Amino Acids (1992) 3: 271–286

Amino
Acids

Absorption of amino acids from the human mouth*

J. V. Vadgama[1] and **D. F. Evered**[2]

[1] Division of Medical Genetics, Department of Pediatrics, UCLA School of Medicine, Torrance, California, U.S.A.
[2] Department of Biochemistry, KQC University of London, U.K.

Accepted March 4, 1992

Summary. Certain amino acids were transported across buccal mucosa in vivo by a carrier-mediated process. Metabolic loss of L-amino acids from the mouth in a 5 min test period was negligible. The buccal mucosal transport process was stereospecific for most L-amino acids tested. The uptake of L-methionine and L-leucine showed a tendency to saturation with increasing substrate concentration. The absorption of L-leucine, L-isoleucine and L-methionine as single amino acids was inhibited in the presence of each other suggesting at least one common transport mechanism. Administration of equimolar amounts of amino acids revealed a specific pattern of absorption that could be classified into fast, intermediate, and slow groups. Absorption of some amino acids was at least partly dependent on the presence of sodium ions in the luminal solution. In conclusion, our studies demonstrate that the human buccal mucosa is permeable to L-amino acids in a selective manner, and may resemble absorption pattern similar to other locations of the gastrointestinal tract.

Keywords: Amino acids – Absorption – Mouth – Buccal mucosa

Introduction

Classical studies by Schanker (Schanker, 1962) demonstrated that the human buccal cavity is lined with mucous membranes, which, like the lining of the entire alimentary canal behaves as a lipoidal barrier to the passage of drugs and other solutes. This finding stimulated the use of human buccal cavity for the administration of several drugs (Beckett and Hossie, 1971). However, only a limited studies have been done to correlate the characteristics of the buccal mucosa

* This work was supported by Grant DK39147 from the National Institutes of Diseases and Digestive and Kidney Diseases, National Institutes of Health, United States Public Health Service, and The Lord Dowding Fund for Humane Research, London, U.K.

with other locations of the gastrointestinal tract, in relation to absorption of substances other than drugs. To date, the buccal mucosa has been shown to exhibit absorption properties for several drugs (Schanker, 1962), sodium dependent uptake of D-glucose, D-galactose, and 3-O-methyl-D-glucose (Manning and Evered, 1976), sodium-dependent uptake of ascorbic acid (Sadoogh-Abasian and Evered, 1979), and facilitated diffusion of nicotinamide and nicotinic acid (Evered et al., 1980). The amino acid antibiotic D-cycloserine was absorbed by passive diffusion (Evered, 1972), a feature consistent with its absorption across the rat small intestine in vitro (Wass and Evered, 1972), and the rat colon (Sprake and Evered, 1979). Here we report the role of human buccal mucosa as a transport site for the uptake of mixtures of protein amino acids, and some single amino acids such as methionine, leucine, and isoleucine.

Methods

Buccal absorption measurement

The method of measuring buccal absorption (Beckett and Hossie, 1971) was modified by using the following buffer solution for preparing the test solution containing amino acids: NaCl, 118.5 mmol/l; KCl, 4.7 mmol/l; KH_2PO_4, 1.2 mmol/l; $MgSO_4 \cdot 7H_2O$, 1.2 mmol/l; citric acid, 1.8 mmol/l; $NaHPO_4 \cdot 12H_2O$, 6.2 mmol/l; $CaCl_2$, 1.9 mmol/l; pH 6.0 $\pm$ 0.05. To study the effect of omitting sodium ions they were replaced by potassium ions in a modified buffer solution: KCl, 123.2 mmol/l; KH_2PO_4, 1.2 mmol/l; $MgSO_4 \cdot 7H_2O$, 1.2 mmol/l; K_2HPO_4, 6.2 mmol/l; citric acid, 1.8 mmol/l; $CaCl_2$, 1.9 mmol/l; pH 6.0 $\pm$ 0.05. Samples (25 ml) of these solutions containing the test amino acids were pre-incubated at 37°C for 5 min and circulated inside the mouth by the cheeks and tongue about once a second for 5 min. The solution was then expelled into a beaker and the mouth rinsed for 5 s with 10 ml of pre-incubated buffer without added amino acids. This washing was expelled into the same beaker and the total volume measured. The pH was checked and the solution diluted to 50 ml with buffer solution. A portion of the mixed solution was centrifuged at 3000 g at room temperature for 20 min, to remove insoluble material. This 5 min contact time was at the upper linear part of a progress curve. At the beginning of each test a buccal "blank" consisting of 25 ml of buffer solution at 37°C but without amino acids, was circulated in the mouth for 5 min. The mouth was rinsed with 10 ml fresh buffer for 10 s. This "blank" solution and the washings were pooled, diluted and centrifuged as before. Buccal "blank" solutions, and saliva collected freshly, were deproteinised using picric acid (Stein and Moore, 1954).

Buccal absorption of amino acids was measured with equimolar mixtures at initial concentrations of 2, 4 and 8 mmol/l. These conditions facilitated comparison with perfusion studies of human small intestine at these concentrations (Adibi and Gray, 1967). Buccal test samples were analyzed as soon as possible after collection but it was necessary to eliminate possible storage artifacts. After dilution to a suitable concentration for analysis (0.1 mmol/l) 0.1 M HCl samples were stored at 4°C and at -20°C and analyzed at intervals of 0, 1, 2 and 3 days. Possible loss by bacterial metabolism was investigated: 25 ml portions of buffer

solution without amino acid were pre-incubated at 37°C for 5 min, circulated in the mouth for 5 min, and expelled into a solution of the amino acid mixtures. The mixed solution, after diluting to 50 ml, was incubated at 37°C and analyzed for individual amino acids at defined intervals.

Amino acid analysis

Amino acid analyses on mixtures were by automatic analyzer (Locarte Scientific Co. Ltd.) using a sodium-buffer gradient recommended by the manufacturers for analyzing protein hydrolysates, with norleucine as internal standard. For separating only methionine, isoleucine, leucine and norleucine an accelerated procedure was devised. Timer settings for the analyzer in min were as follows: pH 4.25 buffer, 35; pH 8.0 buffer, 10; regeneration with 0.2 M NaOH, 20; equilibration with pH 4.25 buffer, 50; with L-ninhydrin pump and recorder off at 80 min. The temperature was 50°C throughout with a total running time of 115 min. Elution times were methionine, 58; isoleucine, 61; leucine, 64; and norleucine, 67.5 min. For separate assay of methionine, a spectrophotometric method was used (Vasantha et al., 1970).

Amino acids

All amino acids were obtained from Sigma London Chemical Co. except L-arginine (Fisons, Loughborough), L-serine, L-tyrosine, L- and D-alanine (British Drug Houses Ltd., Poole, Dorset), D-aspartic acid and L-norleucine (Calbiochem Ltd., Bishops Stortford, Herts.). Purity of the test amino acids was established by automatic analysis as above and one-dimensional thin-layer chromatography on MN 300 cellulose (Mackerey Nagel Co., Donau, Federal Republic of Germany) developed with a solvent consisting of isopropanol/formic acid/water (75:12:5:12.5, by vol) or pyridine/acetone/ammonium hydroxide (58%)/water (45:30:5:20). After drying in hot air for 5–10 min the plates were sprayed with 0.25% ninhydrin (w/v) in 95% ethanol containing 2% (v/v) collidine.

Subjects

The subjects were three Caucasian men aged 26, 32 and 36 years, numbered 1, 2 and 3, respectively, and a Nigerian male aged 26 years (No. 4). None used dentures and all were apparently healthy. Informed consent was obtained from each subject.

Results

Mechanical loss of test amino acid from the buccal cavity was negligible. Using the buccal absorption method outlined here and a plant polysaccharide (inulin), the recovery of this lipid-insoluble macromolecule was 99% or more (Manning and Evered, 1976). Possible metabolic losses of amino acids due to bacterial metabolism were investigated. These studies were compared with either buccal blanks incubated simultaneously with test amino acid solutions, or standard

amino acid solutions, not put in contact with the buccal mucosa but incubated for similar time intervals. Metabolic losses were negligible, from 0.4 to 2.2% (data not shown). Storage of buccal test samples at 4°C gave a loss of <2% with most amino acids after 1 day but greater losses after 2 or 3 days. Losses were greatest with serine, glutamic acid, glycine and valine. Samples stored at −20°C gave smaller losses during 3 days and this procedure was adopted when storage was unavoidable.

The salivary amino acid concentrations determined were similar to those in the literature (Dreyfus et al., 1968) and are not reported here. The amino acid concentrations in buccal blank solutions were negligible compared with buccal absorption and they were neglected in our calculations of absorption rates. Buccal absorption of L-amino acids from equimolar mixtures at initial concentrations of 2, 4 and 8 mmoles/l gave the results presented (Table 1). The uptake of long-chain neutral amino acids, e.g. L-leucine, was the most rapid. Glycine and the L-isomers of serine, threonine and the short-chain neutral amino acids were absorbed more slowly (Table 1). This absorption pattern was consistent with three different experimental subjects though minor individual differences were seen (Table 2). With each subject, the difference in the rate of absorbtion were examined statistically. The rate of absorption of each amino acid was compared with the one which showed a maximum rate. That is, phenylalanine with subject 3, methionine with subject 4 and leucine with subject 2. The results (Table 2) show that with slowly absorbed amino acids, the difference was statistically significant ($p < 0.05$ to $p < 0.005$).

Table 1. Buccal absorption of L-amino acids from equimolar mixtures at concentrations of 2 mM, 4 mM and 8 mM

	μ Moles absorbed/5 min		
L-Amino acids	2 mM	4 mM	8 mM
Aspartic acid	9.44 ± 0.47	13.8 ± 1.50	15.3 ± 2.00 (10)
Threonine	8.73 ± 0.77	12.5 ± 2.12	14.9 ± 1.57 (7)
Serine	6.40 ± 0.36	10.8 ± 0.80	16.3 ± 2.91 (7)
Glutamic acid	11.2 ± 1.06	14.3 ± 1.35	14.8 ± 2.47 (6)
Proline	7.53 ± 0.47	14.9 ± 1.70	16.9 ± 1.43 (8)
Glycine	7.88 ± 0.70	14.3 ± 2.05	16.5 ± 2.86 (9)
Alanine	8.77 ± 1.02	13.3 ± 1.80	15.8 ± 2.44 (8)
Valine	9.31 ± 0.39	11.3 ± 2.02	12.4 ± 1.70 (7)
Methionine	9.89 ± 0.59	15.5 ± 1.90	21.2 ± 2.57 (6)
Isoleucine	9.38 ± 0.35	16.3 ± 1.30	19.6 ± 3.13 (7)
Leucine	11.70 ± 0.54	16.9 ± 1.92	19.0 ± 1.79 (6)
Phenylalanine	12.2 ± 1.01	18.8 ± 2.65	22.7 ± 1.93 (9)
Histidine	8.90 ± 0.55	13.8 ± 2.05	16.5 ± 2.75 (7)
Lysine	7.68 ± 0.92	13.3 ± 1.55	16.2 ± 1.58 (7)
Arginine	8.04 ± 0.28	15.8 ± 2.00	22.2 ± 2.10 (9)

At 2 mM and 4 mM, the values were mean absorption ± S.E.M. for 6 and 4 experiments respectively. At 8 mM the values were for the number of experiments in parenthesis. All studies were with one subject (male 1).

Table 2. Inter-subject variation to show the buccal absorption of L-amino acids from equimolar mixture at a concentration of 8 mM

	μ Moles absorbed/5 min					
L-Amino acid	Subject male 3	Statistical significance[a]	Subject male 4	Statistical significance[a]	Subject male 2	Statistical significance[a]
Aspartic acid	13.1 ± 1.65	$p < 0.025$	8.75 ± 1.50	$p < 0.025$	10.8 ± 0.72	$p < 0.01$
Threonine	11.9 ± 1.35	$p < 0.005$	10.7 ± 0.60	$p < 0.05$	10.1 ± 1.35	$p < 0.025$
Serine	8.10 ± 0.90	$p < 0.005$	7.00 ± 1.00	$p < 0.10$	11.7 ± 0.80	$p < 0.025$
Glutamic acid	7.60 ± 1.85	$p < 0.005$	8.75 ± 1.00	$p < 0.025$	12.6 ± 1.90	$p < 0.05$
Proline	12.8 ± 2.20	$p < 0.025$	7.38 ± 1.92	$p < 0.025$	14.3 ± 0.95	$p < 0.05$
Glycine	12.1 ± 1.65	$p < 0.010$	11.4 ± 1.42	NS	13.7 ± 1.18	$p < 0.05$
Alanine	10.8 ± 1.10	$p < 0.005$	10.4 ± 2.12	NS	15.2 ± 1.92	NS
Valine	13.2 ± 1.05	$p < 0.025$	7.50 ± 1.65	$p < 0.010$	12.4 ± 2.00	$p < 0.05$
Methionine	16.8 ± 2.20	NS	[b]14.8 ± 1.55		17.8 ± 1.82	NS
Isoleucine	16.7 ± 1.30	NS	14.1 ± 2.02	NS	18.3 ± 0.88	NS
Leucine	16.5 ± 1.52	NS	13.5 ± 0.70	NS	19.7 ± 2.05[b]	
Phenylalanine	[b]20.4 ± 2.10		12.6 ± 1.90	NS	17.3 ± 1.44	NS
Histidine	15.3 ± 1.20	$p < 0.05$	10.4 ± 1.38	$p = 0.05$	15.2 ± 1.70	NS
Lysine	13.3 ± 1.60	$p < 0.025$	7.67 ± 2.08	$p < 0.05$	14.3 ± 0.92	$p < 0.05$
Arginine	14.5 ± 1.75	$p < 0.05$	13.4 ± 1.33	NS	16.4 ± 1.85	NS

Mean values S.E.M. are given for five experiments with subject 3 and three for subjects 2 and 4.

[a] The rate of absorption of each amino acid was compared with that which showed a maximum value [b]. That is, phenylalanine with subject 3, methionine with subject 4, and leucine with subject 2

Students' 't' test. *NS* non-significant

The effect of substituting K^+ ions for Na^+ ions in the buccal buffer solution was tested. It is, however, important to note that with in vivo buccal absorption technique it was not possible to exclude Na^+-ions from the bulk medium completely since saliva could contribute from 15 mM Na^+ in the unstimulated state to 45 mM Na^+ in the stimulated state. This data is well documented by Eastoe (Eastoe, 1961). In our studies this contribution of Na^+-ions in addition to 136 mM in Krebs buffer is indicated in both Tables 3 and 4. By replacing the Na^+-buffer with K^+-buffer, uptake of most amino acids at concentrations of 2 mmoles/l was significantly decreased, particularly of isoleucine, leucine, methionine and phenylalanine. The exceptions were threonine and serine (Table 3). However, at amino acid concentrations of 8 mmoles/l, the effect of Na^+ replacement was less marked than that at 2 mmoles/l (Table 4).

Figure 1 shows the buccal absorption of L-methionine and L-leucine at five different concentrations over the range 0 to 10 mmoles/l. Each point is a mean ± S.E.M. of 4 to 6 determinations. Hofstee plots of the same data were linear suggesting a single K_t for both L-methionine and L-leucine (Fig. 2). The lines are drawn through the intercepts of V_{max} and K_t obtained from a computer fit employing weighted least-square regression analysis (Cleland, 1967). For L-methionine: $K_t = 8.57 \pm 0.48$ mmoles/l and $V_{max} = 48.8 \pm 1.53$ μmoles absorbed in 5 min.

Table 3. Effect of replacing sodium ions by potassium ions on the buccal absorption of L-amino acids from equimolar mixtures at 2 mM concentration

L-amino acid	μ Moles absorbed/5 min		% Inhibition	Statistical significance[a]
	Control Na^+ (136 mM Na^+ + 15 mM to 45 mM Na^+)[b]	Test K^+ (15 mM to 45 mM Na^+)[b]		
Aspartic acid	8.92 ± 0.66	6.91 ± 0.74	22.5	$p < 0.025$
Threonine	8.44 ± 0.51	7.05 ± 0.81	16.5	NS
Serine	6.15 ± 0.83	5.91 ± 0.77	3.9	NS
Glutamic acid	10.9 ± 0.98	7.22 ± 0.64	33.8	$p < 0.025$
Proline	7.88 ± 0.55	4.83 ± 0.75	38.7	$p < 0.025$
Glycine	8.02 ± 1.01	5.02 ± 0.88	37.4	$p < 0.05$
Alanine	8.66 ± 0.92	5.41 ± 0.83	37.5	$p < 0.025$
Valine	9.01 ± 0.45	5.98 ± 0.94	33.6	$p < 0.025$
Methionine	10.2 ± 0.62	6.32 ± 0.90	38.0	$p < 0.010$
Isoleucine	9.85 ± 0.80	5.15 ± 0.65	47.7	$p < 0.005$
Leucine	11.4 ± 0.93	6.18 ± 1.01	45.8	$p < 0.005$
Phenylalanine	11.9 ± 0.88	7.42 ± 0.89	37.6	$p < 0.025$
Histidine	8.48 ± 0.78	6.64 ± 0.45	21.7	$p < 0.050$
Lysine	7.78 ± 0.86	5.59 ± 0.68	28.1	$p < 0.050$
Arginine	8.97 ± 0.50	5.82 ± 0.72	35.1	$p < 0.010$

Mean values ± S.E.M. are given for four experiments with Na^+ and without Na^+. Subject: male 1

[a] Students' 't' test. *NS* non-significant

[b] Indicates possible salivary contribution of Na^+ (13, see text for further details)

Table 4. Effect of replacing sodium ions on the buccal absorption of L-amino acids from equimolar mixtures at 8 mM concentration

L-amino acid	μ Moles absorbed/5 min		% Inhibition	Statistical significance[a]
	Control Na^+ (136 mM Na^+ + 15 mM to 45 mM Na^+)[b]	Test K^+ (15 mM to 45 mM Na^+)[b]		
Aspartic acid	15.3 ± 2.00 (10)	10.4 ± 0.86	32.0	NS
Threonine	14.9 ± 1.57 (7)	15.6 ± 1.22	—	NS
Serine	16.3 ± 2.91 (7)	17.8 ± 1.15	—	NS
Glutamic acid	14.8 ± 2.47 (6)	9.35 ± 0.62	36.8	$p < 0.05$
Proline	16.9 ± 1.43 (8)	11.6 ± 0.78	31.4	$p < 0.01$
Glycine	16.5 ± 2.86 (9)	11.9 ± 0.95	27.9	NS
Alanine	15.8 ± 2.44 (8)	10.6 ± 0.77	32.9	NS
Valine	12.4 ± 1.70 (7)	8.82 ± 0.51	28.9	NS
Methionine	21.2 ± 2.57 (6)	12.8 ± 0.60	39.6	$p < 0.01$
Isoleucine	19.6 ± 3.13 (7)	11.4 ± 0.84	41.8	$p < 0.025$
Leucine	19.0 ± 1.79 (9)	10.7 ± 0.90	43.7	$p < 0.01$
Phenylalanine	22.7 ± 1.93 (9)	13.2 ± 0.86	41.9	$p < 0.005$
Histidine	16.5 ± 2.75 (7)	15.8 ± 1.85	4.2	NS
Lysine	16.2 ± 1.58 (7)	19.5 ± 1.92	—	NS
Arginine	22.2 ± 2.10 (9)	15.8 ± 0.78	28.8	$p < 0.05$

Mean values ± S.E.M. are given for five experiments with K^+, and for Na^+, mean values were for the number of experiments in parenthesis

[a] Students' 't' test. *NS* non-significant

[b] Indicates possible salivary contribution of Na^+ (13)

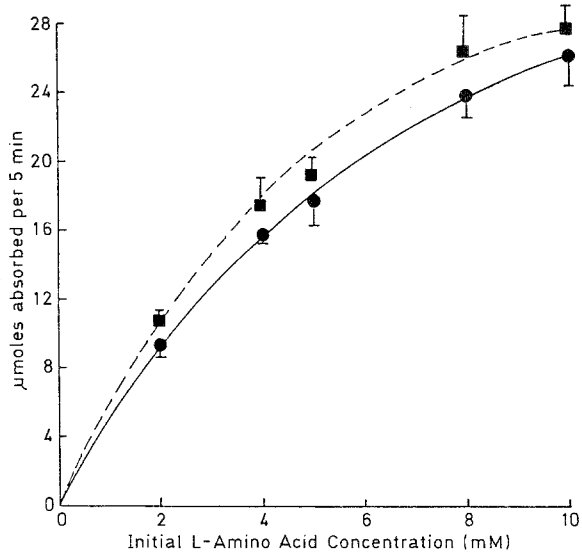

Fig. 1. The effect of initial concentration on the absorption of L-methionine (●) and L-leucine (■) across the buccal mucosa. Mean values ± S.E.M. are given for 4 to 6 determinations at each concentration. For clarity only single bars are drawn

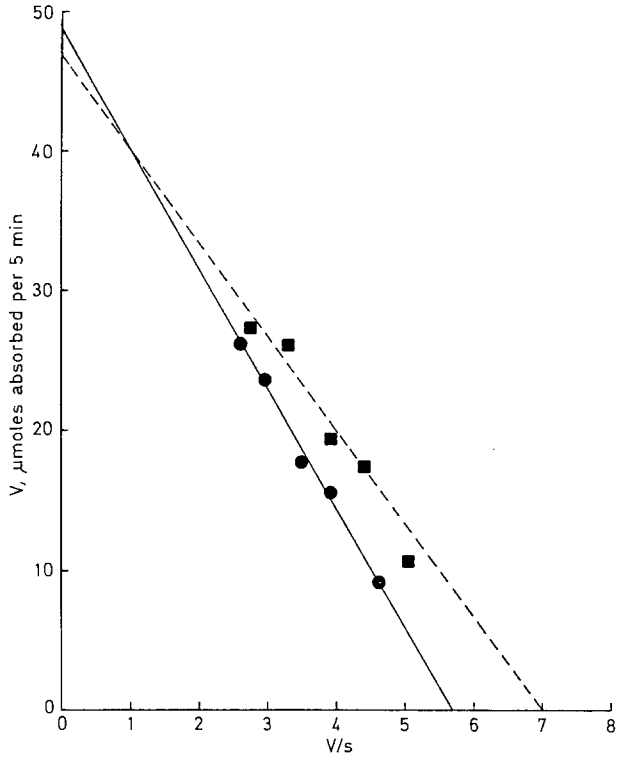

Fig. 2. Hofstee plots of L-methionine (●) and L-leucine (■) absorption across the buccal mucosa. The lines are drawn through the intercepts of V_{max} and K_t obtained from a computer fit employing weighted least-square regression analysis (Cleland, 1967)

Table 5 shows mutual inhibition between L-methionine, L-leucine and L-isoleucine. These inhibitions were not due to possible osmotic changes since 25 mmoles/l of mannitol, a non-absorbable sugar had no effect on the buccal absorption of 5 mM L-methionine. In the absence of mannitol, μmoles of methionine absorbed in 5 min were 18.4 $\pm$ 1.62 and in the presence of mannitol, 17.9 $\pm$ 2.10. In both cases the values are mean $\pm$ S.E.M. of five determinations. Table 6 shows the stereospecific uptake of methionine, leucine and isoleucine across the buccal mucosa. At a concentration of 5 mmoles/l the rate of absorption of the D-isomers was significantly lower than that of the L-isomers ($p < 0.005$). Table 7 shows the stereospecific uptake of other L- or D-amino acids from equimolar mixtures at 2mM. The results demonstrate that, with the exception of threonine and serine, most L-amino acids are better absorbed than their corresponding D-isomers.

Discussion

The results for the buccal absorption of the individual essential L-amino acids, methionine and leucine can be summarized as showing mediated transport, stereospecificity, Na^+-dependence and mutual inhibition of the transport of each other. Kinetic and inhibition studies with rat small intestine indicated a transport system common to L-leucine, L-methionine and other long-chain mono-

Table 5. Inhibition studies with L-methionine, L-leucine and L-isoleucine

Amino acid	Possible inhibitor	μ Moles absorbed/5 min		% Inhibition	Statistical significance[a]
		Control without inhibitor	Test with inhibitor		
5 mM L-Methionine	25 mM L-Leucine	17.2 ± 1.10 (4)	10.3 ± 0.85 (4)	40	$p < 0.005$
5 mM L-Isoleucine	25 mM L-Leucine	22.2 ± 0.96 (4)	14.8 ± 1.01 (4)	35	$p < 0.005$
5 mM L-Leucine	25 mM L-Methionine	19.8 ± 0.88 (6)	15.0 ± 0.90 (6)	24	$p < 0.005$
5 mM L-Isoleucine	25 mM L-Methionine	23.6 ± 1.15 (4)	16.9 ± 1.05 (4)	28	$p < 0.005$
5 mM L-Methionine	25 mM L-Isoleucine	17.8 ± 0.78 (6)	12.5 ± 0.85 (6)	30	$p < 0.005$
5 mM L-Leucine	25 mM L-Isoleucine	20.8 ± 1.22 (4)	12.9 ± 1.08 (4)	38	$p < 0.005$

Mean values ± S.E.M. for the number of experiments in parenthesis. Male subject 1

[a] Students' 't' test

Table 6. Comparison of the rates of absorption of D- and L-isomers of methionine, leucine and isoleucine

Amino acid	μ Moles absorbed/5 min		Statistical significance[a]
	L-isomer	D-isomer	
5 mM Methionine	16.8 ± 0.92 (4)	9.15 ± 0.88 (4)	$p < 0.005$
5 mM Leucine	19.2 ± 0.75 (6)	8.09 ± 0.95 (6)	$p < 0.005$
5 mM Iso-leucine	23.3 ± 1.10 (6)	10.1 ± 1.30 (6)	$p < 0.005$

Mean values ± S.E.M. for the number of experiments in parenthesis
[a] Students' 't' test

Table 7. Comparing the buccal absorption of L- and D-forms of amino acids from equimolar mixtures at 2 mM

Amino acid	μ Moles absorbed/5 min		Statistical significance[a]
	2 mM L-form	2 mM D-form	
Aspartic acid	9.44 ± 0.47	4.25 ± 0.84	$p < 0.005$
Threonine	8.73 ± 0.77	6.00 ± 1.64	NS
Serine	6.40 ± 0.36	5.25 ± 1.25	NS
Glutamic acid	11.2 ± 1.06	4.50 ± 0.79	$p < 0.005$
Proline	7.53 ± 0.47	5.00 ± 0.73	$p < 0.01$
Glycine[b]	7.89 ± 0.70	10.0 ± 1.30	NS
Alanine	8.77 ± 1.02	3.00 ± 0.64	$p < 0.005$
Valine	9.31 ± 0.39	5.00 ± 0.70	$p < 0.005$
Methionine	9.89 ± 0.59	3.50 ± 0.59	$p < 0.005$
Isoleucine	9.38 ± 0.35	3.95 ± 1.02	$p < 0.005$
Leucine	11.7 ± 0.54	4.15 ± 0.87	$p < 0.005$
Phenylalanine	12.2 ± 1.01	6.75 ± 1.21	$p < 0.005$
Histidine	8.9 ± 0.55	6.25 ± 1.00	$p < 0.025$
Lysine	7.68 ± 0.92	3.00 ± 0.39	$p < 0.005$
Arginine	8.04 ± 0.28	4.50 ± 1.02	$p < 0.005$

With both forms, the values were mean absorption ± S.E.M. for six experiments with a single subject (male 1)
[a] Students' 't' test. *NS* non-significant
[b] Glycine was added to both L- and D-amino acid mixtures

amino monocarboxylic acids (Newey and Smyth, 1964; De La Noüe et al., 1971). This system is stereospecific as brush-border preparations from rat small intestine preferentially bound L-leucine compared with D-leucine (Reiser and Chritiansen, 1972). Our results in Table 6 show a similar stereoselectivity. We therefore see a parallelism between the two gastrointestinal sites for transport of these long-chain neutral amino acids.

Absorption of amino acids presented as mixtures to the buccal mucosa also showed a characteristic pattern, Na^+-dependence and stereospecificity. At the highest concentration tested (8 mmoles/l) there was some selectivity in amino acid absorption across buccal mucosa. Arginine, phenylalanine and the long-

chain neutral amino acids were the most rapidly absorbed. Glutamic acid, aspartic acid, threonine and valine were less well absorbed. Similar patterns of absorption were observed at 4 mmoles/l. In this case, in addition to threonine and valine, serine was poorly absorbed. At the lowest concentration tested (2 mmoles/l) the differences in absorption rates for different amino acids were not very obvious with the exception of phenylalanine and leucine which were more rapidly absorbed than serine, proline, glycine and lysine. Apparently, glutamic acid was rapidly absorbed at 2 mmoles/l but not at 4 or 8 mmoles/l. Since L-glutamic acid is metabolized extensively by transamination when being absorbed across mammalian intestine (Neame and Wiseman, 1958) it seems likely that metabolic loss accounts for this discrepancy in the buccal experiments too.

Similar observations have resulted from studies on amino acid absorption from mammalian small intestine. Perfusion of the human jejunum about 30 cm beyond the ligament of Trietz was conducted using a double-lumen tube (Adibi and Gray, 1967). At low concentrations of each amino acid in the mixture (1.2 mmoles/l) the rates of absorption were very similar. Mucosal slices of rat jejunum did not show any obvious competition for uptake among amino acids when the concentration of each was in the range of 0.15 to 1.39 mmoles/l (Bronk and Leese, 1974). However, at concentrations above 2 mmoles/l human jejunum in vivo was found to absorb methionine, leucine, isoleucine and valine more rapidly than serine, proline, alanine, phenylalanine and arginine. Glutamic acid, aspartic acid and threonine were absorbed the slowest (Adibi et al., 1967). Similar observations were made with absorption of amino acids at 9 mmoles/l in a mixture placed in the human ileum (Orten, 1963). With ileum, arginine, isoleucine, leucine and methionine were rapidly absorbed while threonine, histidine, glycine and glutamic acid were poorly absorbed.

In summary, these various locations in the gastrointestinal tract show a similar pattern for amino acid absorption. Table 8 shows a summary of the observations made with the human buccal mucosa and compares with the absorption patterns reported for the small intestine. Buccal mucosa did show a difference of poor absorption for valine compared with intestine. Amino acids with large non-polar side groups were well absorbed. Glycine and amino acids with polar side chains, however, were slowly absorbed.

Buccal absorption of some amino acids was sodium dependent just as it is in the small intestine with the exception of the hydroxyamino acids threonine and serine. Active accumulation of amino acids by mucosal slices of rat jejunum was abolished when K^+ ions were substituted for Na^+ ions in the incubation medium (Bronk and Leese, 1974).

In most cases absorption of L-amino acids was significantly higher than that for D-amino acids at a concentration of 2 mmoles/l. However, serine and threonine were exceptions as the buccal mucosa was not strongly stereospecific for these two hydroxyamino acids. Why the buccal absorption of the hydroxyamino acids threonine and serine should be unresponsive to both Na^+ substitution by K^+ and to stereospecificity is not clear. It is interesting that D-serine was transported against the concentration gradient with rat small intestine in vitro (Randall and Evered, 1964). However, D-methionine too was actively

Table 8. Summary of pattern of absorption of L-amino acids from equimolar mixtures

	Tissue	Fast	Intermediate	Slow
(I)	*Human buccal cavity* 8 mM mixture Absorption period 5 min. (Present study)	Phenylalanine Arginine Methionine Leucine Iso-leucine	Proline Glycine Histidine Lysine Alanine Serine	Aspartic acid Threonine Glutamic acid Valine
(II)	*Human jejunum* Perfusion, with a double-lumen tube. 8 mM mixture Absorption period 55 min. (Adibi et al. [20])	Methionine Iso-leucine Leucine Valine	Proline Arginine Alanine Phenylalanine Tyrosine Serine Cystine Tryptophan	Glycine Threonine Histidine Lysine Glutamic acid Aspartic acid
(III)	*Human ileum* Thirty-loop 9 mM mixture Absorption period 3 hours (Orten [21])	Arginine Iso-leucine Leucine Methionine	Valine Lysine Phenylalanine Cystine Tyrosine Tryptophan Proline Aspartic acid Alanine Serine	Threonine Histidine Glycine Glutamic acid
(IV)	*Rat small intestine* Tied-off loops *in vivo* 2 mM mixture Absorption period 15 min. (Delhumeau et al. [26])	Cysteine Methionine Arginine Iso-leucine Tryptophan Leucine Valine	Phenylalanine Proline Tyrosine Lysine Alanine Histidine Serine Glycine Threonine	Aspartic acid Glutamic acid
(V)	*Domestic fowl small intestine* Tied-off segments *in vivo*. 2.5 mM mixture Absorption period 10 min. (Tasaki and Takahashi [27])	Methionine Iso-leucine Valine Leucine Tryptophan Phenylalanine	Histidine Lysine Alanine Serine Threonine Tyrosine Cystine Proline Arginine Glycine	Aspartic acid Glutamic acid

transported across small intestine from rats (Jervis and Smyth, 1960) and hamsters (Lin et al., 1962).

When speaking of absorption across the buccal mucosa, we cannot discriminate uptake by the epithelium of the tongue and other sites in the mouth. However, from histological studies the dorsal surface of the tongue is known to be highly keratinized (Squire et al., 1976) and therefore unsuitable for absorption. The ventral surface on the other hand, is a thin, non-keratinized stratified squamous epithelium and has an extensive vascular supply. It is likely that some absorption may occur from this region of the tongue, in addition to the floor of the mouth. The buccal mucosa, however, covers the largest surface area in the mouth and is practically non-keratinized and assumed to be the most predominant site for absorption of drugs and nutrients.

Finally, on the basis of our present in vivo studies, it is difficult to delineate the presence of specific amino acid transport systems described in (a) the brush border and basolateral membrane vesicles isolated from rodent intestines (Stevens et al., 1982; 1984; Munck, 1983), and (b) plasma membranes of non-epithelial cells (Christensen, 1984; Kilberg, 1982; Collarini and Oxender, 1987; Vadgama et al., 1987; 1991). We anticipate that future studies directed towards in vitro isolation of buccal mucosal cells, their subsequent membrane vesicle preparations and successful establishment of in vitro culture system may allow us to identify specific transport systems and their response to nutrient regulation (Shotwell et al., 1983; Kilberg et al., 1985; Fafournoux et al., 1990; Christensen, 1990; Ferraris and Diamond, 1989).

The precise physiological significance of the buccal absorption of nutrients is yet unclear. The absolute amounts of nutrients absorbed across the buccal mucosa may not be significant for nutritional needs of the body. However, it is conceivable that the absorption of glucose and certain amino acids into the systemic circulation could cause a brief increase in their plasma concentrations, which could lead to insulin secretion followed by secondary signals to the gastrointestinal tract. For instance, it has been demonstrated that small postprandial changes in plasma glucose concentration (4.4–6.6 mM) serve as a conditional modifier of insulin secretion, and dramatically alter the responsiveness of pancreatic islets to a combination of neurohumoral agonists. These agonists in turn have two functions. Cholecystokinin (CCK) and acetylcholine activate the hydrolysis of phosphoinositides (PIP_2), and gastric inhibitory polypeptide (GIP) and glucagon-like peptide I activate adenylate cyclase. These events have been described by Rasmussen et al (Rasmussen et al., 1990). These authors propose an interesting model which attempts to describe sequential events that lead to insulin secretion in response to oral load of glucose. In principal, the model suggests that insulin secretion occurs in three stages. Cephalic, early enteric, and late enteric. During the cephalic phase of digestion, acetylcholine is released from cholinergic synapses on the β-cell, followed by hydrolysis of PIP_2. The resulting IP_3 (inositol triphosphate) and DAG (diacylglycerol) concentrations are elevated, with an increase in intracellular Ca^{2+} concentration. DAG, a substrate for Protein Kinase C (PKC) increases the kinase activity and induces translocation of PKC to the plasma membrane. During the early enteric stage, enteric neurons are activated by the entry of

nutrients in the intestine, and these release CCK at synapses on β-cell. CCK also activates PIP_2 hydrolysis and causes the second small, transient increase in insulin secretion and translocation of additional PKC to the plasma membrane.

During the later enteric stage, the combination of a small increase in glucose concentration plus GIP and CCK released from the intestinal cells act in concert on the sensitized islets to increase cAMP, the DAG content of the plasma membrane, and Ca^{2+} influx rate. These events bring about enhanced activity of both calmodulin-dependent protein kinases and plasma membrane associated PKC, leading to an increase of both first and second phase insulin release. A detailed measurement of the amount of glucose or amino acids absorbed into the circulating plasma via the buccal cavity, followed by step-wise measurements of the events occurring during cephalic, enteric, and later enteric stages should elucidate the physiological role of buccal absorption of nutrients for transmitting signals to the gastrointestinal tract.

Acknowledgments

We thank our subjects for their participation, for giving their informed consent to conduct the experiments, and Leslie Sugahara for her help in preparing this manuscript.

References

Adibi SA, Gray JJ (1967) Intestinal absorption of essential amino acids in man. Gastroenterology 52: 837–845

Adibi SA, Gray SJ, Menden E (1967) The kinetics of amino acid absorption and alteration of plasma composition of free amino acids after intestinal perfusion of amino acid mixtures. J Clin Nutr 20: 24–33

Beckett AH, Hossie RD (1971) Buccal absorption of drugs. Handbook Exp Pharmacol 28: 25–46

Bronk JR, Leese HJ (1974) The absorption of a mixture of amino acids by rat small intestine. J Physiol (Lond) 241: 271–286

Christensen HN (1984) Organic ion transport during seven decades: the amino acids. Biochim Biophys Acta 779: 255–269

Christensen HN (1991) Role of amino acid transport and countertransport in nutrition and metabolism. Physiol Rev 70: 43–77

Cleland WW (1967) The statistical analysis of enzyme kinetic data. In: Bord FF (ed) Adv Enzymol, vol 29. Interscience, J Wiley, New York, pp 1–32

Collarini EJ, Oxender DL (1987) Mechanisms of transport of amino acids across membranes. Annu Rev Nutr 7: 75–90

De La Noüe J, Newey H, Smyth DH (1971) Transfer of alanine isomers by rat small intestine. J Physiol (Lond) 214: 105–114

Delhumeau G, Pratt GV, Gitler C (1962) The absorption of amino acid mixtures from the small intestine of the rat. J Nutr 77: 52–60

Dreyfus PM, Levy HL, Efron ML (1968) Concerning amino acids in human saliva. Experientia 24: 447–448

Eastoe JE (1961) The chemical composition of saliva. In: Long C (ed) Biochemists handbook. Spon, London, pp 901–911

Evered DF, Sadoogh-Abasian F, Patel PD (1980) Absorption of nicotinic acid and nicotinamide across human buccal mucosa in vivo. Life Sci 27: 1649–1651

Evered DF (1972) Permeability of human buccal mucosa to an amino acid analogue, the antibiotic D-cycloserine. Biochem J 130: 16P
Fafournoux P, Remesy C, Demigne C (1990) Fluxes and membrane transport of amino acids in rat liver under different protein diets. Am J Physiol 259 (Endocrinol Metab 22): E614–E622
Ferraris RP, Diamond JM (1989) Specific regulation of intestinal nutrient transporters by their dietary substrates. Annu Rev Physiol 51: 125–130
Jervis EL, Smyth DH (1960) The active transfer of D-methionine by the rat intestine in vitro. J Physiol (Lond) 151: 51–58
Kilberg MS (1982) Amino acid transport in isolated rat hepatocytes. J Membr Biol 69: 1–12
Kilberg MS, Barber EF, Handlogten ME (1985) Charactersitics and hormonal regulation of amino acid transport in isolated rat hepatocytes. Curr Top Cell Regul 25: 133–163
Lin ECC, Hagihira H, Wilson TH (1962) Specificity of the transport system for neutral amino acids in the hamster intestine. Am J Physiol 202: 919–925
Manning AS, Evered DF (1976) The absorption of sugars from the human buccal cavity. Clin Sci 51: 127–132
Munck BG (1983) Comparative aspects of amino acid transport in guinea pig, rabbit and rat small intestine. In: Gilles-Baillian M, Gilles R (eds) Intestinal transport. Springer, Berlin Heidelberg New York Tokyo, pp 260–283
Neame KD, Wiseman G (1958) The alanine and oxo acid concentrations in mesenteric blood during the absorption of L-glutamic acid by the small intestine of the dog, cat and rabbit in vitro. J Physiol (Lond) 140: 148–155
Newey H, Smyth DH (1964) The transfer system for neutral amino acids in the rat small intestine. J Physiol (Lond) 170: 328–343
Orten AU (1963) Intestinal phase of amino acid nutrition. Fed Proc 22: 1103–1109
Randall HG, Evered DF (1964) Amino acid uptake by living cells. I. Amino group requirement by small intestine of the rat in vitro. Biochim Biophys Acta 93: 98–105
Rasmussen H, Zawalich KC, Ganesan S, Calle R, Zawalich WS (1990) Physiology and pathophysiology of insulin secretion. Diabetes Care 13: 655–666
Reiser S, Christiansen PA (1972) A basis for the difference in the inhibition of uptake of various neutral amino acids by lysine in the intestinal epithelial cells. Biochim Biophys Acta 266: 217–229
Sadoogh-Abasian F, Evered DF (1979) Absorption of vitamin C from the human buccal cavity. Br J Nutr 42: 15–20
Schanker LH (1962) Passage of drug across body membranes. Pharmacol Rev 14: 501–530
Shotwell MA, Kilberg MS, Oxender DL (1983) The regulation of neutral amino acid transport in mammalian cells. Biochim Biophys Acta 737: 267–284
Sprake SA, Evered DF (1979) Transport of cycloserine across the mucosae of rat colon and the human mouth. J Pharm Pharmacol 31: 113–114
Squire CA, Johnson NW, Hopps RM (1976) Human oral mucosa. Development, structure and function. Blackwell, Oxford
Stein WH, Moore S (1954) The free amino acids of human blood plasma. J Biol Chem 211: 915–926
Stevens BR, Ross HJ, Wright EM (1982) Multiple transport pathways for neutral amino acids in rabbit jejunal brush border vesicles. J Membr Biol 66: 213–225
Stevens BR, Kaunitz J, Wright EM (1984) Intestinal transport of amino acids and sugars: Advances using membrane vesicles. Annu Rev Physiol 417: 417–433
Tasaki I, Takahashi N (1966) Absorption of amino acids from the small intestine of domestic fowl. J Nutr 88: 359–364
Vadgama JV, Castro M, Christensen HN (1987) Characteristics of amino acid transport during erythroid cell differentiation. J Biol Chem 262: 13273–13284
Vadgama JV, Chan M, Wu J (1991) Differential expression of amino acid transport systems A and ASC during erythroleukemia cell differentiation. Am J Physiol 260 (Cell Physiol 29): C392–C399

Vasantha MS, Moorjani MN, Sreenivasan KS (1970) A modified calorimetric method based on McCarthy and Paille procedure for estimation of methionine. Biochem Biophys Res Commun 41: 568–578

Wass M, Evered DF (1971) Transport characteristics of the amino acid analogues, cycloserine and α-methyldopa, using rat small intestine in vitro. Life Sci 10 (Part II): 1005–1013

Authors' address: Dr. J. V. Vadgama, Division of Medical Genetics, Bldg. E4, Harbor-UCLA Medical Center, 1124 West Carson Street, Torrance, CA 90502, U.S.A.

Received December 16, 1991