Rapid communications

Erythrocyte sodium-lithium countertransport is not different in Type 1 (insulin-dependent) diabetic patients with and without diabetic nephropathy

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Summary. We studied erythrocyte sodium-lithium countertransport in 33 patients with Type 1 (insulin-dependent) diabetes mellitus with diabetic nephropathy, 18 patients with Type 1 diabetes without diabetic nephropathy and in 42 non-diabetic patients with various other renal diseases. No significant differences were found in sodium-lithium countertransport between these three groups (median

(range) 322 (162–676) vs 321 (189–627) vs 300 (142–655) $\mu\text{mol} \cdot \text{l cells}^{-1} \cdot \text{h}^{-1}$). We conclude, that sodium-lithium countertransport cannot be used as a marker for diabetic nephropathy.

Key words: Type 1 (insulin-dependent) diabetes mellitus, sodium-lithium countertransport, diabetic nephropathy.

Erythrocyte sodium-lithium countertransport (NaLiCT) is regarded as a marker for a predisposition to essential hypertension [1]. In non-diabetic parents of patients with diabetic nephropathy a higher prevalence of essential hypertension is found [2], suggesting a relationship between a familial predisposition to essential hypertension and the occurrence of diabetic nephropathy. Recently, it has been demonstrated that patients with diabetic nephropathy had higher levels of NaLiCT than diabetic patients without nephropathy [3, 4] and non-diabetic patients with other renal diseases [4]. These data suggested that NaLiCT could be a useful marker for diabetic nephropathy. We have measured NaLiCT in diabetic patients with and without nephropathy and in patients with non-diabetic renal diseases.

Subjects and methods

We studied three groups of Caucasian patients.

1. Eighteen patients with Type 1 diabetes mellitus for more than 15 years and without diabetic nephropathy (albumin excretion rate (AER) $< 20 \mu\text{g}/\text{min}$).

2. Thirty-three patients with Type 1 diabetes mellitus with diabetic nephropathy (AER $> 20 \mu\text{g}/\text{min}$; duration of diabetes > 10 years; all patients had evidence of retinopathy).

3. Forty-two patients with various non-diabetic renal diseases.

All patients came to the out-patient clinic between 08.00 and 10.00 hours. A venous blood sample was drawn into two heparin-containing 10 ml vacuum tubes and processed within 2 h for duplicate NaLiCT-measurement. A blood sample was also taken for measurement of serum creatinine and HbA_{1c}. NaLiCT was measured according to the method described by Ibsen [5]. Briefly, erythrocytes were loaded with lithium in a LiHCO₃-medium over 20 min resulting in an intracellular lithium concentration above the maximum transport capacity ($> 5 \text{ mmol} \cdot \text{l cells}^{-1}$). Normal values for NaLiCT in our laboratory, obtained in 24 healthy subjects (9 males, 15 females; mean age 30 ± 9 years, range: 22–56 years) varied from 134–390 with a median value of $244 \mu\text{mol} \cdot \text{l cells}^{-1} \cdot \text{h}^{-1}$. The intra-assay variation of the method is 5%. The inter-assay variation, also representing the biological variability, is 13%.

Of the diabetic patients albuminuria was measured in overnight timed urine samples using laser immunonephelometry [6]. In the patients with non-diabetic renal disease proteinuria was measured in a single urine sample using the biuret method and expressed per mmol of creatinine [7]. HbA_{1c} was measured by HPLC (normal value: 4.3–6.3%). Blood pressure was measured using a standard mercury sphygmomanometer with the patients in supine position after 5 min rest. Hypertension was defined as a mean arterial pressure (MAP) $> 110 \text{ mmHg}$ or the use of antihypertensive drugs.

Statistical analysis

All results are expressed as mean $\pm$ SD or as median (range) when not normally distributed. The Kruskal-Wallis test was used to compare groups. Bonferroni's adjustment for multiple comparisons was applied. Spearman's correlation coefficients are reported.

Table 1. Clinical characteristics and metabolic parameters of patients with Type 1 (insulin-dependent) diabetes mellitus without (DNP -) or with nephropathy (DNP +) and non-diabetic patients with other renal diseases (RD)

	DNP -	DNP +	RD
Number (M/F)	18 (8/10)	33 (19/14)	42 (26/16)
Age (years)	45 ± 8	37 ± 11 ^a	44 ± 15
Diabetes duration (years)	27 ± 9	25 ± 8	-
(range)	(18-44)	(10-37)	
HbA _{1c} (%)	9.0 ± 1.2	9.0 ± 0.7	-
AER (μg/min)	7 (3-19)	271 (30-2846)	-
Creatinine (μmol/l)	83 ± 12	106 ± 41 ^b	399 ± 286 ^c
Protein/creatinine ratio (g/mmol × 10)	-	-	2.6 (0.1-19.7)
MAP (mm Hg)	93 ± 6	101 ± 10 ^d	107 ± 11 ^e
Hypertension (n)	0	15	24

^a $p < 0.02$ compared to DNP - and RD;^b $p < 0.05$ compared to DNP -;^c $p < 0.001$ compared to DNP - and DNP +;^d $p < 0.001$ compared to DNP -;^e $p < 0.02$ compared to DNP + and DNP -

AER - albumin excretion rate;

MAP - mean arterial blood pressure

Results

Clinical data of the patients are given in Table 1. The two groups with diabetes mellitus did not differ with respect to duration of diabetes or HbA_{1c}. The patients with diabetic nephropathy were significantly younger than the patients without nephropathy ($p < 0.01$). In the patients with diabetic nephropathy serum creatinine and MAP were significantly higher ($p < 0.05$ and $p < 0.001$) compared to the diabetic patients without nephropathy. Of the 33 patients with diabetic nephropathy 11 had incipient nephropathy (AER 20-200 μg/min) whereas 22 had overt nephropathy (AER > 200 μg/min). The hypertensive patients in this group ($n = 15$) all had overt nephropathy. In the patients with renal disease the underlying renal disease was glomerular in origin in 26 patients and non-glomerular in 16 patients. NaLiCT levels in the three patient groups are shown in Figure 1. All three groups had elevated levels of NaLiCT compared to healthy subjects (median 244; range 134-390 μmol·l cells⁻¹·h⁻¹), however, no significant differences were found between the patient groups. Median values were 321 (189-627) μmol·l cells⁻¹·h⁻¹ for the diabetic patients without nephropathy, 322 (162-676) in the diabetic patients with nephropathy and 300 (142-655) in the patients with other renal diseases. Furthermore, values of patients with incipient nephropathy did not differ from values in patients with overt nephropathy (284 (217-649) vs 344 (162-676) μmol·l cells⁻¹·h⁻¹). Values above the upper limit of normal (400 μmol·l cells⁻¹·h⁻¹) were found in 5 of 18 (28%), 10 of 33 (30%) and 10 of 42 (24%) of the groups 1, 2 and 3, respectively ($p = \text{NS}$). No correlations were found between NaLiCT and age, HbA_{1c}, creatinine, AER and body weight.

Discussion

We did not find increased levels of NaLiCT in patients with diabetic nephropathy compared to patients without diabetic nephropathy or patients with other renal diseases. Our observations are in contrast to the results reported by Mangili et al. [4] and Krolewski et al. [3]. There are no obvious explanations for this difference. Theoretically, differences in methods of NaLiCT measurement could be responsible. Our patients were not studied in the fasting state and we used LiHCO₃ instead of LiCl for loading of the erythrocytes with lithium. We do not believe that differences in methodology explain the differences in results. First, we were sure to achieve intracellular lithium concentrations above the maximum transport capacity. Secondly, our results in normal volunteers are similar to those reported by Mangili et al. [4] and Carr et al. [8]. Thirdly, we have compared both methods of measurement of NaLiCT in six healthy subjects and found no differences (mean with LiHCO₃: 266 ± 63 μmol·l cells⁻¹·h⁻¹; mean with LiCl 254 ± 66; mean difference 17 ± 9; NS). Lastly, it has been reported that a light breakfast does not influence NaLiCT [9].

If we analyse the different studies and calculate in the various patient groups the proportion of patients with NaLiCT above 400 μmol·l cells⁻¹·h⁻¹ (the upper limit in healthy subjects [4,8, this study]) a similar distribution is found for patients without diabetic nephropathy: 15% [4]; 36% and 40% [3]; 33% [8] and 28% (this study). This similarity is also found for patients with non-diabetic renal disease: 27% [4] and 24% (this study). A major difference is only observed for patients with diabetic nephropathy: respectively 87% [4], 60% [3] and 30% (this study). It should be realized that exogenous factors can influence NaLiCT, especially abnormal lipid profiles [10]. Noticeably, the patients with diabetic nephropathy in the studies of Mangili et al. [4] and Krolewski et al. [3] had respectively a higher degree of proteinuria and a higher level of HbA_{1c}, factors which could have influenced plasma lipids. Further

Fig. 1. Individual and median values of sodium-lithium countertransport (NaLiCT) of Type 1 (insulin-dependent) diabetic patients without (DNP -) and patients with nephropathy (DNP +) and patients with non-diabetic renal disease (RD). The upper and lower limits of NaLiCT in healthy control subjects are indicated by dotted lines

studies are needed to clarify which exogenous factors can influence NaLiCT and could have biased the results of the studies. In conclusion, we could not confirm that NaLiCT is increased in patients with diabetic nephropathy. Therefore, NaLiCT cannot be used as a marker for diabetic nephropathy.

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