Sodium-Lithium Transport in Adolescents With IDDM

Relationship to incipient nephropathy and glycemic control

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OBJECTIVE — The aims of this study were to examine genetic and environmental influences in the development of early diabetic nephropathy and to assess the value of measuring membrane sodium transport as a marker for early nephropathy.

RESEARCH DESIGN AND METHODS — We measured erythrocyte sodium-lithium (Na-Li) countertransport, blood pressure (BP), HbA_{1c}, and microalbuminuria (MA) in 84 adolescents with insulin-dependent diabetes mellitus (IDDM), 29 of whom had MA. Twenty-nine non-MA patients were selected and matched for age, sex, and IDDM duration with the 29 diabetic subjects with MA. The 84 diabetic adolescents were also compared with 85 nondiabetic siblings.

RESULTS — The erythrocyte Na-Li countertransport was significantly greater in the IDDM group than in the sibling group (mean ± SD, 0.41 ± 0.14 vs. 0.30 ± 0.11 mmol Li · liters of erythrocytes^{-1} · h^{-1}, respectively, $P < 0.0001$), but a significant correlation was noted between the results in IDDM subjects and their siblings ($r = 0.42$, $P < 0.0008$). Na-Li countertransport was not different in the diabetic subjects with or without MA (0.43 ± 0.13 vs. 0.37 ± 0.13 mmol Li · liters of erythrocytes^{-1} · h^{-1}, respectively). There was a significant correlation in the IDDM group between recent diabetic control (HbA_{1c}) and Na-Li countertransport ($r = 0.37$, $P < 0.003$). Diastolic BP was significantly higher in the IDDM group with MA than in those without MA (60 ± 6 vs. 55 ± 6 mmHg, respectively, $P < 0.03$).

CONCLUSIONS — These results suggest that erythrocyte Na-Li countertransport is influenced by the diabetic milieu. However, there was also evidence in our subjects of a genetic contribution to Na-Li countertransport as seen by the correlation between levels in the IDDM subjects and their siblings. Using Na-Li countertransport, we were not able to segregate those IDDM adolescents with and without early nephropathy.

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IDDM, insulin-dependent diabetes mellitus; MA, microalbuminuria; BP, blood pressure; AER, albumin excretion rate; BMI, body mass index.

Most patients with insulin-dependent diabetes mellitus (IDDM) develop renal abnormalities within a few years after the onset of the disease (1). These include glomerular hyperfiltration, renal hypertrophy, and morphologic lesions, which include glomerular basement membrane thickening, mesangial expansion, and microalbuminuria (MA) (1,2). However, only 30–40% of these patients exhibit progressive renal disease as manifested by overt proteinuria, hypertension, and declining renal function (3). Although the factors responsible for the development of nephropathy in this group of patients with IDDM are unknown, both genetic and environmental influences are potentially important. Of the environmental influences, glycemic control and alterations in glomerular hemodynamics have been implicated in the development of diabetic nephropathy with added factors such as systemic hypertension and lipid abnormalities contributing to the progression of established nephropathy (4–7).

Recent scientific literature has focused on certain heritable characteristics that may be useful in identifying patients at risk of developing nephropathy, and studies have suggested that the presence of either a family history of hypertension or abnormal membrane sodium transport may be such markers or risk factors (8–10). The rate of sodium-lithium (Na-Li) countertransport and the activity of the sodium-hydrogen (Na-H) exchanger have been widely studied as potential markers of essential hypertension (11–14). Erythrocyte Na-Li countertransport has been reported to be abnormal in normotensive patients with IDDM and nephropathy (9,10), and thus a potential marker for diabetic nephropathy.

The aims of this study were to examine erythrocyte Na-Li countertransport in adolescents with IDDM and their siblings to assess associations of this transporter with MA and blood pressure (BP), and to examine its use as a marker of early diabetic nephropathy. We thus

compared membrane sodium transport, HbA_{1c}, and BP in adolescents with IDDM and their siblings. In addition, we compared IDDM adolescents with and without evidence of incipient diabetic nephropathy as assessed by the presence of MA.

RESEARCH DESIGN AND METHODS

— All subjects were recruited from the diabetes clinic at The Hospital for Sick Children in Toronto. Informed consent was obtained from all study participants and their parents, and the study was approved by the human ethics review committee.

We screened 265 patients 12–18 years of age with IDDM of >2 years duration for the presence of MA by a timed 1-h urine collection (15). This represents >90% of the children with IDDM who attended the diabetes clinic from January to June 1991 and who fulfilled the age and diabetes duration criteria for study entry. Sixty-two (23%) of the 265 patients had MA on the initial 1-h screen. Seven were unwilling or unable to participate in the study. MA was confirmed by two 24-h urine collections in 29 of the remaining 55 subjects. These 29 patients with MA (accounting for 11.1% of the original 265 subjects) were matched for age (within 2 years), sex, and duration of diabetes (within 2 years) with an equal number of subjects with IDDM but without MA (negative 1-h screen and 24-h urine collections). The remaining 26 IDDM subjects who had MA on the initial 1-h screen, but were found to have normal urinary albumin excretion on the subsequent 24-h collections, were included in the IDDM group for a total of 84 diabetic subjects. The latter 26 subjects were added to increase the numbers of subjects for correlations and sibling comparisons. Subjects in this group of 26 did not differ from the 29 subjects without MA in any of the characteristics recorded. Eighty-five nondiabetic siblings (one IDDM adolescent had twin siblings and was compared to both) were also recruited. None of the study subjects were taking antihypertensive medications.

Microalbuminuria

Urine collections were stored at −20°C and analyzed within 3 months of collection. A double antibody radioimmunoassay (Pharmacia AB, Uppsala, Sweden) was used to measure urine albumin concentration. A positive screening test was defined as an albumin excretion rate (AER) >15 μg/min (5) and was confirmed with two 24-h urine collections for AER. MA was defined as urinary AER of 15–200 μg/min on the screen plus at least one of the two 24-h urine collections. Analysis of the data using cut-offs for MA of >20 or >30 μg/min did not alter the study results.

BP

Before BP measurement, subjects were seated at rest for at least 5 min in a quiet room. Measurements were made from the right arm in the sitting position every 2 min for 6 min using a Dinamap Vital Signs Monitor (Critikon, Tampa, FL). The mean of the last two readings was recorded provided that they did not differ by more than 5 mmHg. If so, a further reading was obtained and the average of the final two readings recorded.

Erythrocyte Na-Li countertransport

Erythrocyte Na-Li countertransport was measured by the method of Canessa et al. (11). Samples were collected between 0800 and 0900 in the immediate postprandial state in all subjects. Erythrocytes were separated within 1 h and the assay performed on the same day as the blood collection. In brief, 10 ml of heparinized blood was centrifuged for 15 min at 3,000 rpm at 4°C, and the erythrocytes were separated from plasma and buffy coat and washed four times with an ice-cold isotonic solution at 4°C (75 mM $MgCl_2$, 75 mM sucrose, 10 mM Tris MOPS, pH 7.4). Packed erythrocytes were incubated in a lithium loading solution (140 mM LiCl, 10 mM glucose, 10 mM Tris MOPS, pH 7.4) for 2.5 h at 37°C in a shaking water bath. Removal of extracellular lithium by repeated washings with the isotonic washing solution was followed by suspension of the cells (5% vol/vol) in both a magnesium medium (75 mM $MgCl_2$, 65 mM sucrose, Tris MOPS) and a sodium medium (140 mM NaCl, 10 mM glucose, Tris MOPS). The Tris concentration in the efflux media was 10 mM, and the pH was 7.4 at 37°C. Ouabain (0.1 mM) was added to the two efflux solutions to block the activity of the Na^+-K^+-ATPase. Aliquots were either centrifuged immediately (zero time incubation) or after a 60-min incubation in a shaking water bath at 37°C. Lithium determinations were performed on the supernatant using an atomic absorption spectrophotometer (Model 4000, Perkin-Elmer/Cetus, Norwalk, CT). The net flux of lithium was calculated from the difference between the lithium concentration in the sodium medium (active transport) and that in magnesium medium (passive diffusion or leak) after subtracting the values at zero incubation time. Results are expressed as mmol Li · liters of erythrocytes^{-1} · h^{-1}. The intra-assay and interassay coefficients of variation for this assay were 8.4 and 12.2%, respectively. These are consistent with the recommendations of Canessa et al. (16).

HbA_{1c}

HbA_{1c} was measured by high-performance liquid chromatography after removal of the labile fraction (nondiabetic reference range 4–6%) (17). The HbA_{1c} level at the time of the study was measured (current HbA_{1c}). In addition, a mean value was calculated for the duration of disease from previously measured values at each clinic visit (every 3–4 months) since the diagnosis of IDDM (duration mean HbA_{1c}).

Statistical analysis

The paired Student's *t* test was used to compare differences between groups where data was normally distributed (BP, HbA_{1c}). HbA_{1c} values were log transformed before analysis. The nonparamet-

Table 1—*Characteristics of subjects with IDDM and their nondiabetic siblings*

	IDDM subjects	Siblings
n	84	85
Sex (M/F)	40/44	37/48
Age (years)	16.3 ± 1.5	17.4 ± 4.9
BMI (kg/m^2)	23.0 ± 3	22.0 ± 4
HbA_{1c}, disease mean (%)	9.0 ± 1.0	—
HbA_{1c}, current (%)	9.0 ± 1.4*	4.9 ± 0.3
Duration of IDDM (years)	7.0 ± 3.4	—
Systolic BP (mmHg)	106 ± 10	103 ± 11
Diastolic BP (mmHg)	57 ± 7	58 ± 6
Mean arterial pressure (mmHg)	74 ± 7	73 ± 7
Na-Li countertransport (mmol Li · liters erythrocytes^{-1} · h^{-1})	0.41 ± 0.14*	0.30 ± 0.11

Data are means ± SD. Nondiabetic reference range for HbA_{1c} is 4–6%. *$P < 0.0001$ by paired Student's *t* test.

ric data for Na-Li countertransport were compared using the Wilcoxon signed-rank test and correlations determined by the Spearman rank correlation coefficient. $P < 0.05$ was used to indicate statistical significance. When an IDDM subject had more than one sibling, the sibling who best matched the subject for age (closest aged sibling), sex (same sex, where possible), and body mass index (BMI) (within 2–3 kg/m^2) was chosen for paired Student's *t* test comparison. Results are expressed as means ± SD unless otherwise stated.

RESULTS

IDDM subjects versus nondiabetic siblings

Table 1 outlines the characteristics of the IDDM and sibling groups. The groups were comparable in terms of age, sex, BMI, and BP. The IDDM group had significantly raised HbA_{1c} levels compared with their siblings (9.0 ± 1.4 vs. 4.9 ± 0.3%, $P < 0.0001$).

The erythrocyte Na-Li countertransport was significantly higher in the IDDM subjects than in their siblings (0.41 ± 0.14 vs. 0.30 ± 0.11 mmol Li · liters of erythrocytes^{-1} · h^{-1}, $P < 0.0001$). However, the Na-Li countertransport in the IDDM group did correlate significantly with that of the sibling group ($r = 0.42$, $P < 0.0008$), which suggests a genetic contribution to the activity of this transporter.

Erythrocyte Na-Li countertransport did not correlate with the duration of disease. No sex differences existed in erythrocyte Na-Li countertransport in either the IDDM or sibling group.

IDDM subjects with and without MA

Details of the matched IDDM subjects with and without MA are presented in Table 2. The groups were comparable in terms of age, sex, BMI, HbA_{1c}, and IDDM duration. Although there was a trend for higher Na-Li countertransport in the IDDM group with MA than in those without MA, this difference did not reach statistical significance (0.43 ± 0.13 vs. 0.37 ± 0.13 mmol Li · liters of erythrocytes^{-1} · h^{-1}; $P = 0.11$). Siblings of IDDM subjects with MA had similar Na-Li countertransport activity to the siblings of those without MA (0.31 ± 0.11 vs. 0.27 ± 0.15 mmol Li · liters of erythrocytes^{-1} · h^{-1}).

Elevated Na-Li countertransport

Elevated countertransport has been defined as Na-Li countertransport >0.4 mmol Li · liters of erythrocytes^{-1} · h^{-1} (see CONCLUSIONS). Characteristics of the IDDM groups with normal and elevated Na-Li countertransport are outlined in Table 3. Thirty of 84 (36%) patients in the IDDM groups and 7 of 85 (8%) siblings had Na-Li countertransport above this level ($P < 0.001$). The IDDM groups with elevated and normal Na-Li countertransport did not differ with respect to age, sex

Table 2—*Characteristics of matched subjects with IDDM with and without MA*

	Subjects with MA	Subjects without MA
n	29	29
Sex (M/F)	15/14	13/16
Age (years)	15.9 ± 1.8	16.0 ± 1.6
BMI (kg/m^2)	23.5 ± 2.5	23.1 ± 2.2
Duration of IDDM (years)	7.0 ± 3.6	7.2 ± 2.6
HbA_{1c}, disease mean (%)	9.2 ± 1.4	8.7 ± 1.6
HbA_{1c}, current (%)	9.1 ± 1.5	8.7 ± 1.3
Systolic BP (mmHg)	108 ± 11	105 ± 11
Diastolic BP (mmHg)	60 ± 6*	55 ± 6
Mean arterial pressure (mmHg)	76 ± 7	72 ± 7
Na-Li countertransport (mmol Li · liters erythrocytes^{-1} · h^{-1})	0.43 ± 0.13	0.37 ± 0.13

Data are means ± SD. Subjects without MA are matched to the subjects with MA with respect to sex, age, and duration of diabetes. *$P = 0.03$.

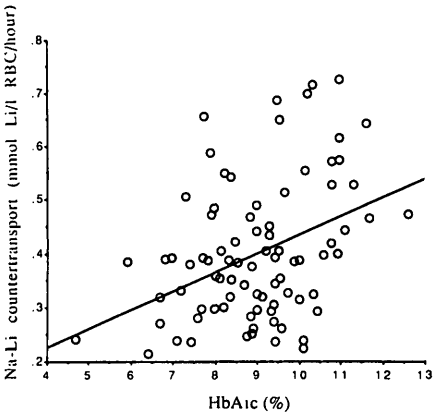

Figure 1—*Erythrocyte Na-Li countertransport of subjects with IDDM plotted against current HbA_{1c} (r = 0.37; P < 0.003).*

Table 3—*Characteristics of IDDM subjects with normal and elevated Na-Li countertransport*

	Na-Li countertransport	
	>0.4	≤0.4
n	30	54
Sex (M/F)	18/12	29/25
Age (years)	15.9 ± 1.8	16.0 ± 1.6
BMI	24 ± 2	23 ± 2
Duration of IDDM (years)	8.0 ± 3.8	7.4 ± 3.6
HbA_{1c}, disease mean (%)	9.4 ± 0.9*	8.9 ± 1.0
HbA_{1c}, current (%)	9.6 ± 1.4*	8.6 ± 1.3
Systolic BP (mmHg)	106 ± 10	106 ± 10
Diastolic BP (mmHg)	58 ± 6	57 ± 7
Mean arterial pressure (mmHg)	74 ± 7	73 ± 7
Subjects with microalbuminuria *n*(%)	14 ± 46%†	15 ± 28%

Data are means ± SD. Na-Li countertransport measured in mmol Li · liters of erythrocytes^{-1} · h^{-1}. Nondiabetic reference range for HbA_{1c} is 4–6%. Microalbuminuria is urinary albumin excretion rate 15—200 μg/min. *P = 0.03; †P = 0.001.

distribution, duration of diabetes, BMI, or BP. Both current and disease duration mean HbA_{1c} were significantly greater in the elevated Na-Li countertransport group than in the group with normal transport ($P < 0.03$). Of the 29 IDDM subjects with MA, 13 (45%) had Na-Li countertransport >0.4 mmol Li · liters of erythrocytes^{-1} · h^{-1} compared with 17 of 55 (31%) of the subjects without MA ($P < 0.0001$).

BP

All IDDM subjects and their siblings were normotensive, the majority having arterial pressures corresponding to the 50th percentile or below for published ranges (18). Arterial pressures did not differ between subjects with normal or elevated Na-Li countertransport levels, whether or not they had MA. Systolic, diastolic, and mean arterial pressures were compared in the IDDM groups with and without MA (Table 2). Only the difference in diastolic pressure was statistically significant (60 ± 6 vs. 55 ± 6 mmHg, $P < 0.03$).

HbA_{1c}

Na-Li countertransport in the IDDM group correlated weakly with the duration mean HbA_{1c} ($r = 0.21$, $P < 0.05$), but more strongly with the current HbA_{1c} ($r = 0.37$, $P < 0.003$) (Fig. 1). Other factors (age, sex, BMI, disease duration, and BP) did not correlate significantly with HbA_{1c}. Furthermore, as stated above, HbA_{1c} levels were greater in the group with elevated Na-Li countertransport activity.

CONCLUSIONS— Increased Na-Li countertransport in erythrocytes was first reported by Canessa et al. (11) as a potential marker for essential hypertension. Similar findings were later reported by Livne et al. (12) studying Na-H exchange in platelets and by Ng et al. (13) using lymphocytes. Schmouder and Weder (14) found elevated platelet Na-H transport activity, but interestingly, found no correlation between Na-H exchange and erythrocyte Na-Li countertransport in patients with essential hypertension.

Studies of Na-Li countertransport measured in individuals with IDDM have yielded conflicting results (9,10,19–24). Krolewski et al. (9) reported the finding of elevated erythrocyte Na-Li countertransport in patients with IDDM, nephropathy, and hypertension, and others have published similar findings in patients with IDDM and nephropathy (10,19,20). These observations led to the suggestion that abnormal Na-Li countertransport could be used as a marker for the risk of developing nephropathy in diabetes. Carr et al. (21) reported elevated erythrocyte Na-Li countertransport in IDDM patients with glomerular hyperfiltration but without nephropathy or hypertension. Jensen et al. (22) found that Na-Li countertransport was elevated in diabetic patients with and without nephropathy and concluded that the diabetic state per se might be responsible for the elevation. Elving et al. (23) studied IDDM subjects with and without diabetic nephropathy, patients with nondiabetic renal disease, and healthy control subjects: Na-Li countertransport was significantly higher in the IDDM group than either the healthy control subjects or those subjects with other renal diseases. However, it was not different in IDDM subjects with and without nephropathy. Gall et al. (24), in a study of non-insulin-dependent diabetes mellitus subjects, reported that erythrocyte Na-Li countertransport was related to the presence of diabetes rather than nephropathy. These same authors commented that a genetic predisposition to hypertension and elevated erythrocyte Na-Li countertransport could not be used to identify patients at risk for clinical nephropathy (23,24).

Our findings are in agreement

with the view that erythrocyte Na-Li countertransport is influenced by the diabetic state, because the Na-Li countertransport level was significantly higher in the IDDM group than their siblings and correlated with the degree of metabolic disturbance as reflected by the current HbA_{1c} level. Furthermore, there was no significant difference in Na-Li countertransport between those IDDM subjects with and without MA, although a greater proportion of MA adolescents had raised countertransport than did those without MA. We did, however, find a correlation between Na-Li countertransport in patients with IDDM and their siblings. This suggests that genetic influences are probably involved in the regulation of this transporter. The use of the nondiabetic siblings provides an informative comparison with the IDDM subjects, because inherited influences such as predisposition to essential hypertension should be similar.

The analysis of abnormal and normal erythrocyte Na-Li countertransport values proved interesting. Na-Li countertransport >0.4 mmol Li · liters of erythrocytes^{-1} · h^{-1} has been used to define elevated transport activity (11, 21,25). Similar to the observation by Carr et al. (21), the percentage of IDDM subjects in our study with an abnormally elevated Na-Li countertransport by this definition (36%) is approximately the same as that of patients who develop diabetic nephropathy. Only 46% of the diabetic subjects with MA had elevated Na-Li countertransport, implying that if one used this as a marker, 54% of these patients would be predicted not to develop nephropathy. However, it may turn out that patients with a mild degree of MA (as was the case in most MA subjects in our study) who also have elevated Na-Li countertransport are those most likely to progress to overt nephropathy. Those with raised Na-Li countertransport but no MA may not have had IDDM of long enough duration to develop sufficient renal damage to produce MA. Lopes de Faria et al. (26) support this latter contention by their finding of a declining frequency of normoalbuminuria in patients with high Na-Li countertransport with duration of the disease. This suggests that prolonged observation of our study cohort may lead to a significant difference in the prevalence of micro- or overt albuminuria in subjects with normal versus raised Na-Li countertransport.

Of importance, we describe here a correlation between erythrocyte Na-Li countertransport and glycemic control as measured by HbA_{1c}. The correlation with recent control as measured by simultaneous HbA_{1c} was greater than that with disease duration mean HbA_{1c}. Furthermore, both current and duration mean HbA_{1c} levels were significantly higher in the group with elevated erythrocyte Na-Li countertransport. Krolewski et al. (9) and Lopes de Faria et al. (26) have also observed a relationship between glycemic control and Na-Li countertransport, although neither group reported the correlation between HbA_{1c} and membrane countertransport measurements. Lopes de Faria et al. (26) reported a higher frequency of albuminuria (micro- and macroalbuminuria) in those IDDM individuals with raised Na-Li countertransport and HbA_{1c} levels above the median for their study group. These results support our belief that at least part of the elevated Na-Li activity is a consequence of the recent metabolic derangement of diabetes.

Most believe that the Na-Li countertransporter represents an activated form of the Na-H antiport (27,28). We speculate that mechanisms that activate the Na-H exchanger may also be responsible for the elevated Na-Li countertransport that has been noted in diabetes. Some of the stimuli that may activate the transporter in diabetes include *1*) nonenzymatic glycation of the exchanger or glycation of other proteins that could indirectly result in increased transporter activity; *2*) intracellular acidosis increasing the number or activity of the exchanger (29); *3*) increased rate of phosphorylation of the transporter by protein kinase C secondary to hyperglycemia; and *4*) alterations in cell volume as a result of activation of the aldose reductase pathway, resulting in an increase in the transporter activity (30).

The methodological issues warrant attention. Canessa et al. (16) have defined the optimum conditions for performance of the Na-Li countertransport assay. Our assay has been well validated and the coefficients of variation fall well within those suggested by Canessa et al. (16). Our studies were performed in the immediate postprandial state. We believe that any systematic error in measurement of countertransport has been eliminated by performing the studies in all subjects at the same time of day in a similar postprandial state. To support the reproducibility of this approach, we repeated Na-Li countertransport measurements in 20 of our subjects in a similar postprandial state some months after the initial study. The correlation between the two readings was 0.95 ($P < 0.0001$) with no subject having a normal measurement on one occasion and a raised level on the other.

We were surprised at the distribution of BP measurements in our study because very few were above the 50th percentile of published ranges with the majority falling between the 10–25th percentiles (18). Careful calibration studies and comparisons with mercury sphygmomanometers confirmed the accuracy of the Dinamap measurements. All measurements were taken by a trained research assistant in quiet surroundings after a rest period of at least 5 min, and in some cases, it was 30 min. The measurements thus represent basal BP values and reflect the relaxed state of the study participants. This may make comparison with published standards inaccurate (18). The lack of difference in systolic BP between the patients with IDDM and their siblings was also somewhat surprising given that the mean duration of IDDM was 7 years and that a number had evidence of incipient nephropathy. Perhaps a more accurate way to measure BP, such as 24-h ambulatory BP monitoring, would permit detection of an earlier de-

rangement of BP regulation (31). The modestly higher diastolic pressure in the patients with MA is in agreement with previous reports (5) suggesting that BP changes develop early in patients with IDDM destined to develop nephropathy.

In summary, our findings support the view that abnormalities of Na-Li countertransport in IDDM are, at least in part, secondary to environmental influences (in particular, abnormal glucose homeostasis) and that the degree of metabolic control influences the activity of this countertransporter. The reasons for the differences between our findings and those of others regarding utility of Na-Li countertransport measurements as a marker of risk for diabetic nephropathy remain speculative. Perhaps full expression of this genetic marker may not occur until well after the completion of puberty.

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