Nephropathy in NIDDM is Associated With Cellular Markers for Hypertension

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OBJECTIVE — To determine if nephropathy in NIDDM is associated with cellular markers for hypertension.

RESEARCH DESIGN AND METHODS — We compared 11 patients with clinical diabetic nephropathy with 15 control subjects with no diabetic nephropathy. The patients were white and had had NIDDM for ≥9 yr and serum creatinine levels ≤177 M (2.0 mg/dl).

RESULTS — Patients with nephropathy were more obese and had higher blood pressures and triglyceride levels, and lower high-density lipoprotein cholesterol levels than control subjects. Erythrocyte sodium lithium countertransport activity, erythrocyte sodium content, and platelet sodium-proton exchange were higher in patients with nephropathy than in control subjects.

CONCLUSIONS — The results of this small study suggest that in white patients with long-standing NIDDM, diabetic nephropathy is associated with cellular markers for hypertension. Measurement of cellular markers for hypertension may be useful to identify patients who are at risk for developing nephropathy.

Increased erythrocyte SLC activity is associated with hypertension in white populations (1,2) and has been proposed as a genetic marker for hereditary predisposition to hypertension. Increased SLC activity also has been described in IDDM patients with glomerular hyperfiltration (3), microalbuminuria (4), or overt diabetic nephropathy (5,6). Thus, increased SLC activity may be a marker for predisposition to nephropathy in IDDM. Only one study has evaluated the relationship of SLC activity to nephropathy in NIDDM and found it not to be specifically associated with nephropathy (7). We undertook this study to determine whether cellular markers for hypertension are associated with nephropathy in NIDDM.

RESEARCH DESIGN AND METHODS

— We selected patients with NIDDM, as defined by National Diabetes Data Group criteria (8), from the adult diabetes clinic. Patients with diabetic nephropathy had proteinuria ≥0.5 g/24 h and no clinical or laboratory evidence of nondiabetic renal disease. Control subjects had urinary protein excretion ≤0.15 g/24 h. Because racial differences in SLC activity have been described (2), we limited our study to white patients. We also selected patients with known disease durations of ≥9 yr and serum creatinine levels ≤177 M (2.0 mg/dl), because duration of diabetes is an important risk factor for nephropathy in NIDDM (9), and renal insufficiency can be associated with changes in BMI, BP, and serum lipid levels.

The University of Michigan Institutional Review Board for Human Research reviewed and approved the protocol. Each study participant gave written informed consent.

We studied 11 patients with diabetic nephropathy and 15 control subjects without nephropathy. We measured platelet sodium-proton exchange in 5 patients with diabetic nephropathy and 8 control subjects in whom assays could be completed within 3 h of blood drawing. This group was younger than the group in which we did not measure platelet sodium-proton exchange (54 ± 2 yr vs. 64 ± 3 yr, $P = 0.01$), but the groups did not differ with respect to sex, known duration of diabetes, diabetes treatment, urine protein, serum creatinine, CCr, BMI, BP, fasting insulin and glucose, GHb, fasting lipids, and potassium.

After each patient sat for 5 min, we measured BP with a standard sphygmomanometer and cuff appropriate to arm size, and obtained a fasting blood sample. Glucose was measured with an

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NIDDM, NON-INSULIN-DEPENDENT DIABETES MELLITUS; SLC, SODIUM LITHIUM COUNTERTRANSPORT; IDDM, INSULIN-DEPENDENT DIABETES MELLITUS; BMI, BODY MASS INDEX; BP, BLOOD PRESSURE; PA, PICRIC ACID; CCr, CREATININE CLEARANCE; RIA, RADIOIMMUNOASSAY; HDL, HIGH-DENSITY LIPOPROTEIN; TG, TRIGLYCERIDE; LDL, LOW-DENSITY LIPOPROTEIN; ANOVA, ANALYSIS OF VARIANCE; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 1— *Clinical characteristics of the study population*

	Patients with nephropathy	Control subjects	*P* value
n	11	15	
Age (yr)	59 ± 3	59 ± 3	0.97
Sex (M/F)	8/3	9/6	0.79
Known duration of diabetes (yr)	16 ± 2	15 ± 2	0.89
Insulin treated (%)	82	73	0.97
24° urine protein (g)	1.79 ± 0.46	0.09 ± 0.01	<0.01
Serum creatinine (μM)	115 ± 18	88 ± 9	0.12
CCr (ml/s)	1.87 ± 0.31	1.67 ± 0.15	0.53

Data are means ± SE.

autoanalyzer using a hexokinase method. Potassium was measured with an ion-selective electrode. Serum and urine creatinine were measured with the PA method, and CCr was calculated with the method of Cockcroft and Gault (10). RIA was used to measure insulin. GHb was measured with boronate affinity chromatography.

Total cholesterol was measured with the cholesterol oxidase method, HDL cholesterol with dextran sulfate-magnesium chloride precipitation, and TG with the glycerol dehydrogenase method. LDL-cholesterol levels were calculated from the measured total cholesterol, HDL, and TG levels. Urine protein was measured with the coomassie blue method. SLC activity was measured with the method of Canessa et al. (1), with minor modifications as described by Weder et al. (2,11). Erythrocyte sodium content was measured with washed erythrocytes (2). Platelet sodium-proton exchange was measured with a volumetric technique using propionic acid activation (12,13).

We measured several parameters related to acid activation of the amiloride-sensitive sodium-proton antiporter, including the amount of amiloride-sensitive platelet swelling during the first 2 min of incubation (initial) and the maximal amiloride-sensitive platelet swelling after 10 min of incubation (total). Both volume-change parameters were expressed as a percentage increase over baseline platelet volume (12). In addition, the slope of the line relating amiloride-sensitive platelet volume increase to the concentration of sodium propionate was calculated over a range of 28–140 mM sodium propionate (slope). All three parameters have been observed to increase in patients with essential hypertension (13).

Statistical analysis

The significance of differences between groups was tested with the unpaired Student's *t* test and the χ^2 test. The relationships among variables within groups were tested with simple linear regression. To assess the joint effects of multiple variables on SLC activity, we made potential confounders discrete at their median values, and used ANOVA with a cell-means model to assess differences. Data are expressed as means ± SE.

RESULTS — The two groups did not differ with respect to age, sex, known duration of diabetes, or diabetes treatment (Table 1). Consistent with our selection criteria, patients with diabetic nephropathy had significantly higher 24-h urine protein excretion rates than control subjects, but did not differ with respect to serum creatinine or calculated CCr (Table 1). On average, patients with nephropathy were more obese and had higher sBP, dBP, and mean arterial pressure than control subjects (Table 2).

Fasting insulin, glucose, and GHb levels did not differ between the

Table 2— *Physical and biochemical characteristics of the study population*

	Patients with nephropathy	Control subjects	*P* value
n	11	15	
BMI (kg/m^2)	36 ± 2	30 ± 1	0.01
sBP (mmHg)	150 ± 8	133 ± 4	0.05
dBP (mmHg)	86 ± 3	75 ± 3	<0.01
Mean arterial pressure (mmHg)	108 ± 4	94 ± 3	<0.01
Fasting insulin (pM)	354 ± 114	306 ± 72	0.73
Fasting glucose (mM)	13.0 ± 2.0	9.7 ± 0.8	0.09
GHb (%)	12.0 ± 1.3	11.2 ± 0.6	0.54
Fasting lipids (mM)			
Cholesterol	5.4 ± 0.4	5.5 ± 0.3	0.94
HDL	0.8 ± 0.1	1.1 ± 0.1	<0.01
LDL	3.0 ± 0.4	3.5 ± 0.3	0.34
TG	8.6 ± 1.2	4.2 ± 0.5	<0.01
Potassium (mM)	4.4 ± 0.1	4.3 ± 0.1	0.27
Erythrocyte SLC (mM/L cells/h)	0.338 ± 0.053	0.214 ± 0.029	0.04
Erythrocyte sodium Content (mM/L cells)	6.47 ± 0.24	5.67 ± 0.28	0.05

Data are means ± SE.

Table 3— *Platelet sodium-proton exchange*

	Patients with nephropathy	Control subjects	*P* value
n	5	8	
Initial Δ (%)	19.0 ± 1.0	15.5 ± 1.1	0.06
Total Δ (%)	58.6 ± 2.3	45.7 ± 1.9	<0.01
Slope (%/mM sodium propionate)	0.40 ± 0.03	0.34 ± 0.02	0.08

Data are means ± SE.

two groups (Table 2). Although total cholesterol and LDL levels did not differ, patients with diabetic nephropathy had significantly higher TG levels and lower HDL levels than control subjects (Table 2). SLC activity and sodium content were significantly higher in the group with nephropathy than in the control group (Table 2). All parameters of sodium-proton exchange were increased in patients with diabetic nephropathy compared with control subjects, but only the difference in maximal amiloride-sensitive change in platelet volume (total) was statistically significant (Table 3).

SLC activity was not significantly associated with BMI or BP. In the nephropathy group, SLC activity was positively correlated with fasting glucose ($r = 0.69$, $P = 0.02$), whereas in the control group, SLC activity was positively correlated with TG ($r = 0.63$, $P = 0.01$) and negatively correlated with HDL cholesterol ($r = -0.69$, $P < 0.01$).

When we constructed a multivariate model to consider potential confounders, SLC activity was associated with fasting glucose ($P = 0.01$) and TG ($P = 0.02$) levels, and a case-control status-HDL interaction term ($P = 0.04$). The latter suggests that among the control subjects, higher SLC activity was associated with lower HDL-cholesterol levels. The model explained 66% of the variance in observed SLC activity ($R^2 = 0.66$, $P = 0.0003$). Maximal amiloride-sensitive platelet volume change (total Δ) was correlated with both SLC activity ($r = 0.55$, $P = 0.05$) and erythrocyte sodium content ($r = 0.68$, $P = 0.01$).

CONCLUSIONS— The results of this small study suggest that in white patients with long-standing NIDDM, nephropathy is associated with obesity, hypertension, hypertriglyceridemia, low HDL cholesterol, increased erythrocyte SLC activity, erythrocyte sodium content, and platelet sodium-proton exchange. Although hyperglycemia and dyslipidemia have been associated with nephropathy in NIDDM, the cellular markers for hypertension have not.

The association between nephropathy and increased SLC activity in NIDDM is consistent with the results of studies in IDDM, where renal hyperfiltration (3), microalbuminuria (4), and diabetic nephropathy (5,6) have been associated with increased SLC activity.

Our finding that nephropathy in NIDDM is associated with increased SLC activity differs from that of Gall et al. (7), who reported increased SLC activity in NIDDM patients both with and without nephropathy, compared with people without NIDDM. In their study, confounding factors may have raised SLC activity in all diabetic patients and affected the association between nephropathy and SLC activity (14). Another possible explanation is that heterogeneity exists between Danish and American populations, because Jensen et al. (15) failed to find increased SLC activity in a group of Danish patients with IDDM and nephropathy.

Our finding that nephropathy in NIDDM is associated with increased erythrocyte sodium content is new. Although Trevisan et al. (16) did not find increased erythrocyte sodium content in NIDDM patients compared with those without NIDDM, their data do not exclude the possibility of a subgroup with nephropathy and high erythrocyte sodium content.

Also new is our finding that nephropathy in NIDDM is associated with increased platelet sodium-proton exchange. The relationship between the erythrocyte sodium-lithium countertransporter and the platelet sodium-proton exchanger is controversial (17), but many hypertensive patients with increased activity of one transporter have increased activity of the other (12,13). We do not regard the weak positive correlations we observed as proof of the homology of the two transport functions, but our data suggest both systems may be markers of predisposition to nephropathy.

Increased erythrocyte SLC activity, erythrocyte sodium content, and platelet sodium-proton exchange are associated with nephropathy in NIDDM, and with hyperglycemia, hypertriglyceridemia, and low HDL-cholesterol levels. Whether the genetic factors responsible for these cellular markers actually contribute to the development of nephropathy is unclear, but because they are present before the development of hypertension, their measurement may help identify patients at risk for nephropathy (18,19). Prospective studies of nephropathy and the insulin resistance syndrome should include measurements of these cellular markers for hypertension.

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