Taurine and Osmoregulation: Platelet Taurine Content, Uptake, and Release in Type 2 Diabetic Patients

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In this study, plasma and platelet taurine content and fluxes were determined in 38 type 2 diabetic patients and in 26 healthy control subjects. Taurine levels in diabetic patients were significantly lower than in control subjects both in plasma (32.1 *v* 48.6 μmol/L, $P = .000$) and platelets (148 *v* 183 nmol/mg protein, $P = .043$). Platelet taurine uptake in diabetic patients was significantly reduced (321.2 *v* 524.9 pmol total taurine 10^8 platelet^{-1} min^{-20}, $P = .000$), whereas taurine release increased in comparison to healthy controls (38.7 *v* 29.5% of platelet ^{3}H taurine at the start of incubation, $P = .000$). These results may reflect modified systems of taurine carriers or a compensatory mechanism in response to an increase of other organic osmolytes.

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ALTHOUGH THE TERM diabetic retinopathy usually refers to changes in retinal blood vessels, vascular cells may not be the only cells affected by diabetes in the retina.[1] Microangiopathy is considered to be the result of combined impairments of the choriocapillaris, the retinal pigmented epithelium (RPE), the nervous layers, the pericytes, and the vitreoretinal interface.[2] More recently a possible dysfunction of Müller cells, the principal glia of the retina, was evidenced.[3] Metabolic and vascular factors are closely related to each other and play important roles in the development of diabetic retinopathy. Among the metabolic factors, the polyol pathway, by which glucose is metabolized to sorbitol, is involved in diabetic complications.[4] The compatible osmolyte hypothesis predicts that organic osmolytes, including taurine, myo-inositol, and sorbitol, respond coordinately to changes in external osmolality to maintain the intracellular milieu.[5,6] Accumulation of intracellular sorbitol in diabetes mellitus may therefore result in depletion of other intracellular osmolytes.

In man, taurine is widely distributed, being found in the highest concentration in tissues that are prone to diabetic complications.[7] In the mammalian central nervous system (CNS), including retina, several roles were suggested for taurine, in addition to its role in osmoregulation: neurotransmission or modulation of neuronal activity, regulatory factor during development, repair and regeneration, antioxidation, and membrane stabilization.[8-10] The biologic importance of taurine in the retina has been recognized ever since the original observation that taurine deficiency in cats results in retinal degeneration and blindness,[11] supporting a role in neuronal survival. In the vertebrate retina, taurine is the most abundant amino acid, accounting for more than 50% of the free amino acid pool, and it is most highly concentrated in RPE and photoreceptor cells.[12-14] High retinal intracellular concentration of taurine (10 to 50 mmol/L) is mainly assured by active uptake from the choroidal blood due to the limited capacity for its biosynthesis.[14] RPE plays a pivotal role in the maintenance of taurine levels in the retinal cells, as it contains sodium-dependent cotransporters with a higher affinity for β- than α-amino acids and chloride-dependent.[15] In addition, RPE has been reported to be involved in a number of ocular lesions, including diabetic retinopathy.[16]

Direct studies in human RPE in vivo are rarely possible, therefore readily accessible tissues are used by several investigators. Here we have investigated the efficiency of taurine uptake and release in platelets of diabetic patients. Platelets had already been used as cell models of taurine homeostasis,[17] as they are rich in taurine and possess carriers with properties similar to those found in CNS and RPE.[15,18-20] In addition, platelets represent 1 of the vascular factors involved in microangiopathy, as has been shown from increased platelet aggregation in diabetic patients.[21] Data obtained in this study show that there is a decreased taurine content in plasma and platelets from diabetic patients. The lower intracellular taurine content is well associated with the reduced uptake and the increased release of the amino acid.

PATIENTS AND METHODS

Subjects

The study was conducted with 2 groups of subjects who gave informed consent. These groups consisted of 26 healthy control volunteers who had no family history of diabetes mellitus and a normal glucose tolerance test and 38 type 2 diabetic patients who were consecutively recruited from the diabetic population attending our outpatient clinic. Characteristics of both groups are reported in Table 1.

Platelet-Rich Plasma and Platelet Pellet Preparation

Platelet-rich plasma (PRP) and platelets were isolated as previous reported by Voaden et al.[19] Polypropylene plastic ware was used for platelet handling. Blood (20 mL) was obtained by venopuncture and added to tubes containing potassium EDTA (final concentration, 0.25%) to prevent coagulation. Approximately 1 hour later, it was centrifuged in 2 $\times$ 10 mL aliquots at 300 *g* for 15 minutes at room temperature. The supernatants, composed of PRP, were drawn off and pooled. Aliquots were either used immediately for assessment of taurine uptake and release or centrifuged at 1,800 *g* for 10 minutes to obtain platelet pellets and plasma.

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Submitted January 19, 2000; accepted June 19, 2000.

Supported in part by grants ex 40% of the Italian Ministry for Scientific and Technological Research (MURST).

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0026-0495/01/5001-0006$10.00/0

doi:10.1053/meta.2001.19432

Table 1. Main Characteristics of Healthy Control Subjects and Type 2 Diabetic Patients

Characteristics	Control Subjects	Type 2 Diabetic Subjects
Number	26	38
Sex (M/F)	13/13	28/10
Age (yr)	61.7 ± 0.45	59.8 ± 1.35
Duration of diabetes (yr)	—	10.5 ± 1.34
Fasting plasma glucose (mmol/L)	4.6 ± 0.11	8.4 ± 0.27*
HbA_{1C} (%)	5.3 ± 0.23	8.0 ± 0.22*
Therapy (diet alone/oral/insulin)	—	4/29/5
Body mass index (kg/m^2)	26.8 ± 0.87	28.4 ± 0.50
Daily energy intake (kj/kg/d)	78.9 ± 3.62	82.3 ± 2.40
Total cholesterol (mg/dL)	208.6 ± 6.56	223.5 ± 7.26
HDL cholesterol (mg/dL)	48.2 ± 1.93	45.5 ± 2.50
Triglycerides (mg/dL)	137.8 ± 18.94	153.4 ± 24.90
Normo-/micro-/macroalbuminuria (no.)	26/0/0	29/7/2
Macroangiopathy (no.)	—	7
Neuropathy (no.)	—	9
Retinopathy (no.)	—	11

NOTE. Data are mean ± SEM.
*P = .000 *v* control subjects.

Determination of Platelet and Plasma Taurine Content

Platelets were lysed by freezing and thawing after resuspension in ice-cold distilled water. Taurine was derivatized with phenylisothiocyanate and its content was determined by high performance liquid chromatography (HPLC) in a Waters (Milford, MA) chromatographic system equipped with a pico-tag column (3.9 × 300 mm), containing a high efficiency reverse phase silica packing. Protein was assayed by the method of Lowry et al.[22]

Uptake of ^3H Taurine

PRP was prewarmed at 37°C in a water-bath. A total of 50 μL Krebs bicarbonate medium (KBM) containing 0.125 μCi ^3H taurine (Amersham, Buckinghamshire, UK; specific activity, 21.0 Ci/mmol) was added to 450 μL PRP, and the ^3H taurine uptake was performed at 37°C with gentle shaking for 20 minutes in an atmosphere of 95% O_2 and 5% CO_2. Additional samples were incubated in an ice bath. Uptake was stopped by placing the tubes in ice bath, and platelets were then recovered by centrifugation at 1,800 × *g* for 15 minutes at 4°C. The supernatant was decanted and the walls of the tubes dried carefully with cotton sticks. Platelet pellets were then washed once with 500 μL fresh KBM. Triton X-100 (0.5 mL, 10% vol:vol, aqueous solution) was then added to solubilize the pellet, followed 30 minutes later by 4.5 mL scintillation fluid. Radioactivity was counted in a Beckman (Fullerton, CA) liquid scintillation spectrophotometer.

Release of ^3H Taurine

A total of 450 μL PRP was preincubated with ^3H taurine 12 nmol/L (50 μL in Ca^{2+}-free KBM) at 37°C for 1 hour in an atmosphere of 95% O_2 and 5% CO_2. After this loading period, platelets were washed twice with KBM (500 μL) to remove the extracellular ^3H taurine, and the resultant pellets obtained at 1,800 × *g* for 15 minutes at 4°C were resuspended in the original volume of fresh KBM and incubated at 37°C for 15 minutes to evaluate taurine efflux. Radioactivity was measured in the incubation medium and release expressed as percent of total ^3H taurine found in platelets at the start of incubation.

Statistical Analysis

The SAS statistical package (SAS Institute, Cary, NC) for personal computers[23] was used. Student's *t* test and simple regression analysis were used. P values ≤ .05 were considered to be significant.

RESULTS

Table 2 shows the mean values obtained from the analysis of the concentration of taurine in plasma from normal subjects in comparison with the data published by Berson et al,[24] Uma et al,[25] Hussain and Voaden,[17] Airaksinen et al,[26] and Franconi et al.[27]

As reported in Table 3, statistically significant changes were found in the plasma and platelet taurine concentrations of diabetic patients with respect to those of controls. The reduction in plasma and platelet taurine levels of diabetic subjects has shown a percent value corresponding to 34% and 19%, respectively, in comparison to controls, making the difference highly significant in plasma (P = .000) and significant in platelets (P = .043).

A preliminary study with platelets obtained from healthy control volunteers established that uptake of ^3H taurine was linear with time for at least 45 minutes (see Fig 1). Platelets from diabetic patients accumulated less taurine, as reported in Table 3, which shows the data (pmol total taurine 10^8 platelets^{-1}) after 20 minutes of incubation. Plasma taurine levels in the control group, as well as in the type 2 patients, however, saturate the 2 carrier systems (higher and lower affinity) of the platelet membrane.[19] A weakly significant correlation was found between plasma taurine and hemoglobin A_{1C} (HbA_{1C}) levels in the diabetic group (r = −.3727, P = .02).

To determine whether or not high glucose per se altered platelet taurine uptake, platelets from healthy subjects were incubated in calcium-free Krebs' medium containing 50 μmol/L taurine (physiologic concentration) and 10 to 25 mmol/L glucose. Taurine uptake decreased proportionally to the increase of glucose levels, reaching the maximum (−30%) with the highest glucose level (25 mmol/L).

To verify how much osmolality changes modify platelet taurine release, experiments were performed using platelets from healthy control subjects. Taurine was retained by the cells in isosmotic condition, and a 50% reduction in osmolality, obtained by decreasing Na^+ concentration of the medium, induced a rapid taurine release (up to 96%). It is the hyposmo-

Table 2. Comparison of Plasma Taurine Concentrations in Normal Subjects

	Plasma Taurine Concentration, μmol/L
De Luca et al (this study)	48.6 ± 4.9 (26)
Berson et al[24]	57.0 ± 8.0 (13)
Uma et al[25]	46.0 ± 8.0 (8)
Hussain and Voaden[17]	62.0 ± 4.0 (24)
Airaksinen et al[26]	43.0 ± 2.0 (10)
Franconi et al[27]	93.3 ± 6.3 (34)*
Franconi et al[27]	103.9 ± 7.2 (29)†

NOTE. Data are mean ± SEM (no.).
*Young subset age (yr), 38 ± 2.6.
†Older subset age (yr), 50.6 ± 1.1.

Table 3. Plasma and Platelet Taurine Content and Platelet Taurine Uptake and Release in Healthy Control Subjects and Type 2 Diabetic Patients

	Healthy Control Subjects (n = 26)	Type 2 Diabetic Patients (n = 38)
Plasma taurine (μmol/L)	48.6 ± 4.9	32.1 ± 1.9*
Platelet taurine (nmol/mg protein)	183.0 ± 12.3	148.0 ± 11.2†
Platelet taurine uptake (pmol total taurine/10^8 platelets^{-1} · min^{-20})	524.9 ± 43.0	321.2 ± 23.9*
Platelet taurine release (% total platelet ^{3}H taurine)	29.5 ± 1.3	38.7 ± 1.0*

NOTE. Data are mean ± SEM.
*P = .000.
†P = .043 *v* control subjects.

lar condition and not the reduction of Na^+ concentration, which induces taurine release, because a medium with low Na^+, but made isosmotic with sucrose, did not elicit the release of ^{3}H taurine. The taurine release from platelets preloaded with ^{3}H taurine for 1 hour and resuspended in fresh isosmotic KBM, linear with time up to 15 minutes, was significantly increased in type 2 patients in comparison to controls (see Table 3).

DISCUSSION

The present results show that plasma from type 2 diabetic patients has a lower taurine content in accordance with the knowledge that plasma concentration of amino acids changes in diabetic patients.[28] A 30% reduction of plasma taurine level in type 1 diabetic patients with good metabolic control was shown by Franconi et al[27] and indirectly by Luzi et al.[29] In diabetes, a higher consumption of taurine by some tissues was proposed as the cause of its reduced plasma level.[27] Indeed, taurine could play a protective role as an antihypoxic agent in those diabetic tissues with reduced oxygenation[30] and as an antioxidant (reducing) agent versus oxygen-free radicals and other oxidants that could be responsible of diabetic complications.[31]

Taurine, with a concentration of 183 nmol/mg protein (Table 3), is a very abundant free amino acid in human platelets. This value is similar to the one reported by Hussain and Voaden[17] and lower than data obtained by Franconi et al.[27] High intracellular concentrations of taurine have been found in other cell types with a limited capacity for taurine biosynthesis,[32] which implies that taurine must be taken up against its concentration gradient. In normal human blood platelets, Voaden et al[19] found 2 carrier systems with apparent K_m values of 8 and 200 mmol/L, corresponding to high- and low-affinity uptake systems, respectively. Lower taurine content that we found in platelets from diabetic subjects could be due to reduced uptake, as well as increased release. The reduced platelet uptake observed in our experiments is not dependent on the lower taurine level in plasma, as plasma taurine concentration in diabetic patients saturates the 2 carrier systems. It could be the consequence of a change in the carrier systems that mediate the passage of taurine across membranes, or a compensatory mechanism in response to an increase of other organic osmolytes, or both. Modified kinetics were observed by Trachtman et al[33] in synaptosomes isolated from hyperglycemic rats, suggesting an adaptive mechanism during chronic hyperglycemia. Kinetic studies on taurine uptake in platelets from diabetic patients were not performed in this study, because to characterize transport kinetics, platelets must be resuspended in a medium other than plasma. This situation does not reflect what happens in vivo, as it is recognized that the kinetics of taurine uptake in platelets may not be the same in plasma as in other medium,[34] and that plasma proteins affect platelet function.[35] In our experiments, the blood platelets respond to the diabetic condition by reducing the uptake and by increasing the release of taurine, with a consequent lower intraplatelet content of the amino acid. The lower taurine level in platelets of diabetic patients was related to an increased platelet aggregation in response to arachidonic acid, which can be reduced by oral administration of taurine.[36] Our results that high glucose levels modify taurine uptake in platelets from healthy subjects suggest that the reduced taurine uptake in platelets from diabetic patients could be the consequence of hyperglycemia.

In diabetes, high glucose drives the aldose reductase (AR) reaction in cells that express the enzyme, including human blood platelets.[37] AR is induced by hyperosmotic stimuli in renal inner medullary cells.[38] Human RPE cells have been shown to exhibit different basal expression and activity of AR in response to 20 mmol/L glucose[39] and to use intracellular organic solutes in an interactive manner to help the intracellular tonicity regulation.[40] This may imply that individual variation in basal activity or expression of AR will determine sorbitol accumulation and secondary compensatory depletion of myo-inositol and taurine in these cells. Taurine, being the major intracellular amino acid in the retina, is also the major amino acid in the efflux in response to osmotic change. High levels of AR activity were associated with the development of chronic diabetes complications.[41-43] In particular, a immunohistochemical study showed enhanced expression of AR in diabetic human RPE and retinas and its correlation with the severity and duration of diabetic retinopathy.[43]

It is known that platelets and RPE possess similar taurine carrier systems.[15,18-20] Smith et al[44] isolated from rat brain a cDNA clone encoding a taurine transporter and showed that the transporter mRNA is located in all the other studied tissues, including retina. The investigators have suggested that a single

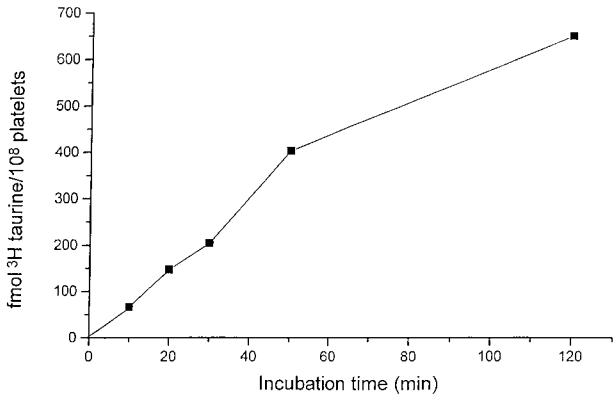

Fig 1. Time course of taurine uptake in PRP. Values are means of separate determinations in 8 healthy control subjects. SEM was lower than 5%.

taurine transport functions in both the brain and peripheral tissues. Therefore, if taurine depletion observed in platelets occurs also in RPE in response to osmotic change, the loss of protection system based on taurine might result in a higher retinal vulnerability in diabetes. It would be reasonable that effects on a long-lived tissue, such as the RPE, might be more severe than on blood platelets with their half-life of only 9 to 11 days. In addition, increased oxidative damage in the pathogenesis of diabetic complications might be exacerbated by taurine depletion, as it acts also as an antioxidant.[9,10]

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