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Absorption of glutathione from the gastro-intestinal tract

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Transport of the peptide glutathione (GSH) has been studied with the rat small intestine in vitro and the human buccal cavity in vivo. Uptake was found to be sodium-independent in both systems. Saturation kinetics were demonstrated and uptake did not require energy in either system. Transport was inhibited by other small peptides. Carrier-mediated facilitated diffusion was postulated as the mode of transport.

Introduction

Evidence of similarities between transport of nutrients by the buccal mucosa in vivo and the mammalian small intestine in vitro has been well documented. Sodium-dependent, carrier-mediated, uptake of monosaccharides by the buccal mucosa [1] resembles uptake by the rat small intestine [2]. Passive diffusion of disaccharides [1,3] and calcium-stimulation of transport of certain sugars [4,5] has also been demonstrated at both gastro-intestinal sites. Human buccal cavity [6] and guinea-pig and human small intestine [7,8] showed sodium-dependent, carrier-mediated uptake of L-ascorbic acid. The vitamins nicotinic acid and nicotinamide are transported across the buccal mucosa [9] and the small intestine [10] by facilitated diffusion which is partially sodium-dependent. Transport of many protein-derived amino acids seems to occur by similar mechanisms in both mucosal systems [11].

Transport of peptides across the mammalian small intestine has been investigated extensively [12]. Some di- and tripeptides are transported against a concentration gradient across the small intestinal mucosa by sodium-dependent and sodium-independent systems [13–15] and some evidence also exists for transport of oligopeptides across the small intestine [16].

Glutathione, L-γ-glutamyl-L-cysteinylglycine, (GSH), occurs in most cells but little is known of its transport across cell membranes. However, GSH is implicated in the γ-glutamyl cycle for transporting amino acids [17]. A large proportion of GSH injected into animals disappeared from the circulation [18]. Whilst human erythrocytes were found to be impermeable to entry of reduced glutathione, oxidised glutathione (GSSG) was transported down a concentration gradient by the rat small intestine [19].

The present study was undertaken to investigate whether GSH is transported across the buccal mucosa and if uptake resembles intestinal absorption of GSH and other peptides.

Materials and Methods

Everted sacs of the rat small intestine were prepared and used as previously described [3]. Alternate sacs were taken down the length of the small intestine with and without inhibitors, respectively, to randomize results. Two sacs, each containing 0.4 ml of sample, were placed into flasks containing 15 ml Krebs-Henseleit buffer (pH 7.4)

gassed with 95% O_2/5% CO_2, and incubated at 37°C for 30 min with constant shaking. Buccal absorption studies were made using a modified Krebs-Ringer buffer (pH 6.0) with 1.8 mM citric acid replacing sodium citrate [20]. 25 ml test solution preincubated at 37°C, were circulated in the mouth for 5 min. The buccal cavity was rinsed with 10 ml buffer at 37°C for 5 s. Samples and rinses were pooled, diluted and centrifuged (10 min at $3000 \times g$), before analysis of the supernatant. Test samples were preceded by blanks of 25 ml buffer and treated as test solutions.

The effect of sodium ions on uptake was investigated by replacing all of the sodium salts in the buffer with potassium or choline salts. Possible effect of inhibitors was studied by pre-treating the buccal cavity as follows: 25 ml buffer at 37°C containing the inhibitor were circulated in the mouth for 5 min before test solutions with inhibitors were treated similarly. Thereafter, at least 30 min were allowed between experiments to avoid contamination by inhibitors. Control experiments were performed to validate this claim. Experiments were carried out in random order, so test solution experiments did not always follow control experiments and vice versa. Possible metabolic loss was monitored by circulating 25 ml buffer at 37°C, in the mouth for 5 min and expelling it into a beaker containing solid GSH. This was incubated at 37°C and 5-ml aliquots were assayed at 5-min intervals. GSH was assayed using a modified version of the method described by Ball [21] with Ellman's reagent, 5,5'-dithiobis(2-nitro)benzoic acid (DTNB) [22]. Ascorbic acid, a protector against the oxidation of GSH, was replaced by ethylenediamine tetra acetic acid (EDTA) since the former reacted with DTNB. Possible oxidation of GSH during experiments was investigated by the electrolytic reduction of GSSG to GSH which was then assayed as before [23].

Validity of buccal experiments was investigated by determining percentage recovery of the plant polysaccharide, inulin, M_r 2000–5000, at a concentration of 25 mg/100 ml buffer.

All chemicals were obtained from the Sigma Chemical Company. Purity of test compounds was tested by overloading samples onto the automatic amino acid analyser, (Locarte Scientific Co., London) and seeking the parent amino acids as possible contaminants. 300 nmol, 10-times the normal loadings, were loaded for analysis.

Results

Commercial GSH used in the experiments had less than 1% amino acid contamination as shown by the automatic amino acid analyser.

Absorption by buccal mucosa

The rate of buccal absorption of GSH as a function of the initial concentration of GSH was curvi-linear over the range 2–20 mM (Fig. 1). Metabolic loss of GSH over a period of 5 min incubation time, as per experimental procedure was 0.6%, a negligible loss in relation to uptake. No significant losses due to the oxidation of GSH were recorded. Intersubject variation in uptake between three healthy Caucasian individuals, none of whom wore dentures, was small (Table I).

Replacement of Na^+ in the buffer by K^+ or choline did not significantly inhibit uptake. Likewise, amytal, a barbiturate, had no significant inhibitory effect on absorption (Table II).

Glycine and the L-isomers of glutamic acid and cysteine inhibited uptake of 5 mM GSH whilst D-isomers of the latter amino acids had no effect. The dipeptides glycyl-L-leucine and glycylglycine inhibited uptake of 5 mM GSH, and glycylglycylglycine inhibited uptake of 5 mM and 10 mM GSH (Table III).

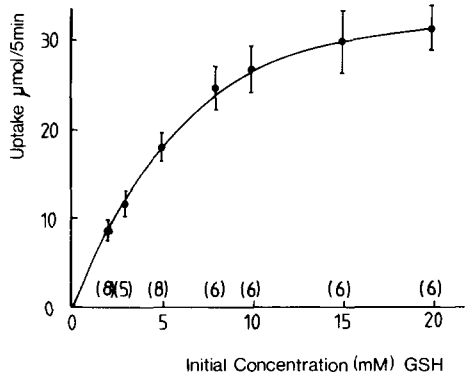

Fig. 1. Effect of initial concentration (mM) of GSH on absorption (µmol/5 min) by human buccal mucosa. Points are mean values with standard errors represented by vertical bars; figures in parentheses are the number of experiments.

TABLE I

INTER-SUBJECT VARIATION IN THE BUCCAL ABSORPTION (μmol/5 min) OF GSH IN HEALTHY SUBJECTS

Mean values with their standard errors are given; the number of determinations in parentheses.

GSH (mM)	Female		Male	P *
Subject No.	1	2	3	
Age (yrs)	28	24	23	
2	8.5 ± 0.7(6)	9.1 ± 0.62(4)	8.1 ± 0.81(6)	n.s. **
5	17.9 ± 0.5(6)	18.7 ± 0.92(4)	18.1 ± 0.87(6)	n.s.

* Statistical significance of difference.

** n.s., not statistically significant when compared with subject 1.

Absorption of GSH by rat small intestine

Analytical recovery of 5 mM GSH from everted sacs was 98.4%. The remaining loss presumably remained in the everted sac tissue. There was negligible metabolic loss of GSH during the experiment. At the initial concentration of 5 mM GSH, the final serosal : mucosal concentration never exceeded 1.0; i.e. transport was not against a concentration gradient. Studies on uptake of 5 mM GSH along the length of the small intestine did not show an optimal site for GSH transport (Fig. 2). Downhill transport of GSH by rat intestinal mucosa exhibited saturation kinetics (Fig. 3) over the concentration range 2–25 mM. Appreciable amounts of glutamic acid and glycine were also detected within the everted sacs.

Replacing Na^+ with K^+ or choline in the buffer had no significant inhibitory effect on uptake nor

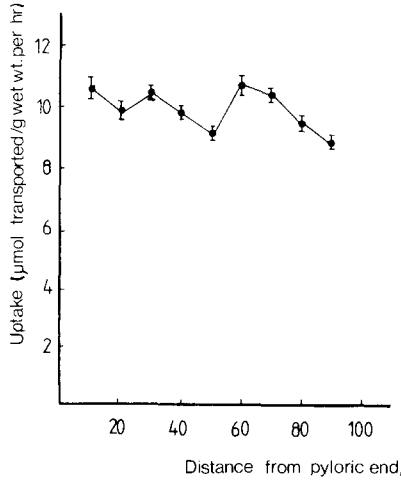

Fig. 2. Downhill transport of 5 mM GSH (μmol/g wet weight tissue per hour) along the rat small intestine. Points are mean values of six experiments with standard errors represented by vertical bars.

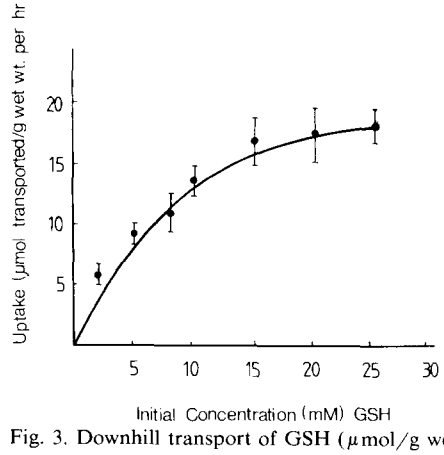

Fig. 3. Downhill transport of GSH (μmol/g wet weight tissue per hour) by rat small intestine as a function of initial concentration. Points are mean values of six experiments with standard errors represented by vertical bars.

TABLE II

EFFECT OF POSSIBLE INHIBITORS ON BUCCAL ABSORPTION (μmol/5 min) OF 5 mM GSH

Mean values with their standard errors are given; the number of determinations in parentheses.

Possible inhibitor	GSH uptake	% inhibition	P *
None (control)	18.4 ± 0.35 (29)		
K^+ buffer	16.4 ± 1.29 (6)	8.69	n.s. **
Choline buffer	19.1 ± 0.9 (6)	–	n.s.
2 mM amytal	16.6 ± 0.63 (6)	9.8	n.s.

* Statistical significance of difference.

** n.s., not statistically different when compared with control value.

TABLE III

INHIBITION OF BUCCAL ABSORPTION OF GSH (μmol/5 min) BY AMINO ACIDS AND OTHER PEPTIDES

Mean values with their standard errors are given; number of determinations in parentheses.

GSH (mM)	Possible inhibitor	GSH uptake		% inhibition	P *
5	None (control)	19 ±0.38	(50)		
	2 mM Gly	13.6 ±0.83	(6)	28.4	< 0.01
	2 mM L-Glu	10.7 ±1.39	(5)	43.7	< 0.01
	2 mM L-Cys	15.0 ±0.33	(5)	21.1	< 0.01
	2 mM D-Glu	16.9 ±0.45	(5)	11.1	n.s. **
	2 mM D-Cys	19.5 ±0.52	(5)	–	n.s.
	2 mM Gly-L-Leu	2.87±0.97	(4)	84.9	< 0.01
	2 mM Gly-Gly	15.7 ±0.88	(4)	18.4	< 0.01
	2 mM Gly-Gly-Gly	4.94±0.94	(4)	74.3	< 0.01
10	None (control)	29.2 ±1.70	(6)		
	2 mM Gly-Gly-Gly	19.9 ±1.8	(6)	31.2	< 0.01

* Statistical significance of difference.

** n.s., not statistically significant when compared with control values.

did the presence in the test solutions of ouabain or 2,4-dinitrophenol. Transport of 5 mM GSH was inhibited significantly in the presence of the tripeptide glycylglycylglycine and the dipeptides glycyl-L-leucine and glycylglycine. As with buccal studies, only glycine and the L-isomers of glutamic acid and cysteine had inhibitory effects on transport of GSH (Table IV).

Discussion

There was negligible loss due to metabolism and the amount of GSH oxidised during the experiments was negligible. Since direct measurements of peptides in the blood supply to the buccal cavity cannot be made, these parameters may be used to define buccal absorption. Loss of GSH from the buccal cavity was assumed to be a measure of mucosal absorption. A high recovery, 98.8%, of the poorly absorbed polysaccharide, inulin, showed the validity of the buccal method.

Saturation kinetics were demonstrated in the downhill transport rate in both systems with an increase in initial concentration of GSH suggesting carrier-mediated transport at both sites.

Uptake of GSH was uninhibited, in the small intestine, when Na^+ was replaced with K^+ or choline or in the presence of ouabain, known to inhibit the membrane pump for Na^+ [24]. Similarly, replacement of Na^+ left buccal absorption unaffected. However, it is difficult to seek a

TABLE IV

INHIBITION OF TRANSPORT OF 5 mM GSH (μmol/g WET WEIGHT PER 60 min) ACROSS EVERTED SACS OF RAT SMALL INTESTINE

Mean values of six experiments with standard errors are given.

Inhibitor	Inhibitor absent	Inhibitor present	% Inhibition	P *
K^+ buffer	9.34±0.28	8.41±0.31	9.89	n.s. **
Choline buffer	9.21±0.24	8.81±0.41	4.3	n.s.
1 mM Ouabain	8.76±0.14	7.12±0.14	7.31	n.s.
0.2 mM DNP	8.81±0.26	7.94±0.61	9.88	n.s.
2 mM Gly-L-Leu	9.02±0.2	4.91±0.35	45.57	s. ***
2 mM Gly-Gly	9.21±0.19	3.84±0.21	58.31	s.
2 mM Gly-Gly-Gly	9.40±0.26	2.42±0.41	74.3	s.
2 mM Gly	9.82±0.33	6.41±0.19	34.8	s.
2 mM L-Glu	8.64±0.16	7.12±0.34	17.6	s.
2 mM L-Cys	9.21±0.33	6.98±0.21	24.2	s.
2 mM D-Glu	8.40±0.25	7.98±0.33	6.2	n.s.
2 mM D-Cys	8.43±0.26	7.94±0.22	5.8	n.s.

* Statistical significance of difference.

** n.s., not statistically different when compared with control value.

*** s., statistically different compared with control; $P < 0.01$.

sodium-free situation in the buccal cavity since there is constant contamination from salivary sodium. Uptake of GSH in both systems appears to be independent of energy. Addition of amytal [25] had little inhibitory effect on buccal uptake and intestinal transport was unaffected in the presence of 2,4-dinitrophenol, an uncoupler of oxidative phosphorylation. These results suggest that uptake of GSH at both sites is by carrier-mediated diffusion.

Inhibition of GSH uptake by glycylglycylglycine suggests the possible existence of a multiple carrier system for tripeptides which may also be employed in the transport of dipeptides. L-Isomers of constituent amino acids also inhibited uptake. Transport of intact peptides has been reported previously [26]. Our studies show the presence of intact GSH within the everted sac.

Transport of GSH at both gastro-intestinal sites appears to be by means of non-energy requiring, sodium-independent, carrier-mediated, diffusion.

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