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Amino acids malabsorption
casein peptides
Hartnup disorder

Intestinal Absorption of Amino Acids and Peptides in Hartnup Disorder

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Extract

This paper reports the investigation of a case of Hartnup disorder, in which tolerance tests were carried out with a mixture of free amino acids simulating casein and a partial enzymic hydrolysate of casein containing small peptides. The investigation also included measurement of renal clearances of amino acids. The patient, a girl aged 26 months, was given by mouth 5 g amino acid mixture in water, blood samples being taken before the dose and at 15, 30, and 45 min after the dose. The procedure was repeated with an equivalent dose (4.7 g) of the enzymic hydrolysate, containing the same amount of nitrogen. The results were compared with those from six normal adults who were given 10 g amino acid mixture and on another occasion an equivalent dose of an enzymic hydrolysate of casein containing small peptides. In the adults, the increments in the plasma level of a given amino acid were similar whether the amino acid mixture or the enzymic hydrolysate was given. In the patient, a different pattern was seen. In some cases (Lys, Arg, and Pro) there was a large increase in plasma levels after both the amino acid mixture and the enzymic hydrolysate, as in the adult controls. In others (Gly, His, Tyr, Ser, Glu) there was no increase or a decrease in plasma levels after the amino acid mixture, but an increase after the enzymic hydrolysate. In a third category (Ala, Val, Leu, Ile, Met, Phe), increments after the enzymic hydrolysate were greater than after the amino acid mixture. The results with Thr were unexpected: after the amino acid mixture there was an initial rise in plasma levels and after the enzymic hydrolysate an initial fall. Renal clearances of Pro, Glu, Asp, and Lys were normal and those of Arg and Gln were slightly increased. The clearance of Gly was slightly increased and the clearances of His, Tyr, Ser plus Asn, Ala, Val, Leu, Ile, Met, Phe, and Thr were greatly increased. The results suggest the following. Absorption of free Lys, Arg, and Pro was normal. Absorption of free Gly, His, Tyr, and Ser was probably severely impaired and that of Val, Leu, Ile, Met, and Phe probably subnormal. Absorption of Glu and Ala was probably also subnormal. We cannot account for the results obtained with Thr. The results indicate that many neutral amino acids are malabsorbed in Hartnup disorder, and that free Glu may also be malabsorbed. Amino acids which are poorly absorbed in the free form are better absorbed from peptides. Most of the amino acids with a substantial increase in renal clearance also provided evidence of malabsorption. The finding of an increased renal clearance of Gly agrees with several previous observations in Hartnup disorder.

Speculation

It is now known that di- and tripeptides are taken up intact by the absorptive cells of the small intestine, in addition to free amino acids. After uptake, the peptides undergo hydrolysis. Previous investigations in Hartnup disorder and cystinuria, combined with physiologic investigations, have shown that mucosal uptake of small peptides is independent of that of amino acids. The present investigation of a case of Hartnup disorder suggests that although many neutral amino acids are poorly absorbed in this condition when given in the free form, absorption of all these "affected" amino acids is better when given in peptide form. It appears that the peptide uptake system or systems of the intestinal mucosa are unaffected by the transport defect of Hartnup disorder, and that this is the explanation of the ability of the patients to maintain a reasonable state of nutrition when on an adequate diet, and of the frequent absence of obvious evidence of intestinal disturbance.

It is now established that in addition to mucosal uptake of free amino acids, there is a second mode of protein absorption in which small peptides are taken up by the absorptive cells, subsequently undergoing intracellular hydrolysis (18). The importance of this mode of absorption in Hartnup disorder, in which there is a congenital transport defect for many neutral amino acids in the small intestine and the kidney (14, 19, 20), has been suggested by investigations in which the ability of patients to absorb free amino acids and dipeptides have been compared. In the first case to be investigated in this way, it was shown by means of tolerance tests that absorption of free histidine, free tryptophan, and free phenylalanine was grossly impaired, but that absorption of these amino acids from the dipeptides β-alanylhistidine, glycyltryptophan and phenylalanylphenylalanine was relatively normal (2, 21). In a second case, tolerance tests indicated that absorption of free tyrosine was defective, but that this amino acid was well absorbed from glycyltyrosine, and studies of uptake by a jejunal biopsy showed that uptake of free histidine was poor whereas histidine was taken up well from glycylhistidine (26).

In this paper we report the investigation of a third case in which tolerance tests were carried out with a mixture of free amino acids simulating casein, and a partial hydrolysate of casein containing small peptides. After protein meals, the lumen of the small intestine contains a mixture of peptides, probably mainly small peptides, and free amino acids (1, 18), so that this procedure appeared to approximate to physiologic conditions more closely than the administration of large doses of individual peptides and of free amino acids. It was hoped that the procedure would provide an indication of how many amino acids were involved in the absorptive defect of this particular patient, and whether all of these or only some were better absorbed from the preparation containing peptides. A preliminary report of this work has appeared (15). The investigation also included measurement of renal clearances of amino acids.

CASE REPORT

The patient, *SR*, a girl, the second child of healthy unrelated parents, was born on April 27, 1971. The mother's weight gain during pregnancy was poor and labor started spontaneously after 36 weeks of gestation. It lasted for 6 hr and there were no perinatal

problems. At birth the patient weighed 1,875 g and was thought to be both small for dates and premature. Her progress was slow from birth and this was the main presenting feature. At the age of 11 months her developmental age was 4–5 months. Hartnup disorder was diagnosed at 14 months by thin layer chromatography of urine amino acids and the characteristic aminoaciduria involving neutral amino acids demonstrated by the same method on several subsequent occasions. She has never had a rash and the only neurologic sign has been a generalized hypotonia which has slowly improved. On admission to the Hospital for Sick Children, Great Ormond Street, at the age of 26 months, she was well nourished; her height was 85.5 cm (25th centile) and her weight 10.1 kg (3rd–10th centile). Her developmental age on the Bayley scales was 8 months. Measurement of the renal amino acid clearances confirmed the diagnosis of Hartnup disorder (see Results and Discussion). All other diagnostic investigations were normal. On discharge from hospital she was started on oral nicotinamide, 50 mg daily. Her general health has subsequently remained satisfactory but developmental delay persists.

MATERIALS AND METHODS

The amino acid mixture (Table 1) was made up from L-amino acids and glycine from a formula based on the amino acid composition of casein as given by Ling *et al.* (16). The enzymic hydrolysate of casein, which was readily soluble and intended for oral administration to human subjects, was prepared by hydrolysis with papain followed by further hydrolysis with hog kidney peptidases according to the method described by Clegg *et al.* (6, 7). It contained about 50% free amino acids and 50% small peptides of mean chain length estimated to be 2–3 amino acid residues (5).

After an overnight fast from 6 pm, at 8 am the patient was given by mouth 5 g amino acid mixture in 180 ml water. A capillary blood sample was taken immediately before the dose, and further samples taken at 15, 30, and 45 min after the dose. On the following day at 8 am, after an overnight fast from 6 pm, the procedure was repeated with an equivalent dose (4.7 g) of the enzymic hydrolysate, which contained the same amount of nitrogen. After deproteinization with an equal volume of salicylsulfonic acid (60 g/liter), plasma amino acids were estimated on a Locarte automatic-loading amino acid analyzer.

It was not possible to obtain control data from normal children, and the results were compared with those from six normal adults, who had been given 10 g amino acid mixture and on another occasion an equivalent dose (9.4 g) of an enzymic hydrolysate of casein prepared by a different method, i.e., tryptic hydrolysis (8) but also consisting of a mixture of free amino acids and peptides of 2–3 amino acid residues. The results obtained in normal adults have been fully reported elsewhere (17).

Measurements of renal clearance were based on analysis of a 24-hr urine collection and of a plasma sample obtained during the collection period, before the mid-day meal. Urine and plasma amino acids were estimated on a Technicon TSM amino acid analyzer.

Table 1. *Composition of amino acid mixture simulating casein*

Amino acid	mmol/5 g dose	Amino acid	mmol/5 g dose
Ala	1.5	Lys	2.4
Arg	1.0	Met	0.9
Asp Cys	2.1	Phe	1.5
Cys	0.1	Pro	4.6
Glu	6.9	Ser	2.6
Gly	1.2	Thr	1.6
His	0.8	Trp	0.3
Ile	2.2	Tyr	1.2
Leu	3.4	Val	2.7

RESULTS

The results of the tolerance tests in normal adults showed that at each time interval (15, 30, and 45 min) the increments in the plasma level of a given amino acid were similar (no significant differences by paired *t*-test) whether the amino acid mixture or the enzymic hydrolysate was given. In the patient, however, a different pattern was seen (Table 2). In some cases (Lys, Arg, and Pro (30)) there was a large increase in plasma levels after both the amino acid mixture and the enzymic hydrolysate, as in the adult controls. (In the case of Pro, 30- and 45-min concentrations were much lower after the enzymic hydrolysate than after the amino acid mixture. This was not seen in the normal adults, but it is possible that it was accounted for by the entry of oligopeptides of Pro into the blood, which is known to occur in man (18). A large number of such peptides, at low concentrations, would not be detected by the analytic procedure used). In others (Gly, His, Tyr, Ser, Glu) there was no increase or a decrease in levels after the amino acid mixture, but an increase after the enzymic hydrolysate. In a third category (Ala, Val, Leu, Ile, Met, Phe) increments after the enzymic hydrolysate were greater than after the amino acid mixture. The results with Thr were quite unexpected: after the amino acid mixture there was a rise in plasma levels at 15 and 30 min followed by a fall at 45 min, but after the enzymic hydrolysate there was a fall at 15 and 30 min followed by a return at 45 min to the resting value. These results were confirmed by a repeat analysis.

The results of the renal clearance test are given in Table 3. The clearances of Pro, Glu, Asp, and Lys were normal, and those of Arg and Gln were slightly increased. The clearance of Gly was slightly increased and the clearances of His, Tyr, Ser plus Asn, Ala, Val, Leu, Ile, Met, Phe, and Thr were greatly increased. In most cases the amino acids with a substantial increase in renal clearance and those apparently involved in the intestinal transport defect (see Discussion) were the same. Exceptions were Glu, which had a normal clearance but was probably malabsorbed when given in the free form, and Thr, which had a raised clearance but appeared to be readily absorbed in the free form.

DISCUSSION

Although "tolerance curves" in peripheral plasma do not ordinarily give a reliable indication of the relative rates of absorption of different substrates from the intestine, being influenced also by renal and tissue clearance of the substrates studied, a special case arises when the same amount of the same amino acid is given (*1*) in the free form and (*2*) in peptide-bound form. In these circumstances, mucosal uptake occurs in different forms, but entry into the blood is in the same form (the free amino acid) whether the oral dose is free or peptide bound. This simplifies interpretation of the results. To take a specific example, the failure of the plasma concentration of His to rise after free His in our patients might be contributed to by increased renal and tissue clearance (12), but the large rise in the concentration of this amino acid after the peptide-containing preparation indicates that these factors are unlikely to be entirely responsible, and suggests that malabsorption of the free amino acid plays an important part.

Bearing these considerations in mind, the results suggest the following. Absorption of free Lys, Arg, and Pro was normal. Absorption of free Gly, His, Tyr, and Ser was probably severely impaired, and that of Val, Leu, Ile, Met, and Phe probably subnormal. These results confirm and extend previous observations indicating that a wide range of neutral amino acids (His, Val, Leu, Ile, Met, Phe, Tyr, Trp, Thr) are malabsorbed in Hartnup disorder (2, 12, 19, 21–23, 25, 26, 29), and they support the hypothesis that these amino acids are more satisfactorily absorbed when given in peptide form. It is of particular interest that there was nothing to suggest malabsorption of Pro. This supports the hypothesis (24) that in human intestine and kidney this amino acid is transported largely by a system or systems distinct from the

Table 2. *Resting levels of plasma amino acids and increments in plasma amino acids after patient had ingested amino acid mixture simulating casein and enzymic hydrolysate of casein containing peptides*

Material fed	Amino acid	Resting levels, μmol/liter	Increments, μmol/liter		
			15 min	30 min	45 min
Amino acid mixture	Lys	164	149	189	181
Enzymic hydrolysate		152	176	186	135
Amino acid mixture	Arg	78	74	94	50
Enzymic hydrolysate		94	53	57	44
Amino acid mixture	Pro	137	372	401	210
Enzymic hydrolysate		230	274	126	89
Amino acid mixture	Gly	272	−18	−13	−62
Enzymic hydrolysate		187	38	32	−9
Amino acid mixture	His	71	−2	−3	−2
Enzymic hydrolysate		71	68	83	70
Amino acid mixture	Tyr	42	−6	−5	−10
Enzymic hydrolysate		31	29	34	40
Amino acid mixture	Ser	251	−84	−83	−106
Enzymic hydrolysate		237	55	82	50
Amino acid mixture	Glu	119	−7	−13	−20
Enzymic hydrolysate		78	34	38	35
Amino acid mixture	Ala	204	30	46	12
Enzymic hydrolysate		181	58	65	42
Amino acid mixture	Val	135	30	49	22
Enzymic hydrolysate		110	79	145	123
Amino acid mixture	Leu	95	35	56	70
Enzymic hydrolysate		76	95	132	124
Amino acid mixture	Ile	49	13	34	21
Enzymic hydrolysate		36	64	89	72
Amino acid mixture	Met	16	18	18	14
Enzymic hydrolysate		14	25	32	47
Amino acid mixture	Phe	50	9	11	4
Enzymic hydrolysate		36	33	35	31
Amino acid mixture	Thr	294	77	156	−104
Enzymic hydrolysate		282	−48	−44	7

Table 3. *Renal clearance of amino acids (milliliters per min 1.73 m²) in normal children aged 2–18 years (3) and in patient SR*

Amino acid	Normal values (mean ± SD)	Values in patient	Amino acid	Normal values (mean ± SD)	Values in patient
Lys	1.2 ± 0.4	2	Val	0.2 ± 0.1	41
Arg	0.3 ± 0.1	1	Leu	0.5 ± 0.1	5
Pro	0	0	Ile	0.3 ± 0.1	6
Gly	4.2 ± 1.4	8	Met	0.8 ± 0.3	8
His	9.5 ± 2.6	86	Phe	1.5 ± 0.3	13
Tyr	2.0 ± 0.8	81	Thr	1.0 ± 0.2	14
Ser + Asn	2.4 ± 0.5	24	Gln	0.1–2.3[1]	5
Glu	0.1–2.4[1]	2	Asp	trace–8.8[1]	2
Ala	0.8 ± 0.4	10			

[1] range.

system or systems utilized by most other neutral amino acids (although shared to some extent by Gly).

Interpretation of the results with Glu and Ala is complicated by the fact that much Glu (whether free or initially peptide bound) (4, 17) is transaminated during absorption, yielding Ala, which is absorbed into the blood (28). The results may represent malabsorption of both free Glu and free Ala; however, a possible although unlikely interpretation is that absorption of free Ala was normal, the low curve for Ala following the amino acid mixture actually reflecting only malabsorption of Glu. Although Glu is not involved in the renal transport defect of Hartnup disorder, the possibility that it might share the transport system for neutral amino acids in the small intestine is suggested by the observation that intestinal transport of Glu is strongly inhibited by neutral amino acids in both experimental animals and normal human subjects (4, 10, 11, 27). We are unable to account for the observation that the plasma concentration of Thr initially rises after the amino acid mixture, but initially falls after the enzymic hydrolysate.

The pattern of aminoaciduria in the patient was characteristic of Hartnup disorder, showing a large increase in the excretion of many neutral amino acids, but little or no increase, in the excretion of Pro and the basic and acidic amino acids. The finding of a raised clearance of Gly agrees with several previous observations (9, 25), though some authorities, *e.g.* (14), state that the clearance of Gly is not increased in Hartnup disorder.

SUMMARY

Absorption of free and peptide-bound amino acids was investigated in a girl with Hartnup disorder aged 26 months. Plasma levels of amino acids were followed after oral administration of (*1*) an amino acid mixture simulating casein and (*2*) an equivalent dose of a partial enzymic hydrolysate of casein containing oligopeptides in addition to free amino acids. The results suggested that many neutral amino acids were poorly absorbed when given in the free form, but much more readily absorbed when given as peptides. Unexpectedly, the results also suggested that glutamic acid was poorly absorbed when given in the free form. The results obtained with threonine could not be interpreted. There was an increased renal clearance of many neutral amino acids, including glycine, but clearance of proline was not increased. Most amino acids with an increased renal clearance also appeared to be poorly absorbed when given by mouth in the free form.

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30. Standard abbreviations are used for amino acids (13).
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