

Sodium-lithium countertransport in children with diabetes and their families

P N Houtman, F M Campbell, V Shah, D B Grant, D B Dunger, M J Dillon

Abstract

Abnormalities of sodium-lithium countertransport have been extensively implicated in adult primary hypertension and a relationship between sodium-lithium countertransport and family history of hypertension in children has been previously found. More recently it has been suggested that increased sodium-lithium countertransport may play a part in the pathogenesis of nephropathy in insulin dependent diabetes mellitus (IDDM). Children and adolescents with IDDM and their family members were studied. In those with IDDM (n=36, median age 14.6 years, range 9.5-19.2 years) there was no relationship between sodium-lithium countertransport (range 0.098-0.585 mmol/l red blood cells/hour) and age, blood pressure as expressed by systolic or diastolic SD scores, glycated haemoglobin, serum lipids, or intracellular sodium concentration. A positive relationship ($r_s=0.44$) was found between sodium-lithium countertransport and early morning urinary albumin to urinary creatinine ratio (UA/UC), expressed as the logarithm of the geometric mean of two consecutive samples, for each individual (range 0.4-22 mg/mmol). Sodium-lithium countertransport was increased in those with IDDM compared with their non-diabetic siblings, in a paired analysis (n=26). There was no relationship between UA/UC in the children with diabetes and sodium-lithium countertransport in their parents. These studies in this population of diabetic children indicate that increased sodium-lithium countertransport may play a part in the early stages of the development of nephropathy in IDDM.

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Nephropathy accounts for a significant part of the morbidity associated with insulin dependent diabetes mellitus (IDDM).¹ However, only a certain percentage of patients with diabetes seem to be susceptible to this complication.^{2,3} It appears that although environmental factors such as poor glycaemic control have an influence on nephropathy, genetic factors also play a major part. Diabetic nephropathy clusters within certain families and it has been shown that the frequency of nephropathy in diabetic siblings of diabetic probands with nephropathy is considerably higher than that in

diabetic siblings of diabetic probands without nephropathy.^{4,5}

Hypertension is an interacting factor in determining susceptibility to diabetic nephropathy.^{2,6} Sodium-lithium countertransport activity in red blood cells, known for some time to be a marker of risk for essential hypertension,⁷⁻⁹ has more recently come to be associated with diabetic nephropathy. Sodium-lithium countertransport has been found to be increased in those diabetics with established nephropathy^{10,11} or microalbuminuria,¹² and this increased activity has also been found in their parents.¹³

Overt renal disease is unusual in young people with IDDM, but the propensity to develop later manifestations may be studied using urinary albumin excretion as an early marker of nephropathy.¹⁴ The main aim of the present study was to determine whether it was possible to show an association between sodium-lithium countertransport and urinary albumin excretion, when measured at an early age, and to show any relationships with countertransport in family members.

Patients and methods

Thirty six patients with IDDM were recruited from those attending the paediatric diabetic clinic at the John Radcliffe Hospital, Oxford and the Hospital for Sick Children, Great Ormond Street, London. Parents and siblings also agreed to take part in the study. Sixteen of these patients were about to undergo a study to assess changes in proteinuria with treatment, and were therefore selected from a larger group for the presence of microalbuminuria. All patients and parents were of European origin, and none of the patients were on any medication apart from insulin treatment. There was no evidence of overt renal or cardiovascular disease in any patient. However, two parents were on drug treatment for hypertension.

Urine albumin excretion was determined using the urinary albumin to urinary creatinine ratio (UA/UC) and expressed as the geometric mean of two consecutive early morning urine samples. Urinary albumin was measured by radioimmunoassay and urinary creatinine by the Jaffe reaction.

The method used for sodium-lithium countertransport was similar to that of Canessa *et al*⁷ incorporating some modifications as reported by Rutherford *et al*.¹⁵ Further modifications in our assay were necessary in view of the smaller quantities of blood available from young children and the concomitant need for greater precision. Venous blood, minimum 5 ml, was collected into tubes containing

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lithium heparin, centrifuged, and the erythrocytes incubated in lithium loading solution (140 mmol lithium chloride, 10 mmol/l lithium carbonate, 10 mmol/l glucose, 10 mmol/l Tris-acetate, gassed with 95% oxygen/5% carbon dioxide, pH 7.5, 290 mosmol/kg) for 90 minutes at 37°C. The erythrocytes were then washed once with magnesium chloride (MgCl₂) (290 mosmol/kg) and twice with choline medium (139 mmol/l choline chloride, 1 mmol/l MgCl₂, 10 mmol/l glucose, 10 mmol/l Tris-acetate, pH 7.4, 290 mosmol/kg). After the final washing 0.05 ml portions of erythrocytes were incubated at 37°C in four tubes containing 0.6 ml of choline medium (as above) including 10⁻⁴ mol/l ouabain and four tubes containing 0.6 ml of sodium medium (145 mmol/l sodium chloride, 1 mmol/l MgCl₂, 10 mmol/l glucose, 10 mmol/l Tris-acetate, 10⁻⁴ mol/l ouabain, pH 7.4, 290 mosmol/kg). The packed cell volume of the erythrocytes was 0.80±0.03 and this figure was used in further calculations. The tubes were cooled after 30, 60, 90, and 120 minutes for both sodium and choline-containing media, and centrifuged at 2000 g for 5 minutes. An aliquot of 200 µl supernatant was mixed with 2.075 ml of 1.63 mmol/l caesium chloride in duplicate tubes and the lithium content was measured using an IL 943 flame photometer (Instrumentation Laboratory). The analyst undertaking this determination was unaware of the identity of the subject. The sodium-lithium countertransport activity (mmol lithium/l red blood cells/hour) was determined as the difference between lithium efflux from erythrocytes in the sodium and choline media. The between assay coefficient of variation using this technique for countertransport was 11%, and to maintain validity a control sample was assayed in parallel during each experimental run.

Blood pressure was recorded using a random zero mercury sphygmomanometer, with the subject resting in the sitting position. Three readings were taken and the average measurements of systolic and diastolic (Korotkov sound IV) blood pressures used. Family history of high blood pressure was recorded from the parents of the children in the study. A ranked score was derived from this information using a five tiered scale as previously described.¹⁶

Analyses between means of groups were by unpaired *t* tests. Parametric regression was used for comparisons except those involving family history score and body mass index for which a non-parametric statistic was obtained (Spearman's coefficient).

The study was approved by the ethics committee of the Hospital for Sick Children and Institute of Child Health, London and the Oxford district ethical committee.

Primary details of the study groups

	Diabetics (n=36)	Siblings (n=35)	Parents (n=54)
M:F	14:22	16:19	27:27
Median age (range)	14.6 (9-19)	13.2 (5-21)	43 (32-59)

Results

Primary details of the patients, their parents, and their non-diabetic siblings are shown in the table.

In 27 families both parents were studied, and only these families were used for analyses involving parents. Siblings were available in 26 families, and when more than one non-diabetic sibling from a family was studied, their mean sodium-lithium countertransport was used.

In the diabetic patients the sodium-lithium countertransport activity in the red blood cells ranged from 0.06 to 0.58 mmol lithium/l red blood cells/hour (mean 0.33). There was no relationship between sodium-lithium countertransport and age, duration of diabetes, blood pressure as expressed by systolic or diastolic SD scores, glycated haemoglobin, serum lipids, or intracellular sodium concentration, in these patients. No significant relationship was found with body mass index (mean 21.2 kg/m², range 14-33). There was also no relationship found between sodium-lithium countertransport and family history score for hypertension.

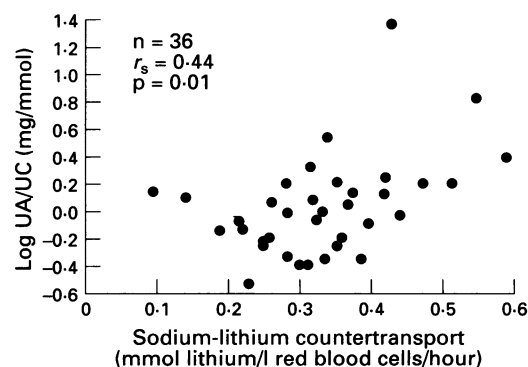
In the non-diabetic siblings of the diabetics, sodium-lithium countertransport ranged from 0.08 to 0.53 mmol lithium/l red blood cells/hour (mean 0.27). In a paired analysis, sodium-lithium countertransport in the diabetics was significantly higher than that in the non-diabetic siblings (n=26, p=0.025). There was no difference in age between these two groups.

Mean UA/UC ranged from 0.3 to 22 mg/mmol in the diabetics. There was a positive correlation between sodium-lithium countertransport and mean UA/UC (log adjusted to account for distribution)¹⁷ (n=36, r_s=0.44, p=0.01; figure), not accounted for by age or body mass index in multiple regression.

In the parents sodium-lithium countertransport ranged from 0.11 to 0.62 mmol lithium/l red blood cells/hour (mean maternal 0.28 and mean paternal 0.35). Using both mid-parental and the higher of parental sodium-lithium countertransport values, there was no relationship between parental sodium-lithium countertransport and UA/UC in their diabetic children (n=27).

Discussion

Cell membrane transport has been linked to both hypertension and diabetic nephropathy



Relationship between UA/UC and sodium-lithium countertransport activity in children with diabetes.

for some time. Much more difficult has been the task of determining precise links between susceptibility to disease and genetic variability in these cell membrane transport systems. It is with sodium-lithium countertransport that the strongest links have been established,¹⁸⁻²⁰ and we believe that there are distinct advantages to studying this system at an early age.

One of the main aims of such research is to predict the secondary complications of disease, such as diabetic nephropathy. We chose UA/UC as a continuous variable because there is increasing evidence that not only is urinary albumin excretion a good predictor of later nephropathy and cardiovascular disease,^{1,2} but also that even very low degrees of microalbuminuria are significant in terms of later renal and cardiovascular complications.^{21,22} This approach allowed us to use a young diabetic population with little overt evidence of any renal damage, but precisely the sort of population which would benefit most from disease prevention. Thus our main finding concerns the positive relationship between sodium-lithium countertransport and urinary albumin excretion in children and adolescents with diabetes. This finding has previously been seen in adults,¹² but the importance of this relationship being discernible so early in the course of the diabetes lies in its significance regarding genetic susceptibility to diabetic complications.

Another reason which makes it a distinct advantage to study a young population is in terms of the potentially reduced influence of the long term secondary effects of morbidity. For example, it has been difficult in this area of research to disentangle the influence of blood pressure on susceptibility to diabetic nephropathy. This area is, in fact, now quite complex, and it is considered that sodium-lithium countertransport is important in both hypertension and diabetic nephropathy, but in different ways according to differing kinetic changes in the transport system.²³⁻²⁵ These factors do not concern us directly, and children rarely manifest essential hypertension. Whereas it has been possible to find a relationship between sodium-lithium countertransport and family history of hypertension at a very early age,¹⁶ we would not expect actual variability in blood pressure at this age to be perceptible in terms of any relationships with disease variables. Therefore we were not surprised that we did not find relationships between sodium-lithium countertransport and systolic and diastolic blood pressure scores, or indeed with serum lipids or glycated haemoglobin concentrations. It may have been more likely to have found a relationship with family history of hypertension in our diabetic population. However, our previous work in this area had involved a larger screening pool. A similar argument applies to body mass index, known to be associated with sodium-lithium countertransport,²⁶ but our negative results may also be concerned with the confounding effects of diabetes on body habitus.

We failed to find a relationship between microalbuminuria in the children and their

parents' countertransport. Using older populations such relationships have been found,¹³ and so it was integral to our study to measure countertransport in the family members. Our failure to define a relationship is likely to be related to the youth of the diabetic patients rather than the youth of their parents. Whereas variability in sodium-lithium countertransport activity is thought to be largely genetically determined,²⁷ microalbuminuria would be expected to be slowly evolving in the early years of diabetes so that any genetic influence from parents would be unlikely to be sufficiently defined in a marker such as urinary albumin to creatinine concentration.

We found that sodium-lithium countertransport in the diabetic patients was greater than that in their non-diabetic siblings. This has not been a common finding in the literature²⁸ and appears to implicate sodium-lithium countertransport in the diabetic process itself as well as being part of the propensity to develop complications.²⁹ Sodium-lithium countertransport is known to be affected by lipids,³⁰ glucose,³¹ and insulin concentrations,²⁴ but only to a small extent in clinical practice, and it would be unlikely that this would account for our finding. Rather it may be that sodium-lithium countertransport is involved in diabetic propensity, and that this difference between diabetic children and their healthy siblings is more likely to be discernible at this early age than later, before other complicating factors muddle any delineation.

In summary we have found that microalbuminuria is associated with increased sodium-lithium countertransport in young diabetic children, before the onset of overt nephropathy. Countertransport was higher in diabetics than their non-diabetic siblings, but we found no relationship between parental countertransport and diabetic albumin excretion. These studies are further evidence of a relationship between cell membrane transport and diabetic nephropathy that can be found at an early age.

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