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ORIGINAL ARTICLE

Sodium-Lithium Countertransport activity in first degree families of diabetics, susceptible persons for diabetes and diabetic patients; a possible biomarker for prognosis of diabetes

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ABSTRACT
Sodium-lithium countertransport (SLC) is a membrane ion transport system. SLC activity is elevated in many metabolic disorders and genetic epidemiologic studies suggest that 50-80 % of SLC activity can be explained by inheritance. The aim of this study was to investigate the correlation between SLC activity and family history of diabetes. Eighty subjects in four equal experimental groups were used for this study. They were classified as diabetic patients, first degree families, susceptible persons and a control group. SLC activity was measured in their red blood cells. Preliminary experiments showed that plasma glucose as well as insulin levels were significantly higher in diabetics compared with other experimental groups. This group also showed higher activity of SLC. The activity of SLC in first degree relatives of diabetic patients was much higher than control subjects and diabetic patients. This study showed that the activity of SLC is changed in diabetes. It also indicated that their first degree relatives, even before the onset of the disease, had SLC activity higher than controls. This result is helpful in the prediction of diabetes and paves the way for the introduction of new strategies for handling the outcome of this life threatening disorder.
Keywords: *Sodium-Lithium Countertransport, Diabetes, Impaired glucose tolerance,HOMA-IR,HOMA-B*

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INTRODUCTION

Sodium-lithium countertransport (SLC) across the red cell membrane was first reported by Tosteson in 1975 [1].SLC is a membrane ion transport system that exchanges sodium for sodium or lithium [2, 3]. Since then many clinical and epidemiological studies have confirmed that SLC activity is enhanced in many metabolic disorders [4]. Thus abnormalities in the rates of Li transport mediated by SLC are reported in several diseases including essential hypertension [5], hyperlipidemia [6] and diabetes (7). Rutherford et al. reported a relation between plasma insulin levels and SLC kinetics in non-diabetic subjects [8, 9]. It is suggested that insulin resistance, rather than insulin concentration, is associated with an increased SLC activity [10-12]. This correlation seems to be independent of the metabolic disorder, because it is reported in both IDDM [11] and NIDDM [13]. SLC activity was found to be significantly higher in IDDM patients without nephropathy than in healthy control subjects. An increased SLC activity has also been reported to be linked to essential hypertension, diabetic nephropathy and hyperlipidaemia and is considered as a risk marker for the development of these diseases [14].

Although some acquired conditions such as pregnancy and obesity can modulate the SLC activity, genetic epidemiology studies suggest that 50-80% of the SLC activity can be explained by inheritance [15]. Even though the protein responsible for the erythrocyte SLC activity has not yet been cloned or identified and

its physiological role in vivo has not been fully clarified and the gene(s) that affect SLC activity have not been identified but elevated Na-Li flux is viewed as a marker of inherited pre-disposition to hypertension and other disorders [16].

Previous studies about the activity of SLC in diabetes focused on the relation of it's activity with hypertension and/or microalbuminuria in both types 1 and type 2 diabetes [14]. The aim of this study is to investigate the correlation between SLC activity and the family history of diabetes. Thus the first degree relatives of diabetic patients as well as the diabetics and susceptible persons were monitored for SLC activity.

MATERIALS AND METHOD

Experimental design

Eighty subjects in four equal experimental groups were used for the study (10 men, 10 women in each group, age 40-54 years).They had no smoking, hypertension, hyperlipidemic, and were not taking antipsychotic, lithium carbonate drugs and medications for metabolic diseases. These groups were classified as first degree family of diabetics, susceptible persons for diabetes (2 hppG: 140-190 mg/dl), parallel diabetic patients and a control group (FBS≤126 mg/dl). Blood samples were collected at 8:00AM following an overnight fasting and poured into test tubes containing lithium heparin(125IU/10ml blood) [17].

Heparinized blood sample were centrifuged for 10 minutes in 2000g in 4oC (18) and packed cells were used to measure the SLC activity. The plasma of each sample was used to measure biochemical parameters.

Analytical procedures

Biochemical parameters such as plasma insulin, glucose, urea, creatinine, cholesterol, triglyceride, HDL, urine albumin and creatinine were determined by enzymatic appropriate biochemical methods using a instrument (BT 3000 plus biotecnica instruments,MD Italia). Serum LDL cholesterol was calculated using the Friedwald formula; LDL cholesterol was not calculated when the triglycerides concentration was>400 mg/kg. Insulin was measured using a commercially available gamma counter kit (Biosource,Bellics).

HOMA-IR and HOMA-B calculated as an insulin resistance and beta cell function respectively by following formula (19, 20).

$$\text{HOMA} - \text{IR} = \frac{\text{Insulin}(\frac{\mu\text{IU}}{\text{ml}}) \times \text{FBS}(\frac{\text{mg}}{\text{dl}})}{405}$$

$$\text{HOMA} - \text{B} = \frac{2\ 0 \times \text{Insulin}\left(\frac{\mu\text{IU}}{\text{ml}}\right)}{\text{FBS}\left(\frac{\text{mmol}}{\text{ml}}\right) - 3.5}$$

Measurement of SLC

SLC was measured in red blood cells according to the method of Canessa (21) with slight modification. Briefly, two milliliters of packed erythrocytes were re-suspended in 8 ml of lithium loading solution containing 140 mmol/l lithium chloride, 10 mmol/l lithium carbonate, 10 mmol/l glucose, and 10 mmol/l TRIS- MOPS acetate (pH 7.5) and incubated at 37 °C for 3 hours. A mixture of 95% oxygen and 5% carbon dioxide was bubbled through the solution during the incubation. Erythrocytes were then washed three times with choline medium (139 mmol/l cholin chloride, 1 mmol/l $MgCl_2$, 10 mmol/l glucose, and 10 mmol/l TRIS-MOPS (pH 7.4) with the osmolality of 290 mmol/kg. After the final washing, packed cell volume was measured using a micro-hematocrit. Aliquots of 0.2 ml of packed cells were incubated in 4 mL of choline-ouabain medium (choline medium as above but containing 1 mmol/l ouabain). Another aliquot of 0.2 ml of packed cells were incubated in 4 ml of sodium-ouabain medium (150 mmol/l NaCl, 1 mmol/l $MgCl_2$, 10 mmol/l glucose, and 10 mmol/ TRIS-MOPS [pH 7.4] with the osmolality of 290 mmol/kg, and 1 mmol/l ouabain). After incubating for 60 min at 37 °C, the incubation mixtures were centrifuged at 2000g, for 3 minutes and 1 ml of the supernatant was removed and mixed with 1 ml of distilled water. The lithium content was measured using an atomic absorption spectrophotometer (Varian: spectra 250). The SLC activity was corrected by subtracting the rate of lithium efflux from erythrocytes in the choline medium from that measured in the sodium medium. As a baseline, hypertension was defined as blood pressure ≥ 140/90 mmHg or the use of anti-hypertensive drugs. Diabetes was defined as fasting plasma glucose level ≥ 126 mg/dl (7 mmol/l) or the use of medication for diabetes[28].

Statistical analyses

Statistical analysis was performed with SPSS 13 and data are presented as means ± SD. Differences between groups were examined by ANOVA and post Hoc tests. Values of $P<0.05$ were considered statistically significant in all analyses.

RESULTS AND DISCUSSION

The activity of SLC in different groups is shown table 1. As seen in this table, relatives of diabetic patients showed SLC activity much higher than control subjects and is comparable to the diabetic patients. The activity of SLC in patients with impaired glucose tolerance (IGT) who are susceptible to diabetes is not significantly different from that of controls.

The biochemical parameters were also measured, the results of which are shown in table 2. As shown in this table plasma glucose as well as insulin levels are significantly different from control values.

Table1. The activity of SLC in patients with impaired glucose metabolism compared with control subjects.

Groups	Diabetics	First family of diabetics	IGT	Control
SLC activity (μmol Li^+/L RBC/h)	352 ± 26* [54.4%]	319 ± 22* [39.5%]	234 ± 21	228 ± 19

Data is reported as mean ± SD of results obtained in 20 patients in each group. IGT,impaired glucose tolerance;the percent increase in SLC activity is shown in bracket and stars indicate that the values are statistically significant($p<0.05$).

Tabale2. The concentrations of different biochemical parameters in four studied groups

	Diabetics	First degree family of diabetics	IGT	Control
FBS(mg/dl)	179.4± 93.5*	100.7 ±20.8	106± 5.7	88.9± 6.2
2 hPG(mg/dl)	219.7±91*	140.7±68.5*	150.5±11.5*	106.1±13.3
Cholesterol(mg/dl)	176.1±35.7	203.5±43.6	193.9±39.8	189.1±31
Triglyceride(mg/dl)	172.5±129	156.9±78	181.5±127.9	140±66.4
LDL (mg/dl)	95.8±20	119.8±36.1	111±32.4	112.7±24.7
HDL (mg/dl)	45.5±13	52±10.7	46.6±16.2	50.2±10.8
Urea(mg/dl)	30±11.1	29.3±5.7	30.4±6.7	28.1±5.4
Creatinine(mg/dl)	1.08±0.3	1.02±.19	1.07±0.32	0.97±0.16
Urine albumin (mg/dl)	23.9±22.3	8.9±7.2	22.8±44.3	10.1±4.5
Urine creatinine (g/dl)	0.16±0.08	0.21±.12	0.17±0.06	0.16±0.04
Insulin(µIU/ml)	17.5±9.9	13.8±3.2	14.7±6.7	14.2±2.6
HOMA-IR	3.28±0.96*	1.45±0.06	1.62±0.03	1.31±0.01
HOMA-B	22.91±12.95*	55.76±12.89*	52.07±23.29*	83.53±15.25

Values are Mean ± SD obtained from 20 subjects in each group. IGT,impaired glucose tolerance; HOMA-IR, homeostasis model of assessment index for insulin resistance; HOMA-B, homeostasis model of assessment index for β cell function; Stars show that the values are significantly different ($p<0.05$).

This study shows that the activity of SLC is changed in diabetic patients and their first degree relatives. This result is helpful in the prediction of diabetes and paves the way for the introduction of new logical theraputic strategies. SLC has been extensively studied in relation to hypertension and related diseases and is currently viewed as a marker of some inherited disorders [16]. Previous investigations regarding the activituy of SLC in diabetes have focused on the relationship of this ion transport system with consequent hypertension and/or diabetes nephropathy [22,23]. This finding that the higher activity of SLC activity is present years before the onset of diabetic symptomes is clinically very important and may indicate that the enhanced SLC activity is not caused by hyperglycemia or its methods of treatments. Moreover it suggests that the activity of this ion transport system may be related to key pathogenic mechanisms that underly the development of type 2 diabetes, thus opening up new perspectives for the understanding of the molecular events leading to the metabolic abnormalities of diabetes. This is a good evidence indicating that the role of genetic is important in inhanced SLC activity. To our knowledge there is no evidence of a relationship between SLC activity and impaired insulin secretion. Familial aggregation and high hereditability of the SLC activity in red cells have been reported in general population [24]. Previous studies indicated that as much as 80-90% of the individual variance in this activity can be

accounted for by inheritance and a model with a major gene or a polygenic transmission, or both, has been proposed [25-27].

As indicated, increased SLC activity may be viewed as a preclinical, possibly genetic, marker of pre-disposition to type 2 diabetes. Although the mechanisms underlying the association between high SLC activity and diabetes are far from clear, but cell membrane abnormalities influencing SLC activity under diabetic situations may be responsible. For example insulin might affect the kinetics of SLC by stimulating a phosphorylation-dephosphorylation-linked mechanism, or it may decrease the viscosity of membrane lipid core which could allow more rapid transport. Whatever the mechanism, this study casts light on the predictive importance of high SLC activity in progression and incidence of many metabolic disorders including diabetes mellitus.

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