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PRELIMINARY REPORT

Amino Acid Profile in Platelets of Diabetic Patients

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Platelet levels of 19 amino acids were measured in 20 outpatients with type 1 (age [mean ± SE], 35.5 ± 2.0 years) and 27 with type 2 (age, 58.4 ± 1.4 years) diabetes, and 20 young (age 33.7 ± 1.3 years) and 20 older (age 57.4 ± 1.5 years) healthy volunteers. Platelet levels of most amino acids tended to be lower in patients with type 1 diabetes than in healthy controls. In particular, asparagine, glycine, taurine, alanine, valine, cysteine, leucine, phenylalanine, and lysine levels, expressed as nmol/10^8 platelets, were significantly lower. Only taurine significantly decreased in patients with type 2 diabetes, whereas threonine, alanine, and isoleucine increased.

ALTHOUGH IN PLASMA,[1-4] erythrocytes, and leukocytes[5,6] the free amino acid content has been determined, in platelets, apart from neurotransmitter amino acids[7-9] and taurine,[10,11] it has received relatively little attention. Changes in platelet taurine content have been reported in various dysfunctions, including diabetes mellitus.[11,12]

It is well known that carbohydrate as well as protein and lipid metabolism appears to be modified in diabetes mellitus and that the absolute or relative lack of insulin leads to defective amino acid metabolism, which may be a more important factor than hyperglycemia in the etiology of some diabetic complications.[13]

The aim of this study was to investigate platelet amino acid concentrations in subjects with type 1 and type 2 diabetes mellitus. Platelets were chosen because their function is somewhat changed in diabetes,[14,15] and altered platelet function may play a role in the pathogenesis of microvascular and macrovascular complications.[16]

PATIENTS AND METHODS

Patients

The study was conducted with groups of subjects who gave informed consent. These groups consisted of 20 patients with type 1 and 27 with type 2 diabetes and healthy control volunteers who had no family history of diabetes mellitus and normal glucose tolerance test results. The healthy group was divided according to age into young (age [mean ± SD] 33.7 ± 1.3 years) and older (age 57.4 ± 1.5 years) subsets to match the type 1 (age 35.5 ± 2.0 years) and type 2 (age 58.4 ± 1.4 years) diabetic groups. Blood was collected by venopuncture after overnight fasting. The mean glycated hemoglobin and glucose were, respectively, 7.5% ± 0.2% and 9.9 ± 1.4 mmol/L in type 1 and 7.5% ± 0.3% and 8.2 ± 0.4 mmol/L in type 2 diabetic groups.

Determination of Platelet Amino Acid Content

Platelet pellets, obtained from platelet-rich plasma as previously reported,[12] were washed once with fresh Krebs bicarbonate medium and then broken by freezing and thawing after resuspension in ice-cold distilled water. Amino acids were derivatized with phenylisothiocyanate, and their content was determined by high-performance liquid chromatography in a Waters chromatographic system equipped with a pico-tag column (3.9 × 300 mm), containing a high-efficiency reverse-phase silica packing.

Statistical Analysis

The SAS statistical package for personal computer[17] was used. Significance was calculated by the Student *t* test; *P* values of ≤.05 were considered significant.

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RESULTS AND DISCUSSION

In this study, patients with type 1 and type 2 diabetes were metabolically controlled, as suggested by hemaglobin A_{1c} values and by the nonsignificant differences from controls in alanine and in the sum of branched-chain amino acid plasma levels (data not reported). In addition, no significant differences among the groups were observed for plasma phenylalanine/tyrosine ratio, a parameter indicating the relationship between essential and nonessential amino acids. The concentration of free amino acids in blood platelets of healthy volunteers and diabetic patients is shown in Table 1. The 2 control groups, which differed in age, had similar amino acid levels. In particular, taurine is the most abundant free amino acid, accounting for more than 50% of the total free amino acid pool. The biochemical role of taurine in platelets has not been fully elucidated, although its antiaggregating effects have been demonstrated;[11] however, its presence in such large quantities in almost all tissues, including platelets, may indicate a more universal role, as in ion movement or in osmotic regulation.[18,19] Taurine is an unusual compound with many characteristics that differentiate it from almost all other amino acids. It is a β-amino acid containing a sulfur group instead of a carboxyl group, and it is frequently the most abundant free amino acid in the intracellular space. In both control platelets, aspartate, glutamate, glycine, glutamine, isoleucine, and leucine were present at concentrations ranging from 2.4 to 4.4 nmol/10^8 platelets (Table 1); all other free amino acids were present in lower concentrations. Glutamate, glycine, and aspartate represent approximately 14% of the total free amino acid pool and are present in relatively high levels in other blood cellular types, whereas the branched-chain amino acid content in platelets is higher than that in lymphocytes, monocytes, and polymorphonucleated cells.[6] The significance of the relatively high levels of glutamate, glycine, and aspartate in platelets remains to be better clarified. It has been demonstrated that platelets as well as red cells contribute to the regulation of plasma levels of excitatory amino acids[20] and that glutamine, glutamate, GABA, and aspartate levels are decreased in platelets from autistic children.[3] The platelet amino acid pattern in control subjects agrees with that previously reported by D'Andrea et al,[8] except for methionine, phenylalanine, isoleucine, and leucine, which are detectable by our method, whereas values reported by Mauri et al[9] are higher.

The concentrations of almost all free amino acids in platelets of patients with type 1 diabetes tend to be lower than control values. The same profiles were also obtained when the contents of free amino acids were calculated as nanomoles per milligram of protein (data not reported). In particular, in type 1 diabetes, asparagine, glycine, taurine, alanine, valine, cysteine, leucine, phenylalanine, and lysine were significantly lower than in controls. In type 2 diabetes, a significant decrease of taurine and a significant increase of threonine, alanine, and isoleucine were observed. The different changes in platelet free amino acid content in subjects with type 1 and type 2 diabetes, compared with their respective controls, can be added to the differences between the 2 types of diabetes, such as the greater degree of insulin resistance in type 2 diabetes mellitus.

The cause and significance of decreased levels of some amino acids in platelets from diabetic patients require further studies. Several lines of evidence have shown the involvement of taurine in osmoregulation. Less information exists about a similar role of the other amino acids, although it is known that human lymphocytes contain high concentrations of taurine and other amino acids, which may make a contribution as volume regulatory osmolytes.[6] The decreased content of some amino acids in platelets of diabetic

Table 1. Free Amino Acid Content in Human Platelets of Diabetic Subjects

Amino Acid	Controls (n = 20)	Type 1 (n = 20)	Controls (n = 20)	Type 2 (n = 27)
Aspartic acid	2.69 ± 0.28	2.01 ± 0.20	3.55 ± 0.33	2.40 ± 0.31
Glutamic acid	4.16 ± 0.40	3.34 ± 0.32	4.43 ± 0.35	5.28 ± 0.34
Serine	2.00 ± 0.24	1.49 ± 0.17	1.53 ± 0.21	2.03 ± 0.20
Asparagine	0.59 ± 0.07	0.35 ± 0.04*	0.97 ± 0.17	1.27 ± 0.09
Glycine	3.76 ± 0.43	2.63 ± 0.18†	3.80 ± 0.39	2.97 ± 0.22
Glutamine	2.45 ± 0.26	2.47 ± 0.24	2.69 ± 0.31	3.17 ± 0.22
Taurine	44.32 ± 1.38	30.90 ± 1.36*	46.54 ± 1.45	30.71 ± 1.49*
Histidine	2.26 ± 0.28	1.72 ± 0.20	1.50 ± 0.23	1.89 ± 0.14
Threonine	1.11 ± 0.12	1.27 ± 0.11	2.02 ± 0.25	3.71 ± 0.37*
Alanine	2.99 ± 0.24	1.96 ± 0.16*	2.04 ± 0.30	3.68 ± 0.23*
Arginine	1.01 ± 0.13	0.71 ± 0.08	1.29 ± 0.14	1.31 ± 0.09
Tyrosine	1.10 ± 0.12	1.16 ± 0.12	1.08 ± 0.15	1.31 ± 0.09
Valine	1.74 ± 0.14	1.36 ± 0.08†	1.62 ± 0.14	1.74 ± 0.10
Methionine	0.92 ± 0.15	0.71 ± 0.08	0.72 ± 0.13	0.68 ± 0.07
Cysteine	0.85 ± 0.16	0.41 ± 0.06†	0.74 ± 0.11	0.87 ± 0.14
Isoleucine	3.45 ± 0.20	2.96 ± 0.45	2.41 ± 0.26	5.14 ± 0.48*
Leucine	3.06 ± 0.29	2.07 ± 0.19*	2.72 ± 0.34	2.05 ± 0.19
Phenylalanine	1.55 ± 0.19	0.94 ± 0.08*	1.22 ± 0.20	0.96 ± 0.09
Lysine	1.02 ± 0.17	0.30 ± 0.02*	1.25 ± 0.19	1.01 ± 0.09

NOTE. Data are means ± SE (nmol/10^8 platelets).

* $.000 \leq P \leq .01$.

† $.01 \leq P \leq .05$.

patients could be considered in this context; it could also be an induced metabolic adaptation in diabetes or, as demonstrated for excitatory amino acids,[20] a mechanism of regulation of plasma amino acid levels.

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