
Age (yr)	61.2 ± 11.1	55.6 ± 7.35*
BMI (kg · m^{-2})	25.6 ± 4.4	28.2 ± 3.9†
Systolic blood pressure (mm Hg)	131.3 ± 18.2	144.1 ± 22.5†
Diastolic blood pressure (mm Hg)	79.4 ± 15.0	84.3 ± 11.8
AER (μg · min^{-1})	6.4 (2.4-158.3)	8.2 (0.2-191.3)
Fasting plasma glucose (mmol · L^{-1})	4.6 ± 0.6	10.1 ± 4.0†
Fasting plasma insulin (pmol · L^{-1})	37.8 (13.8-157.0)	59.0 (2.6-183.2)
Total cholesterol (mmol · L^{-1})	5.55 ± 0.99	5.49 ± 1.07
HDL cholesterol (mmol · L^{-1})	1.34 ± 0.36	1.12 ± 0.33*
Triglycerides (mmol · L^{-1})	1.3 (0.7-3.5)	1.65 (0.4-6.9)*
SLC (mmol · L^{-1} · h^{-1})	0.387 ± 0.195	0.399 ± 0.147
Platelet buffering capacity (mmol · L^{-1} · pH $unit^{-1}$)	22.5 (1.2-82.6)	24.7 (11.6-164.0)
K_m for sodium-hydrogen ion exchange (mmol · L^{-1})	51.4 (21.8-406.1)	49.1 (13.2-182.6)
Vmax for sodium-hydrogen ion exchange (mmol · L^{-1} · min^{-1})	0.81 (0.06-5.02)	0.70 (0.19-4.04)

NOTE. Values are the mean ± SD for normally distributed data and the median (range) for skewed data.
*$P < .05$.
†$P < .01$.

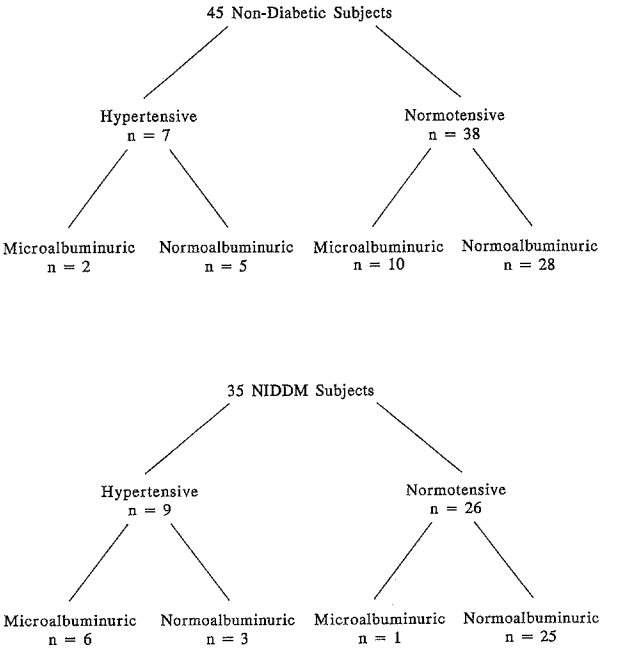

Fig 1. Breakdown of study groups according to AER and blood pressure status.

Table 2. Comparisons of Indices of Membrane Sodium Transport Between Subjects With and Without Hypertension and With and Without Microalbuminuria

Variable	Normotensive (n = 64)	Hypertensive (n = 16)	*P*	Normoalbuminuric (n = 61)	Microalbuminuric (n = 19)	*P*
Erythrocyte SLC (mmol · L^{-1} · h^{-1})	0.38 ± 0.18	0.43 ± 0.17	.37	0.39 ± 0.17	0.42 ± 0.21	.71
Platelet Na^+-H^+ Vmax (mmol · L^{-1} · min^{-1})	0.80 (0.14-5.02)	0.60 (0.06-2.83)	.41	0.77 (0.14-4.04)	0.88 (0.06-5.02)	.88
K_m for extracellular Na^+ (mmol · L^{-1})	50.7 (13.2-406.2)	49.4 (18.0-156.0)	.31	46.9 (13.2-375.9)	53.5 (24.1-406.2)	.09
Platelet buffering capacity (mmol · L^{-1} · pH^{-1})	24.4 (3.5-82.6)	22.1 (1.2-164.4)	.36	24.9 (3.5-82.6)	18.4 (1.2-164.4)	.16

NOTE. Subjects were dichotomized into normotensive and hypertensive (cutoff, 160/95 mm Hg) and normoalbuminuric and microalbuminuric (cutoff urinary AER, 20 μg · min^{-1}) groups. *P* values are given for ANOVA with the presence of diabetes as a covariate. Data are the mean ± SD or the median (range).

between hypertensive and normotensive subjects, or between those with and without microalbuminuria. Table 2 shows these variables in the whole group, adjusting for the presence of diabetes. Separate analyses of diabetic and nondiabetic subjects again showed no differences. There remained no significant difference between diabetic and nondiabetic subjects in any of the sodium transport variables when microalbuminuric or hypertensive subjects were excluded from the analysis.

Correlations were sought between sodium membrane-transport variables and cardiovascular risk factors, treating blood pressure and AER as continuous variables. This analysis was performed on the combined group after adjustment for the presence of diabetes and for age and BMI. Table 3 shows the data for all 80 subjects. SLC was negatively correlated with Vmax for Na^+-H^+ exchange ($r = -.24$, $P = .04$). There were no other relationships between the two transport-system variables. SLC was related inversely to age ($r = -.30$, $P < .01$) and positively to triglyceride ($r = .23$, $P = .05$), but correlations with systolic blood pressure ($r = .21$, $P = .06$), diastolic blood pressure ($r = .19$, $P = .10$), and BMI ($r = .15$, $P = .19$) failed to reach significance. Neither platelet buffering capacity nor Na^+-H^+ kinetics were related to age, BMI, or any of the

Table 3. Correlation Matrix Showing Relationships Between Sodium-Transport Indices and Cardiovascular Risk Factors in the Combined Group of Diabetic and Nondiabetic Subjects

	Total Triglyceride	HDL Cholesterol	Total Cholesterol	Systolic BP	Diastolic BP	Fasting Insulin	Urinary AER	Buffering Capacity	Na/H K_m	Na/H Vmax
SLC										
r	.23	.02	−.03	.21	.19	.06	−.03	.04	−.15	−.24
P	.05	.90	.90	.06	.10	.63	.83	.73	.20	.04
Na^+-H^+										
r	−.04	−.09	−.07	−.12	−.15	.14	−.03	.25	.55	
P	.71	.45	.55	.29	.20	.26	.90	.03	<.001	
Na^+-H^+ K_m										
r	−.07	−.05	−.15	−.19	−.06	−.08	.01	−.03		
P	.53	.66	.22	.10	.59	.51	.93	.82		
Buffering capacity										
r	.08	−.22	−.06	−.05	−.13	−.01	−.09			
P	.51	.06	.59	.64	.28	.93	.45			
Urinary AER										
r	−.122	−.02	.04	.28	.23	.12				
P	.30	.89	.73	.01	.04	.30				
Fasting insulin										
r	.07	−.07	.10	.10	.07					
P	.57	.59	.41	.41	.58					
Diastolic BP										
r	.03	.07	.07	.61						
P	.56	.57	.56	<.001						
Systolic BP										
r	.07	.14	.05							
P	.56	.24	.69							
Total cholesterol										
r	.35	.10								
P	.002	.40								
HDL cholesterol										
r	−.56									
P	.56									

NOTE. Correlation coefficients (*r*) are adjusted for age, BMI, and the presence of diabetes; two-tailed *P* values are given.
Abbreviation: BP, blood pressure.

other variables studied (Table 3). However, Vmax for Na^+-H^+ exchange was positively related to platelet buffering capacity and K_m ($r = .25$, $P = .03$ and $r = .55$, $P < .001$, respectively). In the whole group, age, BMI, and systolic and diastolic blood pressure were associated with AER. The correlation analysis was repeated with the inclusion of an interaction term for diabetes and SLC, with little difference in the correlation coefficients, suggesting similar relationships in diabetic and nondiabetic subgroups. In the two separate subgroups, there were no significant independent correlations of SLC or Na^+-H^+ variables with either blood pressure or AER (data not shown).

DISCUSSION

We examined the relationships between cardiovascular risk factors and two different cellular ion-transport systems in nondiabetic and NIDDM subjects. The data suggest that neither erythrocyte SLC nor platelet Na^+-H^+–exchange Vmax and the K_m for extracellular sodium differ between NIDDM and nondiabetic subjects, and that they are not strongly related to cardiovascular risk factors.

No difference was observed in SLC rates between the two groups. Although our estimate of SLC in normotensive normoalbuminuric nondiabetics appeared high, the sample size of 28 produces a 95% confidence interval of 0.323 to 0.467 mmol $\cdot$ L^{-1} $\cdot$ h^{-1}, a figure compatible with that observed in several other studies,[29,30] especially in similar age groups.[7] The results of previous studies in subjects with NIDDM have been conflicting. Two studies have reported no difference in SLC rates between uncomplicated NIDDM and nondiabetic control subjects.[31,32] A third study demonstrated increased SLC in both nephropathic and uncomplicated NIDDM subjects,[20] and a fourth report showed increased SLC in black hypertensive subjects.[19] One small study found elevated SLC in NIDDM patients with nephropathy, but did not study nondiabetic controls.[33] We have also previously reported no differences in SLC between nondiabetic and NIDDM subjects in a largely separate group of subjects.[34] In this study, we were unable to demonstrate any relationship between SLC rates and AER in 35 NIDDM subjects, 45 nondiabetic subjects, or the combined group of all subjects.

In the combined group, there were borderline correlations of SLC with systolic blood pressure and triglyceride concentrations. However, there was no relationship between SLC and AER, serum insulin, and HDL cholesterol. In a previous large population study,[7,35] SLC activity was significantly related to both systolic and diastolic blood pressure and to associated factors including BMI, HDL cholesterol, plasma uric acid, and glucose, with correlation coefficients ranging from .11 to .23. It is possible, then, that the insignificant relationships between SLC and the risk factors we studied are a consequence of a type II error,[36] although a previous study found significant correlations with glucose and triglyceride concentrations in just 26 NIDDM subject.[33]

Few data have yet been published concerning platelet Na^+-H^+ exchange in NIDDM. In this study, a wide variation was observed in the indices of platelet Na^+-H^+ exchange, and although interrelated, there were no differences between diabetic and nondiabetic groups. Moreover, none of these indices showed any relationship with the cardiovascular risk factors examined, including blood pressure and AER. The relationships between platelet buffering capacity, Na^+-H^+–exchange Vmax, and K_m result from the method used in their calculation. Platelet buffering capacity is measured directly and is used in the calculation of H^+ efflux rate, with both Vmax and K_m being subsequently derived from the slope and intercept of a Lineweaver-Burke plot. In a previous study, we found a reduction in buffering capacity in microalbuminuric NIDDM subjects but no difference between diabetic patients and nondiabetic controls.[37] A small study using a cell-swelling method to estimate Na^+-H^+ exchange reported an increase in nephropathic versus normoalbuminuric NIDDM patients,[33] but the method used is a crude technique that does not dissect out the separate kinetic components of the exchange mechanism we have studied.

The relationship between SLC and Na^+-H^+ exchange is controversial, and even when these are measured in the same cell type, they may not be linearly related.[38] A positive relationship between the two transport systems measured in different cell types would therefore appear unlikely. Paradoxically, we found a weak negative association between erythrocyte SLC rates and Vmax for platelet Na^+-H^+ exchange, which may simply represent a type I error.[36]

In the present study, kinetic analysis of Na^+-H^+ exchange was performed for extracellular sodium binding. The results do not exclude the possibility of differences in intracellular hydrogen ion binding kinetics. Furthermore, we examined only standardized SLC rates, as opposed to the K_m for extracellular sodium ions or the Vmax for the sodium-lithium exchanger. Conditions in which increased SLC has been described have been associated with changes in K_m or Vmax for the sodium-lithium exchanger,[39] and it is possible that changes in both of these variables could occur without a net effect on the countertransport rate. However, we chose to study SLC as the best-defined sodium membrane transport abnormality associated with hypertension and other cardiovascular risk factors. It is nevertheless possible that kinetic studies of SLC might prove more informative than measures of crude SLC activity.

In summary, we observed no differences between nondiabetic and NIDDM subjects in terms of either erythrocyte SLC rates or Na^+-H^+–exchange kinetics for extracellular sodium. A weak relationship between SLC and triglyceride was observed in the combined group, but no other relationships were seen with the cardiovascular risk factors examined. Thus, SLC appears to be weakly associated, if at all, with these cardiovascular risk factors. In contrast, no relationship was observed between platelet Na^+-H^+ kinetics and the cardiovascular risk factors studied. Our data suggest that neither of these ion-transport systems are specifically disturbed in NIDDM subjects.

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