Preliminary Report

FREE AMINO ACIDS IN LIVER, PLASMA, AND MUSCLE OF PATIENTS WITH CIRRHOSIS OF THE LIVER

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A statistically valid increase in clearance from plasma of branched-chain amino acids and threonine in cirrhotic patients as compared to normal subjects has recently been reported in this journal [1]. A significant increase in rate of disappearance of branched-chain amino acids was observed after an oral protein load or following infusion of pure L-amino acids; the significant increase in threonine clearance was observed only after intravenous administration of a balanced mixture of essential amino acids. Arguments in support of the premise that this phenomenon may be indicative of an increased need by the cirrhotic patient for these amino acids have been presented. In order to seek further evidence for this proposal, the distribution of free amino acids in liver, plasma, and muscle has been determined at the time of operation in cirrhotic patients and in patients with normal hepatic function undergoing cholecystectomy.

METHODS

"Control" subjects were seven patients of comparable age undergoing elective cholecystectomy; in each instance, preoperative studies of liver function were within normal limits. Ten patients with cirrhosis of the liver all had well-compensated liver disease and portal hypertension and were undergoing elective portacaval shunt.

Blood specimens were obtained just prior to induction of anesthesia in order to eliminate variables introduced by administration of intravenous fluids or blood. A portion of each liver specimen obtained at biopsy was sent for histological confirmation of "normality" or of the presence of cirrhosis. All muscle specimens were obtained at biopsy from the right rectus abdominis muscle within the confines of the incision.

The processing of blood specimens has been previously described. Tissue specimens were immediately chilled, freed of fat and connective tissue, weighed, and converted to a 20% homogenate in 1% picric acid. A small portion of each specimen was reserved for analysis of water and nitrogen. Concentrations of free amino acids in the picric acid filtrates were determined with the Piez modification of the amino acid analyzer [3].

Limited sample size and methodological difficulties prevented the acquisition of sufficient data on tryptophan content of these specimens of tissue.

The data were analyzed statistically by the nonparametric rank-sum test of Wilcoxon.

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Table 1. Concentration of Free Essential Amino Acids in Liver, Plasma, and Muscle of "Controls" and Patients with Cirrhosis

Amino Acids, Water, Nitrogen	Liver		Muscle		Plasma	
	Controls	Cirrhotics	Controls	Cirrhotics	Controls	Cirrhotics
Threonine	59.8 ± 13.1	52.2 ± 7.1	90.9 ± 24.3	81.4 ± 17.3	14.7 ± 1.3	14.2 ± 1.8
Valine	27.0 ± 2.8	24.3 ± 3.1	24.3 ± 0.4	18.1 ± 1.7[a]	22.0 ± 1.1	15.9 ± 1.6[b]
Isoleucine	10.0 ± 1.2	10.2 ± 1.4	8.3 ± 0.9	5.9 ± 0.6[a]	6.0 ± 0.6	4.6 ± 0.6
Leucine	22.6 ± 2.8	21.4 ± 1.6	15.8 ± 1.3	11.4 ± 1.3[a]	12.0 ± 0.8	9.1 ± 1.3
Lysine	28.8 ± 3.6	30.6 ± 2.6	124.4 ± 21.4	100.2 ± 10.9	18.3 ± 2.2	14.1 ± 1.0
Methionine	3.6 ± 0.9	3.1 ± 0.6	5.3 ± 0.6	4.6 ± 1.3	2.0 ± 0.2	1.8 ± 0.4
Phenylalanine	10.7 ± 1.3	12.1 ± 1.4	10.7 ± 2.0	10.9 ± 2.0	6.0 ± 1.0	7.2 ± 0.5
Water	72.0 ± 0.7	76.8 ± 1.1[b]	71.7 ± 3.5	75.0 ± 1.4		
Nitrogen	2.71 ± 0.11	2.46 ± 0.19	2.86 ± 0.20	2.77 ± 0.12		

Note: Values for amino acids in liver and muscle are reported as mean plus or minus standard error in micromoles per 100 gm. of tissue. Values for plasma from fasting patients are reported in micromoles per 100 ml. Content of water and nitrogen is in grams per 100 gm. of tissue.

[a] $P < 0.05$.

[b] $P < 0.01$.

RESULTS

The data with accompanying standard errors are reported in Table 1. A significantly lower concentration of branched-chain amino acids (valine, isoleucine, leucine) in the muscle of cirrhotic patients, as compared to "control" subjects, was found. A valid difference in concentration of free valine in plasma from fasting patients was also observed.

In the statistical analysis of these data, nonparametric methods have been used, since the form of the distribution of the population may not agree with the assumptions of the classical statistical methods. However, since the "t" test for groups of unequal size is somewhat more "powerful," this statistic has also been derived, and an equivalent order of significance was found by this method as well.

DISCUSSION

These findings might be explained on the basis that the increased need for branched-chain amino acids is supplied by protein catabolism in muscle, bringing about a secondary depletion of amino acid pools in muscle. This might provide an explanation for the profound muscle-wasting observed in the more advanced forms of cirrhosis.

In a study of the chemical pathology of amino acid deficiency in rats force-fed diets devoid of a single amino acid, Sidransky and Verney [4] have found a decrease in protein synthesis in skeletal muscle as measured by rate of incorporation of a radioactive amino acid. The content of free amino acids in the livers of these rats was normal, including the amino acid absent from the diet; they also postulate that breakdown of muscle protein takes place to satisfy obligatory requirements for synthesis of more essential proteins by the liver.

Miller *et al.* [2], in studies involving perfusion of the eviscerated carcass of rats with a labeled amino acid, have shown that nonhepatic tissues rapidly remove amino acids from circulating blood. The increased rate of disappearance of branched-chain amino acids from the plasma of patients with cirrhosis may, on the basis of findings reported here, be related to an increased uptake by muscle relatively deficient in these amino acids; in turn, the decreased concentration in muscle of these amino acids may be secondary to increased catabolism of muscle protein so that adequate amounts of these amino acids are available to the liver for the synthesis of essential proteins by this organ.

SUMMARY

A significant decrease in concentration of branched-chain amino acids in skeletal muscle of cirrhotic patients has been observed. These data are presented in further support of the concept that a relative deficiency of these amino acids may be present in cirrhotic patients.

REFERENCES

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