Erythrocyte sodium-lithium countertransport activity is related to membrane fluidity in IDDM patients

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Summary Sodium-lithium countertransport (SLC) activity at a standard physiological sodium concentration is raised in uncomplicated IDDM, for which the kinetic mechanism is a raised maximum velocity ($V_{\max}$). Diabetic patients with nephropathy do not have raised values for $V_{\max}$ but a low Michaelis constant (k_m). Transporter activity could be influenced by its membrane lipid environment. This was assessed in 21 control subjects, 32 uncomplicated diabetic patients, 17 patients with diabetic nephropathy and 11 patients with non-diabetic nephropathy by measuring the fluorescence anisotropy of DPH and TMA-DPH to assess different membrane regions. Standard SLC was higher in all the patient groups compared to the control subjects: 0.307 ± 0.020 mmol Li/h $\times$ 1 cells in uncomplicated IDDM; 0.300 ± 0.032 in diabetic nephropathy patients and 0.276 ± 0.019 in non-diabetic nephropathy patients vs 0.216 ± 0.011 mmol Li/h $\times$ 1 cells in control subjects ($p < 0.001$, $p < 0.05$, $p < 0.05$, respectively). This was due to raised $V_{\max}$ values in the uncomplicated group: 0.528 ± 0.035 vs 0.385 ± 0.022 mmol Li/h $\times$ 1 cells in control subjects ($p = 0.001$) and low values for k_m in the diabetic nephropathy group: 58 (27–170) vs 106 (81–

161) mmol/l in control subjects ($p < 0.001$). Raised SLC in the non-diabetic nephropathy group was largely due to raised $V_{\max}$: 0.460 ± 0.030 mmol Li/h $\times$ 1 cells; $p = 0.053$, with no difference in k_m : 99.5 (74–137). DPH anisotropy was lower in the uncomplicated diabetic patients (0.210 ± 0.0009) compared to the control subjects (0.214 ± 0.0007 ; $p = 0.006$) and was related to both standard SLC ($rs = -0.68$, $p < 0.001$) and $V_{\max}$ ($rs = -0.77$, $p < 0.001$). Though DPH anisotropy in the diabetic nephropathy group was not different from normal control subjects, it also correlated with SLC ($rs = -0.72$, $p < 0.001$) due to the same relationship with $V_{\max}$ ($rs = -0.73$, $p < 0.001$) as in uncomplicated diabetes. TMA-DPH anisotropy showed no difference between the four groups and was not related to either standard SLC, $V_{\max}$ or k_m . Therefore, changes in membrane fluidity in the hydrophobic regions of the erythrocyte membrane may be responsible for some of the differences in SLC in IDDM. [Diabetologia (1994) 37: 394–400]

Key words Sodium, lithium, erythrocyte, membrane fluidity, fluorescence polarization, DPH

Received: 29 June 1993
and in revised form 4 November 1993

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Abbreviations: SLC, sodium-lithium countertransport; DPH, 1,6-diphenyl-1,3,5-hexatriene; TMA-DPH, 1,4-trimethylammonium-3,5-hexatriene; IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus.

Several studies have shown increased sodium-lithium countertransport activity (SLC) in IDDM [1–4]. Membrane fluidity has also been studied in diabetes but patient groups have varied and the results have been inconsistent [5–13]. The sodium-lithium countertransporter is a transmembrane protein with increased activity in some diabetic patients but its character has not been studied in relation to membrane fluidity in diabetes.

In IDDM, a raised SLC has been suggested as a possible marker for those patients at risk of nephropathy [14, 15]. Measured under standard conditions of

150 mmol/l external sodium concentration, SLC could be raised due to either a low Michaelis constant with respect to external sodium (low k_m) or a raised maximum velocity (V_{max}). In uncomplicated IDDM there appears to be an increase in the V_{max} of the transporter [4], whereas patients with diabetic nephropathy do not have raised values for V_{max} but have low values for k_m [16].

The sodium-lithium countertransporter has no known physiological role and no satisfactory explanation has been proposed as to how a raised activity could directly influence the development of nephropathy in diabetes. A raised activity may be a marker of a more fundamental membrane abnormality. It has been shown that membrane lipids can regulate certain membrane proteins (such as transporters and receptors) either through specific interactions or as a result of more generalised properties of the membrane lipids such as their fluidity [17–21]. An altered fluidity could increase the rate of transporter turnover and therefore ion transport, with a resulting increase in V_{max} . Lipid properties around the transporter could affect ion binding and result in a change in k_m , although changes in ion translocation steps could also affect k_m .

Membrane fluidity can be assessed using fluorescent dyes which localise to particular regions of the membrane [22, 23]. DPH is very hydrophobic and positions itself in the deep hydrophobic regions of the membrane lipids. TMA-DPH is more hydrophilic and localises to the more superficial, less hydrophobic regions of the membrane. The fluidity of the regions in which the dyes are situated affects their rate of rotation and hence the degree of depolarisation of fluorescent light. The anisotropy value is a quantitative expression for this effect.

Therefore the relationship of SLC activity to membrane fluidity, assessed from anisotropy measurements of DPH and TMA-DPH in erythrocyte membranes, was determined in IDDM patients with and without diabetic nephropathy, non-diabetic patients with nephropathy and a group of healthy control subjects.

Subjects and methods

Patients

Thirty-two uncomplicated patients with IDDM (initial presentation with weight loss and ketosis, and aged less than 30 years) were studied. These patients had diabetes of relatively short duration (less than the median for the onset of nephropathy). As previous studies have shown that SLC is not raised in uncomplicated IDDM of long duration [16, 24], it is necessary to investigate membrane fluidity in relation to raised SLC in diabetic patients of relatively short duration. Criteria for patient selection to uncomplicated diabetes were: (i) no retinopathy on retinal photography; (ii) urine albumin excretion (measured by radioimmunoassay) of less than 15 µg/min in timed overnight collections; (iii) no impairment of glycaemic awareness; (iv) no symptomatic neuropathy. Patients with other factors known to

affect SLC were excluded and no regular medication other than subcutaneous insulin was being taken. A group of 21 healthy control subjects, matched for age, sex and body mass index was studied for comparison. None was taking any regular medication or had a family history of essential hypertension.

A group of 17 patients with diabetic nephropathy was also studied. The diagnosis of diabetic nephropathy was made on the basis of established criteria, i.e. duration of diabetes of at least 10 years, persistent proteinuria as the first clinical feature, concomitant retinopathy (by retinal camera), no evidence of other renal or urinary tract disease and absence of cardiac failure. Renal biopsies had been performed in two patients and showed only common features of diabetic nephropathy. Of these 17 patients, 2 had end-stage renal failure and were on continuous ambulatory peritoneal dialysis, 10 had renal impairment with serum creatinine raised above 120 µmol/l, and 5 had persistent proteinuria (Albustix positive on repeated occasions for at least 2 years and urinary protein excretion of more than 500 mg/24 h, but serum creatinine less than 120 µmol/l). In addition to insulin, 10 patients were also receiving anti-hypertensive medication: 6 patients on angiotensin converting enzyme inhibitors, 5 on calcium antagonists, 4 on diuretics and 2 on prazosin. Some patients were taking more than one anti-hypertensive drug.

A group of 11 non-diabetic patients with nephropathy (chronic pyelonephritis, adult polycystic kidney disease, obstructive uropathy, chronic glomerulonephritis or single kidney) was studied for comparison with the diabetic nephropathy group. Of these 11 patients, 2 had end-stage renal failure and were on continuous ambulatory peritoneal dialysis and 9 had serum creatinine above 120 mmol/l. The patients were matched with the diabetic patients with nephropathy on the basis of age/sex distribution, body mass index and degree of renal impairment. Of these 11 patients, 3 were not receiving any anti-hypertensive medication, 4 were on calcium antagonists, 2 on diuretics, 2 on beta-blockers and 1 was on an angiotensin converting enzyme inhibitor.

Ethical approval for this study was granted by the Newcastle Health Authority/Newcastle University Joint Ethical Committee, and all subjects gave their informed consent.

Evaluation of patients and subjects

Details of patients and subjects are given in Table 1. Blood pressure (Korotkoff phases 1 and 5) was measured in the sitting position on at least three separate occasions and is presented as the mean arterial pressure (diastolic pressure plus one-third of the pulse pressure). The mean of the last three glycated haemoglobin concentrations (Ciba-Corning electroendosmosis, Halstead, UK; reference range 5.0–7.5%) was used to assess glycaemic control. The plasma cholesterol and triglyceride concentrations were determined by enzymatic techniques using a centrifugal analyser with a kit supplied by Boehringer Mannheim (Mannheim, Germany). The samples were also examined for chylomicrons and if present, suggesting a non-fasting state, the data were discarded.

Assay for the determination of standard SLC activity and its kinetic characteristics

The details of the assay used to measure standard SLC activity, k_m and V_{max} are fully described in our previous reports [4, 16, 25]. Briefly, SLC activity was measured as sodium-stimulated lithium efflux from washed, lithium loaded erythrocytes (internal lithium concentration > 7.0 mmol/l). Erythrocytes were incu-

Table 1. Clinical details of control subjects, diabetic patients and nephropathy patients

	Control subjects	Diabetic patients		Non-diabetic nephropathy patients
		Uncomplicated	Nephropathy	
<i>n</i>	21	32	17	11
Age (years)	35.9 ± 2.0	32.8 ± 1.7	46.8 ± 1.4 ^a	51.2 ± 6.1 ^a
Sex (male/female)	9/12	16/16	10/7	7/4
Duration of diabetes (years)	–	8.5 ± 1.0	24.4 ± 1.9 ^e	–
Body mass index (kg/m ²)	25.2 ± 0.5	23.6 ± 0.6	25.5 ± 1.1	26.5 ± 1.1
Glycated haemoglobin (%)	–	8.1 ± 0.3	7.4 ± 0.3	–
Plasma triglyceride (mmol/l)	1.2 (0.4–2.0)	1.1 (0.4–2.3)	2.3 (0.6–5.3) ^b	2.3 (1.2–4.0) ^b
Plasma cholesterol (mmol/l)	5.24 ± 0.19	4.82 ± 0.19	6.16 ± 0.48 ^d	6.32 ± 0.49 ^d
Mean arterial pressure (mm Hg)	91.1 ± 1.7	90.2 ± 1.9	112.8 ± 2.3 ^c	106.5 ± 2.3 ^c

Values are mean ± SEM.

^a*p* ≤ 0.01, ^b*p* < 0.02, ^c*p* < 0.001 vs control subjects and uncomplicated diabetic patients;

^d*p* < 0.02, ^e*p* < 0.001 vs uncomplicated diabetic patients

bated for up to 2 h (haematocrit of 10–16 %) at 37°C in choline medium and six sodium containing media with external sodium concentrations of 37.5, 62.5, 75.0, 100.0, 125.0 and the standard 150.0 mmol/l. SLC activity was determined by linear regression analysis as the difference between lithium efflux from erythrocytes in the sodium and choline media. The Eadie-Hofstee transformation of the Michaelis-Menten equation was used to calculate *k_m* and *V_{max}*. The mean standard error of the slope of the lines from which *k_m* and *V_{max}* was determined was 5.6 %.

Membrane preparation

Erythrocyte ghosts were prepared using a method based on that of Dodge et al. [26]. Venous blood was collected in tubes containing lithium-heparin, centrifuged and the erythrocytes washed with isotonic phosphate buffered saline (290 ± 2 mosmol/kg, pH 7.4) by repeated centrifugation (3,000 rev/min, 4 min). The erythrocytes were then lysed in hypotonic phosphate buffer (20 ± 2 mosmol/kg, pH 7.4) containing 4 mmol/l MgSO₄ to avoid alterations in membrane phospholipid alignment [27, 28]. The ghosts were washed repeatedly with the hypotonic buffer to remove the haemoglobin. All steps in the preparation of the membranes were carried out at 4°C. The protein concentration in the resulting membrane suspension was determined by the intensity of fluorescence emission of tryptophan [29] using bovine serum albumin as a standard and 2 mg of membrane protein was then suspended in 3 ml of isotonic phosphate buffer for fluorescence measurements.

Fluidity measurements

Membrane fluidity was assessed by the anisotropy of the fluorescent probes DPH and TMA-DPH [30, 31] (Sigma Chemical Company Ltd., Poole, Dorset, UK). Stock solutions of the probes were prepared in tetrahydrofuran and N,N-dimethyl-

formamide respectively at a concentration of 2 × 10^{−3} mol/l and stored in the dark at 4°C. For DPH, labelling was carried out on the erythrocyte membranes prior to hypotonic lysis by adding DPH stock solution to vigorously stirred isotonic phosphate buffer to make a suspension of concentration 2 × 10^{−6} mol/l DPH. An aliquot (10 ml) of this DPH suspension was mixed with 0.5 ml of erythrocytes and incubated in the dark at 37°C for 30 min. After labelling with DPH, the erythrocyte ghosts were prepared as described above. For TMA-DPH, labelling was carried out on the ghost membranes by adding 3 µl of the stock solution of TMA-DPH to 3 ml of the final membrane suspension and incubating in the dark for 5 min before making the fluorescence measurements.

Steady-state fluorescence polarization intensity was measured with a Perkin Elmer LS50 spectrometer equipped with integral polarizers (Perkin Elmer, Beaconsfield, Bucks., UK). Excitation and emission wavelengths were 359 nm and 440 nm respectively for DPH, and 361 nm and 454 nm for TMA-DPH. The measured anisotropies were obtained from the intensities of emission polarized parallel (*I_{||}*) and perpendicular (*I_⊥*) to the polarized excitation beam. Standard formulae were used including an appropriate grating correction (*G*):

$$\text{Anisotropy} = \frac{I_{||} - I_{\perp} \cdot G}{I_{||} + 2I_{\perp} \cdot G}$$

Light scattering errors were minimised by use of a 390 nm emission filter and by measuring the anisotropy at several dilutions and extrapolating to zero membrane concentration to obtain readings that were independent of concentration [32].

Statistical analysis

Results that had a normal distribution are given as mean ± SEM, or where data were not normally distributed as median and range. The significance of differences between groups was assessed using the unpaired Student's *t*-test on normally dis-

Table 2. Standard SLC activity, SLC kinetics and DPH and TMA-DPH anisotropy in control subjects, diabetic patients and nephropathy patients

	Control subjects	Diabetic patients		Non-diabetic nephropathy patients
		Uncomplicated	Nephropathy	
<i>n</i>	21	32	17	11
Standard SLC activity (at 150 mmol/l Na) (mmol Li/h × 1 cells)	0.216 ± 0.011	0.307 ± 0.020 ^a	0.300 ± 0.032 ^b	0.276 ± 0.019 ^b
Michaelis constant (<i>k_m</i>) (external site) (mmol/l)	106 (81–161)	115 (91–256)	58 (27–170) ^c	99.5 (74–137)
Maximum velocity (<i>V_{max}</i>) (mmol Li/h × 1 cells)	0.385 ± 0.022	0.528 ± 0.035 ^a	0.414 ± 0.056	0.460 ± 0.030
DPH anisotropy	0.214 ± 0.0007	0.210 ± 0.0009 ^d	0.214 ± 0.0014	0.214 ± 0.0010
TMA-DPH anisotropy	0.234 ± 0.0012	0.234 ± 0.0009	0.236 ± 0.0020	0.235 ± 0.0007

Values are mean ± SEM or median (range).

^a $p \leq 0.001$, ^b $p < 0.05$ vs control subjects;

^c $p < 0.001$ vs control subjects, uncomplicated diabetic and non-

diabetic nephropathy patients;

^d $p < 0.001$ vs control subjects, $p < 0.02$ vs diabetic and non-diabetic nephropathy patients

Fig. 1. Relationship between standard SLC activity and DPH anisotropy in normal control subjects (□) and diabetic patients: uncomplicated (●) and with nephropathy (○) **Fig. 2.** Relationship between *V_{max}* and DPH anisotropy in normal control subjects (□) and diabetic patients: uncomplicated (●) and with nephropathy (○)

tributed values, or the Mann-Whitney U-test where data were not normally distributed. Calculation of correlation between variables was made using Spearman's rank correlation coefficients (r_s). The best fit line of y on x was determined by least squares regression analysis.

Results

No difference was observed in body mass index or glycaemic control between patients with uncomplicated diabetes and those with diabetic nephropathy. However, as may have been expected, diabetic patients with nephropathy were older, had a longer duration of diabetes and had raised serum lipids and mean arterial blood pressure compared to uncomplicated diabetic patients (Table 1). Non-diabetic patients with nephropathy also had raised levels of serum lipids and mean arterial pressure to a similar degree to patients with diabetic nephropathy.

The mean standard SLC activity (measured at 150 mmol/l external sodium concentration) was higher in both groups of diabetic patients compared to the control subjects (Table 2). However, there was a large region of overlap with 38 % of uncomplicated and 41 %

of diabetic nephropathy patients having values greater than the control range. In the uncomplicated patients, raised standard SLC was due to an increase in the *V_{max}* but in the patients with nephropathy, the raised SLC was due to a lower *k_m*. There was no evidence that the values for *k_m* of SLC were affected by renal failure as the non-diabetic patients with nephropathy had values for *k_m* that were not different to the control group. Standard SLC however, was raised in the patients with non-diabetic nephropathy compared with control subjects and was largely due to raised values for *V_{max}* which reached borderline statistical significance ($p = 0.053$).

The anisotropy value for the fluorescence of DPH in the erythrocyte membrane was lower in the uncomplicated diabetic patients than in the control subjects and in these patients was correlated with both standard SLC ($r_s = -0.68$, $p < 0.001$: regression slope = -15.08 ± 2.67 , intercept = 3.47 ± 0.56 ; Fig. 1) and *V_{max}* ($r_s = -0.77$, $p < 0.001$: regression slope = -30.54 ± 3.80 , intercept = 6.93 ± 0.80 ; Fig. 2). DPH anisotropy in patients with diabetic nephropathy was not different from the control group but was correlated with standard SLC ($r_s = -0.72$, $p < 0.001$: regression slope = -18.05 ± 3.88 , intercept = 4.16 ± 0.83) and *V_{max}* ($r_s =$

-0.73 , $p < 0.001$: regression slope = -32.51 ± 6.49 , intercept = 7.36 ± 1.39). DPH anisotropy and $V_{\max}$ were also correlated in the control subjects ($r_s = -0.48$, $p = 0.028$: regression slope = -15.89 ± 6.22 , intercept = 3.78 ± 1.33) and the non-diabetic nephropathy group ($r_s = -0.80$, $p = 0.003$: regression slope = -21.88 ± 7.76 , intercept = 5.13 ± 1.66). There was no relationship however, between k_m for SLC and DPH anisotropy in any of the groups studied ($r_s = -0.06$ to $+0.12$, $p > 0.1$ in all four groups).

The anisotropy value for TMA-DPH was not different between the uncomplicated diabetic patients and those with nephropathy. There was also no difference between the diabetic patients and either normal control subjects or non-diabetic patients with nephropathy. The anisotropy value for TMA-DPH was not related to SLC or either of its kinetic parameters.

Standard SLC and $V_{\max}$ were both correlated with serum triglycerides ($r_s = 0.35$, $p = 0.002$ and $r_s = 0.29$, $p = 0.01$, respectively). This has been reported previously [25, 33]. However, no correlations between membrane fluidity and serum lipids were seen. In addition, there were no significant relationships between glycated haemoglobin, age, sex, body mass index or mean arterial pressure and membrane fluidity or SLC.

Discussion

The group of patients with uncomplicated IDDM of relatively short duration had raised standard SLC. The kinetic mechanism for the high values was an increase in $V_{\max}$ of the transporter. In addition, patients with diabetic nephropathy were found to have a low k_m . These findings are similar to those of previous studies [4, 16]. However, $V_{\max}$ was not different in the diabetic nephropathy group compared to the control subjects which differs from a previous report [16] in which $V_{\max}$ was lower in diabetic nephropathy patients. The reason for this may be that the high plasma triglyceride levels of the patients with nephropathy in the present study would have the effect of raising $V_{\max}$ [25, 33] which would offset any reduction in $V_{\max}$ as a result of the presence of diabetic nephropathy alone.

The erythrocyte sodium-lithium countertransporter is a transmembrane protein and like other such proteins is probably closely associated with lipid molecules of the lipid bilayer. Its function may be regulated by interactions with bilayer lipids. In support of this, the present results show a strong relationship between membrane fluidity in the deep hydrophobic regions and SLC in uncomplicated IDDM. This relationship between SLC and fluidity would seem to be specific to the deep hydrophobic regions of the membrane however, as there was no correlation between TMA-DPH anisotropy and SLC. Therefore, the raised $V_{\max}$ of SLC in uncomplicated IDDM is associated with increased

membrane fluidity in the deep hydrophobic regions of the erythrocyte membrane.

SLC was also related to DPH anisotropy in the diabetic nephropathy group. It is clear from the relationship between DPH anisotropy and the kinetic parameters k_m and $V_{\max}$, that the relationship between membrane fluidity and $V_{\max}$ is the same in both uncomplicated diabetic patients and those with nephropathy. This strongly supports a primary relationship between $V_{\max}$ and membrane fluidity. As the diabetic nephropathy patients have lower k_m s, then they would be expected to have higher values of standard SLC for a given $V_{\max}$. Although standard SLC was raised in this group to a similar level as the uncomplicated diabetic patients, $V_{\max}$ was not raised, and neither was membrane fluidity as assessed by DPH anisotropy. Therefore, the relationship between standard SLC and DPH anisotropy is different from that in uncomplicated diabetic patients in that for a given value for DPH anisotropy, the SLC tends to be higher.

SLC was also raised in the non-diabetic nephropathy group. This may be due to the associated hyperlipidaemia as SLC is known to be raised during hyperlipidaemia, for which the kinetic mechanism is a raised $V_{\max}$ [25, 33]. A similar degree of hyperlipidaemia in the diabetic nephropathy group would also tend to have the effect of raising $V_{\max}$. However, $V_{\max}$ was not raised in the group of patients with diabetic nephropathy. Therefore, raised plasma triglycerides cannot explain the difference in $V_{\max}$ between patients with uncomplicated diabetes and those with diabetic nephropathy.

The anisotropy value for DPH was lower in uncomplicated IDDM patients than normal control subjects. As anisotropy and fluidity are inversely related, this indicates increased fluidity in the deep hydrophobic regions of the erythrocyte membrane. This has been reported in previous studies [5–8], although others have also reported normal [9, 10] or lower fluidity [11–13] as assessed by DPH in diabetes. The reason for these differences is not entirely clear. However, it should be noted that the patients studied in these reports often had different characteristics. The patients studied by Baba et al. [13] were mostly NIDDM patients. One group studied by Watala et al. [11] were juvenile IDDM patients of short duration and another [12] consisted of a mixed group of IDDM and NIDDM patients many of whom also had diabetic complications and had a longer duration of diabetes compared to the uncomplicated patients in the present study. The group studied by Birlouez-Argagon et al. [9] also contained some NIDDM patients but duration of diabetes and presence/absence of complications were not mentioned. The patients studied by Hill et al. [10] had a similar duration of diabetes but were younger (9–19 years) and some patients had microvascular disease. The patients studied by Testa et al. [5], Mazzanti et al. [6], Juhan-Vague et al. [7] and Rahmani-Jourdheuil et al. [8] however, all had IDDM

of similar duration and age to those patients in the present study, but some of the patients in these previous studies had diabetic complications. The results in the present study demonstrate that the presence of diabetic complications could affect membrane fluidity as those patients with diabetic nephropathy had lower membrane fluidity compared to their uncomplicated counterparts although this was not different when compared to normal control subjects. It has also been previously suggested that diabetic patients with microangiopathy might have an elevation of membrane cholesterol and consequently, lowered membrane fluidity [11]. In addition, if raised SLC is a marker for those diabetic patients at risk of nephropathy (as has been suggested) then studying patients with a relatively long duration of diabetes would eliminate those with a raised SLC since if development of nephropathy were likely, it would already have occurred. In fact previously described patients with uncomplicated diabetes of relatively long duration do not have raised values for SLC [16, 24]. Therefore since the present study shows a relationship between SLC and membrane fluidity, uncomplicated diabetic patients with a relatively long duration of diabetes are also unlikely to have high values of membrane fluidity. These differences in patient characteristics may therefore partly explain some, but certainly not all, of the discrepancies in results of erythrocyte membrane fluidity in diabetes.

Our results show a relationship between the fluidity in the deep hydrophobic membrane regions and the $V_{\max}$ of SLC such that, higher membrane fluidity was associated with higher $V_{\max}$. In broad terms an increase in $V_{\max}$ could be due to a greater number of SLC transporter units in the membrane or an increase in the turnover number (ions transported per second) of each transporter unit. These cannot be distinguished at present because the number of transporters cannot be determined. Membrane fluidity could increase $V_{\max}$ by either of these proposed mechanisms. Firstly it has been suggested that at any one time, the transporters are not all present at the surface of the membrane but a change in the fluidity of the membrane could allow more of them to travel to the surface and be available for ion transport [34]. The second hypothesis suggests that a change in membrane fluidity could affect any conformational change in the transporter necessary for ion transport and so affect the turnover number.

In summary, therefore, raised SLC activity in uncomplicated IDDM may be due to altered physical properties of the membrane lipid bilayer. Such membrane alterations may be important in the subsequent development of nephropathy.

Acknowledgements. We are grateful to the Northern Counties Kidney Research Fund for supporting this work.

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