Volume 216, number 2, 287-290 FEB 04731 June 1987

Effects of protein-free diet in amino acid homeostatis of rat blood plasma and gut contents

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Received 11 March 1987

Amino acids in rat systemic and portal vein plasma and jejunal and ileal gut contents after 7 days of feeding normoprotein (NP) and protein-free diet (PF) are investigated. Amino acid analyses revealed that ingestion of PF diet resulted in unusual amino acid patterns in both plasmas. Thus, while the levels and/or molar ratios of all indispensable amino acids were significantly decreased, those of several gluconeogenetic amino acids, especially of glycine and alanine, were increased in both plasmas, but particularly in portal. By contrast, the molar ratios of the majority of amino acids in jejunal and ileal contents were not changed by PF diet.

Amino acid homeostatis; Protein-free diet; (Rat plasma, Gut)

1. INTRODUCTION

In animal species studied, the free amino acid composition of the luminal contents of small intestine differs significantly from that of the dietary protein fed [1]. Ingested protein is diluted 4–6-fold with endogenous protein released into the alimentary canal as a result of mucosal cell renewal and digestive secretions [1,2]. Therefore, analysis of the amino acid composition of the contents of the entire small intestine after feeding protein-free diet to rats [3] provided results similar to those obtained after feeding various proteins.

On the other hand, examination of free amino acid concentrations on both sides of the mucous membrane provided evidence that the amino acid pattern in the gut was considerably altered in passing through the mucosa [1–4]. Therefore, the portal vein amino acid composition is influenced by anabolic and catabolic reactions that occur in cells of gut wall [1,2,5,6].

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While the limits of homeostatic regulation of many constituents of blood are quite well-defined, for gut contents the information is very scanty. For that reason, it seemed worthwhile to determine how much ingestion of protein-free diet interferes with amino acid homeostasis in systemic and portal blood plasma and jejunal and ileal gut contents.

2. MATERIALS AND METHODS

Experiments were performed using male and female 60-day-old rats of the Wistar strain weighing 190–210 g. The rats were individually caged in wire-bottomed cages at 22–25°C and fed stock laboratory diet ad libitum until their body mass had plateaued. After that, two groups of rats were separated, 8–10 in each group. The control group were fed ad libitum isoenergetic normoprotein (15% casein) diet (NP group); the experimental group were fed protein-free high-carbohydrate (0% casein) diet (PF group). Food consumption and body mass were recorded daily. After 7 days of that treatment rats were anesthetized with ether between 8 and 9 a.m. Following laparatomy, two blood samples were taken; one from the inferior

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

vena cava (systemic plasma) and the other from the clamped portal vein. The entire small intestine was delivered with ligatures at pilorus and ileocecal junction. The excised small intestine was chilled immediately on ice, and a ligature was placed to mark the midpoint of the small gut. Gut contents of proximal (jejunum) and distal (ileum) half of small intestine were washed out with about 15 ml cold isotonic glucose solution, and then hydrolyzed with dilute HCl, as described by Nasset and Lu [2]. Amino acid concentrations in plasma and final filtrate of gut contents were determined by ion-exchange chromatography utilizing a 119C Beckman amino acid analyser. Student's t-test was used for statistical analysis of the data.

3. RESULTS

For purposes of this presentation, the total of free amino acids found in jejunum vs ileum with NP and FP diets was used to compute the molar ratios of individual amino acids (mol/1000) as shown in table 1. As can be seen, the differences between amino acid composition of jejunal and ileal contents, in both groups, were minimal with significant variations ($p < 0.05$) only for glutamic acid, glycine and lysine in the NP group and for glycine and lysine in the PF group. It was also noticed that significant differences were observed for glutamic acid, alanine, valine and lysine in both jejunal and ileal contents and for glutamine, glycine and leucine only in that of jejunum between control and experimental animals.

The results of amino acid analysis of systemic and portal blood plasma are presented in table 2. The results are expressed in μmol/l (column I) and in mol/1000 mol total free amino acids (column II). It was noticed that the normal NP systemic/portal plasma ratio, that is with significantly higher portal values for the majority of amino acids, was maintained in the PF group with only a few exceptions. Only glutamic acid in the NP group and glutamine in the PF group showed an opposite tendency with highly signifi-

Table 1

Molar ratios (mol/1000 amino acid residues; means ± SE) of amino acids in jejunal and ileal gut contents of rats fed normoprotein (NP) and protein-free diet (PF)

Amino acid	NP diet (control)		PF diet	
	Jejunum	Ileum	Jejunum	Ileum
Asp	54 ± 5.6	61 ± 7.2	38 ± 4.0	44 ± 5.0
Thr	45 ± 3.5	46 ± 3.1	47 ± 2.0	41 ± 4.5
Ser	56 ± 3.3	53 ± 4.1	49 ± 5.2	54 ± 2.9
Glu	113 ± 6.4	132 ± 10.8	66 ± 4.9[c]	79 ± 3.8[b]
Gln	59 ± 4.1	42 ± 3.7[d]	44 ± 3.1[a]	38 ± 5.2
Pro	66 ± 3.0	77 ± 7.7	60 ± 5.8	73 ± 3.5
Gly	51 ± 6.1	75 ± 7.5[d]	73 ± 6.8[a]	95 ± 3.2[d]
Ala	73 ± 3.7	84 ± 3.4	104 ± 9.5[b]	114 ± 5.7[b]
Val	55 ± 1.8	55 ± 1.6	74 ± 4.7[b]	69 ± 5.8[a]
Met	22 ± 1.6	17 ± 1.8	22 ± 3.2	20 ± 2.1
Ile	36 ± 2.6	33 ± 2.7	36 ± 3.5	33 ± 2.3
Leu	88 ± 4.5	80 ± 5.7	67 ± 6.5[a]	71 ± 3.8
Tyr	43 ± 3.0	34 ± 2.4	36 ± 2.5	30 ± 2.3
Phe	47 ± 3.0	44 ± 3.8	46 ± 4.2	47 ± 5.2
Lys	103 ± 6.3	83 ± 5.6[d]	138 ± 7.8[b]	105 ± 6.8[a,d]
His	38 ± 4.0	35 ± 4.1	40 ± 4.1	37 ± 4.1
Arg	52 ± 2.5	49 ± 3.9	60 ± 4.6	50 ± 5.0

There were 8–10 rats (NP diet) or 5–6 rats (PF diet). According to the t-test, [a,b,c] indicate $p < 0.05$, $p < 0.01$, $p < 0.001$, in comparison of NP vs PF; [d] significantly different $p < 0.05$ between jejunal and ileal content

Table 2

Amino acids in systemic and portal blood plasma [expressed in μmol/l (I) and mol/1000 amino acid residues (II); means ± SE] of rats fed NP or PF diet ($n = 5$–6)

Amino acid	NP diet				PF diet			
	Systemic		Portal		Systemic		Portal	
	I	II	I	II	I	II	I	II
Asp	36 ± 2	11 ± 2.6	49 ± 4[d]	12 ± 1.4	38 ± 2	15 ± 0.7	48 ± 2[d]	18 ± 3.2
Thr	444 ± 28	140 ± 9.8	498 ± 28	114 ± 8.3	87 ± 6[c]	37 ± 2.4[c]	88 ± 8[c]	36 ± 3.5[c]
Ser	210 ± 15	66 ± 5.3	298 ± 22[d]	72 ± 3.5	281 ± 14[b]	114 ± 3.7[c]	274 ± 8	102 ± 1.2[c,d]
Glu	198 ± 9	63 ± 5.9	152 ± 12[d]	37 ± 2.9[e]	234 ± 22	96 ± 3.1[c]	185 ± 15	69 ± 8.8[b,d]
Gln	257 ± 22	81 ± 7.0	341 ± 26[d]	78 ± 4.5	348 ± 28[a]	127 ± 9.1[b]	225 ± 15[c]	82 ± 3.9[e]
Pro	252 ± 16	80 ± 8.6	388 ± 36[d]	90 ± 6.7	138 ± 19[b]	59 ± 5.4	172 ± 14[b]	65 ± 9.8
Gly	123 ± 7	39 ± 1.5	210 ± 16[e]	50 ± 1.6[d]	314 ± 25[c]	127 ± 5.4[c]	387 ± 17[c]	138 ± 10.0[c]
Ala	352 ± 30	111 ± 5.8	690 ± 38[f]	164 ± 7.6[f]	398 ± 39	170 ± 10.0[c]	559 ± 43[a,d]	202 ± 15.2[a]
Val	204 ± 14	65 ± 3.7	297 ± 20[e]	69 ± 4.8	65 ± 5[c]	27 ± 0.9[c]	86 ± 9[c]	32 ± 1.2[c]
Met	53 ± 9	17 ± 3.5	57 ± 7	13 ± 2.0	17 ± 1[c]	7 ± 0.6[a]	23 ± 2[b,d]	8 ± 0.6
Ile	90 ± 6	28 ± 2.9	109 ± 9[d]	26 ± 1.8	30 ± 4[c]	13 ± 1.4[b]	50 ± 5[c,d]	18 ± 2.1[a]
Leu	124 ± 8	39 ± 2.8	172 ± 14[d]	42 ± 2.5	60 ± 5[c]	24 ± 1.6[c]	82 ± 8[b,d]	30 ± 1.3[b,d]
Tyr	92 ± 7	29 ± 3.3	116 ± 8	28 ± 2.4	31 ± 2[c]	13 ± 2.9[b]	34 ± 3[c]	12 ± 1.5[c]
Phe	55 ± 4	17 ± 1.6	79 ± 5[e]	19 ± 0.8	40 ± 4[a]	16 ± 2.7	39 ± 2[c]	15 ± 1.9[a]
Lys	500 ± 41	156 ± 13.0	557 ± 28	132 ± 5.9	262 ± 12[c]	106 ± 5.8[b]	327 ± 10[c,e]	120 ± 7.0
His	58 ± 4	19 ± 1.7	84 ± 5[e]	20 ± 1.2	63 ± 4	26 ± 1.3[a]	82 ± 3[e]	31 ± 2.1[b]
Arg	114 ± 7	36 ± 3.5	153 ± 10[d]	35 ± 0.4	57 ± 7[c]	23 ± 4.8	58 ± 6[c]	22 ± 4.1[b]

[a,b,c] Significantly different ($p < 0.05$, $p < 0.01$, $p < 0.001$) between NP (control) and PF (experimental) group
[d,e,f] Significantly different ($p < 0.05$, $p < 0.01$, $p < 0.001$) between systemic and portal plasma

cant values in systemic plasma. Generally, feeding PF diet resulted in decreasing values of essential amino acids (Thr, Val, Met, Ile, Leu, Phe, Lys and Arg) but in increasing those of non-essential (Ser, Glu, Ala) especially of glycine, in both systemic and portal plasma (see table 2).

4. DISCUSSION

In view of the known role of the digestive system in amino acid homeostasis [1], we investigated the overall effects of protein-free diet on the amino acid composition of jejunal and ileal gut contents and systemic and portal plasma in the period of maximal digestibility of rat. The results showed generally that the amino acid composition of gut contents was not changed significantly by ingestion of protein-free meal (table 1). These results agree generally with those of other, e.g. Nasset and co-workers [1,2]. They found that a single test meal fed to adequately nourished fasting animals yielded a mixture of amino acids in the gut lumen that was qualitatively relatively constant [3]. This was true whether the meal contained egg albumin or zein, or when devoid of protein. These results indicate that the digestive system provides a preabsorptive step in amino acid homeostasis by the hydrolysis of the mixture of luminal, dietary and endogenous proteins, derived from shed mucosal cells and digestive secretions. By contrast, the results presented in table 2 show that it may be useful to differentiate between luminal or digestive homeostasis and extraluminal or metabolic homeostasis [2]. It is possible that under the condition of exogenous protein ingestion cessation, a large availability of carbohydrates could maintain and/or increase concentrations of non-essential amino acids, whereas the endogenous protein sources seemed to be inadequate to prevent the level of essential amino acids decreasing in portal blood [7]. According to Fernstrom et al. [6], the plasma concentrations of the neutral amino acids, aromatic and branched-chain amino acids varied directly with the protein content of the diet. In contrast, the relations between dietary protein content and the plasma concentrations of glycine and

alanine, two small neutral amino acids, were inverse. It is obvious that the amino acid homeostasis in the gut lumen particularly prevents wide fluctuations in the amino acid pattern of gut content and blood plasma [1,7]. In consideration of our investigations on the effects of only 7 days of ingestion of PF diet, further work is required to establish the long-term effects during early growth and under various physiological states and nutritional conditions.

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