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CHOLINE INFLUX ACROSS THE BRUSH BORDER OF GUINEA PIG JEJUNUM

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Summary

Choline uptake across the mucosal border of guinea pig jejunum was measured to determine the characteristics of this step in intestinal absorption. Unidirectional influx of [^{14}C]choline appears to proceed primarily by a saturable, carrier-mediated process at low mucosal choline concentrations; at high concentrations (>4 mM) the influx rate is approximately linearly related to the mucosal choline concentration, suggesting that absorption by passive diffusion predominates. Influx was only minimally reduced by elimination of Na^+ from the mucosal test solution or by reduction of the intracellular Na^+ concentration. Preincubation of tissue samples with metabolic inhibitors or with ouabain did not markedly reduce influx. These results are consistent with a model of choline transport across the brush border membrane by a carrier-mediated mechanism which is similar to that involved in fructose absorption but different from the Na^+-dependent mechanism which participates in active transport of sugars and amino acids. At low lumenal choline concentrations, influx into colonic mucosa is slower than in jejunum and appears to be attributed solely to simple diffusion.

Introduction

Choline is frequently classified as a water-soluble vitamin because of its structure and because most animal species require it in the diet in small quantities. The size and solubility properties of choline tend to limit its intestinal absorption in the absence of a specific transport mechanism. Experimental evidence in support of such a mechanism is not easily obtained because choline is metabolized within intestinal cells [1,2]; therefore, studies in vitro of net transmural transport and intracellular accumulation by intestinal mucosa provide results which are difficult to interpret.

Absorption of other water-soluble nutrients, including glucose [3], fructose

[4], alanine [5] and ascorbic acid [6—8] occurs in small intestine by specific carrier-mediated processes at the brush border membrane. In the case of actively transported sugars, amino acids and ascorbic acid, transport across the brush border proceeds simultaneous with absorption of sodium, and therefore, influx is greatly reduced in sodium-free media. Fructose influx, in contrast, demonstrates saturation kinetics but is independent of mucosal sodium. Thus, it was of interest to examine the properties of unidirectional choline transport across the brush border membrane of guinea pig jejunum with the methodology previously employed to study absorption of other nutrients [3—6,9—12]. Experiments were performed specifically to examine the influx process for saturation kinetics, sodium dependence and cellular metabolic energy dependence.

Materials and Methods

Guinea pigs which had been maintained on normal dietary intake were killed by intraperitoneal injection of pentabarbitol. A section of jejunum was resected, opened along the mesenteric border, and washed free of intestinal contents with cold Ringer. A segment of the intestine was mounted, mucosal surface up, in a Lucite chamber which consisted of six isolated perfusion ports and was similar to the original design of Schultz et al. [12]. In each port, 0.265 cm^2 of mucosal surface was exposed to a bathing solution which was stirred by a fine stream of humidified 95% O_2/5% CO_2. The serosal surface of the tissue rested on moistened filter paper and was not exposed to the solutions bathing the mucosal surface. The tissue was preincubated at 37° C in Ringer of the following composition: NaCl, 142 mM; $KHCO_3$, 10 mM; K_2HPO_4, 4.2 mM; KH_2PO_4, 1.7 mM; $CaCl_2$, 1.2 mM; $MgCl_2$, 1.2 mM; pyruvate, 5.5 mM; pH 7.2. Na^+-free buffer solutions were prepared from Ringer by replacing NaCl with choline chloride, Tris · HCl, or KCl. A test solution containing [^{14}C]choline and [^{3}H]inulin (New England Nuclear Corp., Boston, Mass.) in Ringer solution was introduced into each port. This solution bathed the mucosal surface for 2, 5, or 10 min and was then removed; the port was flushed briefly with cold (4° C) choline buffer. The time interval from injection of the test solution to injection of the cold choline buffer was taken as the duration of exposure of the mucosal surface to the isotope. The cold choline buffer served to terminate the influx of isotope both by diluting the test solution remaining in the port and by cooling the tissue. The exposed area of tissue was cut out of the port with a steel punch, washed briefly in cold choline buffer, blotted and extracted in 0.1 M HNO_3 for 18—24 h. Aliquots of tissue extract and test solution were assayed for ^{3}H and ^{14}C activity by liquid scintillation spectrometry. Uptake of [^{14}C]choline by the tissue was calculated after correction for the inulin space. Previous studies have shown that inulin does not enter intestinal cells to a significant extent; thus, inulin space is a measure of the volume of adherent test solution which was not removed by the cold choline wash. The results of these experiments provide a reliable measure of unidirectional influx since choline does not appear to be metabolized within the intestinal lumen [2,10] and the methodology of the present experiments is not influenced by intracellular metabolism of choline provided that the ^{14}C-labeled products of the reactions are retained within the tissue for the duration of the influx.

Results

Unidirectional choline influx. The time course of choline influx was determined by exposing individual tissue samples to a concentration of 0.024 mM [^{14}C]choline for 2-, 5-, or 10-min periods. Uptake of isotope across the mucosal border was found to be linear over a 10 min period (Fig. 1). Linear uptake with time indicates that there is no significant loss of ^{14}C activity from the tissue during this period. Linear extrapolation of the data points yields an intercept on the y axis of 0.03 nmol/cm^2 which is not statistically different from a zero intercept ($P > 0.2$). 5-min exposure periods were used in subsequent experiments.

Kinetics of choline influx. Choline influx was determined as a function of the choline concentration in the test solution over the range 0.024—20 mM (Fig. 2). At low mucosal concentrations, influx is not a linear function of the initial choline concentration but shows a tendency toward saturation, which is characteristic of a carrier-mediated process. At mucosal concentration above 4 mM the data points do not differ significantly from a linear relationship and entry by passive diffusion is indicated. The saturable component of choline influx was further evaluated following correction of the original data for the diffusion component of influx indicated by the approximate linearity observed at choline concentrations above 4 mM. From a plot of choline concentration/choline influx versus choline concentration [13] (Fig. 3) values were obtained for K_m of 0.6 mM and V of 132 nmol/cm^2 per h.

Effect of Na^+ depletion on choline influx. Choline influx from Na^+-free mucosal solutions was determined using Tris buffer, potassium buffer or mannitol buffer as the test solution. Tissue samples in alternate (experimental) ports were rinsed with the Na^+-free test solution for 1 min immediately prior to influx to insure a low Na^+ concentration in the port during the experiment. The results in Table I show that Tris and potassium substitution resulted in modest decreases in choline influx, but mannitol substitution had no significant effect.

The influence of intracellular Na^+ on choline influx was determined by preincubating paired tissue samples at 0°C for 30 min either in Ringer or in Tris buffer to lower the intracellular Na^+ concentration. Choline influx in the two groups of tissues did not differ significantly (Table I). The combined effect on

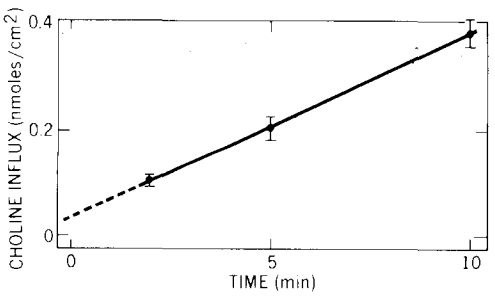

Fig. 1. Influx of choline across the mucosal border of jejunum as a function of exposure time of mucosa to 0.024 mM [^{14}C]choline. Each bracket is the mean (±S.E.) of 11—14 influx determinations. The slope represents choline influx of 2.3 nmol/cm^2 per h.

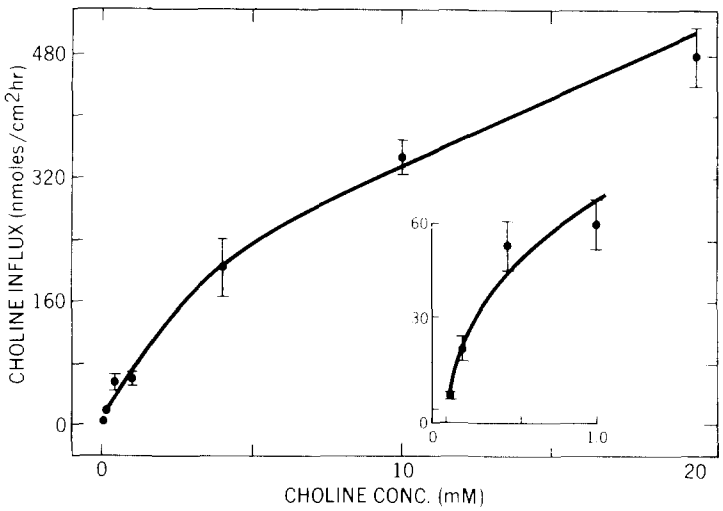

Fig. 2. Choline influx into jejunum from mucosal choline concentrations of 0.024—20 mM. Exposure times were 5 min.

choline influx of a reduced cellular Na^+ concentration and a Na^+-free musocal solution was determined in paired groups of tissues. Test samples were preincubated at 0°C for 30 min in potassium buffer to maintain the normal high intracellular K^+ concentration; Tris buffer was used as the test solution in experimental tissues. Control tissues were exposed only to Ringer. Choline influx was not significantly different between test and control tissue samples.

Effect of transport inhibitors on choline influx. The rate of choline influx was determined in jejunal mucosa in which cellular metabolism was inhibited. Tissue samples were preincubated at 0°C for 20 min in Ringer with 2,4-dinitrophenol (0.1 mM) and cyanide (2.0 mM). Influx determined in Ringer with dinitrophenol and cyanide did not differ from influx in tissue samples not exposed to metabolic inhibitors (Table II).

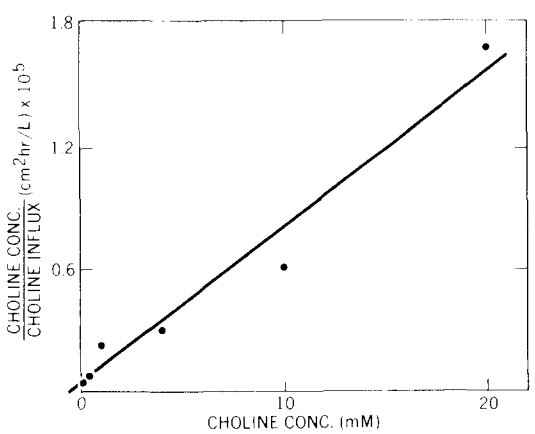

Fig. 3. Plot of choline concentration/choline influx vs. choline concentration. Values derived from Fig. 2 following correction of original data for a linear diffusion component ($y = 15.7\ x$).

TABLE I

EFFECT OF Na^+-FREE CONDITIONS ON CHOLINE INFLUX

Control and experimental tissues were preincubated for identical times in the solution indicated. *n* is the number of influx determinations. Test solutions contained 0.024 mM choline.

Preincubation solution (time)	Test solution	Choline influx ($nmol/cm^2$ per h ± S.E.)	*n*	*P*
	Ringer	5.2 ± 1.0	9	>0.2
	Tris buffer	3.6 ± 0.7	9	
	Ringer	4.9 ± 0.9	8	>0.2
	Mannitol buffer	5.4 ± 1.1	9	
	Ringer	6.3 ± 0.7	9	<0.01
	Potassium buffer	3.9 ± 0.4	9	
Ringer Tris buffer (30 min)	Ringer	2.3 ± 0.2	9	>0.2
	Ringer	2.0 ± 0.1	9	
Ringer Potassium buffer (30 min)	Ringer	3.8 ± 0.2	6	>0.2
	Tris buffer	3.8 ± 0.4	6	

The effect of ouabain was determined by preincubating tissue samples for 30 min at 0°C in Ringer containing the cardiac glycoside (0.1 mM). Influx determined in Ringer with ouabain did not differ from control tissue samples (Table II).

Choline influx in colon. For purposes of comparison, choline influx was measured in guinea pig colon; this tissue does not possess the intricate brush border mechanisms of the small intestine for transport of organic nutrients. Influx was determined as a function of the choline concentration in the test solution over the range 0.024—10 mM. The data points (Fig. 4) are adequately fit by a straight line corresponding to a rate of diffusional entry approx. 45% higher than the diffusional entry rate in jejunal tissue. Thus, diffusional absorption in colon is faster than in jejunum, but colonic tissue does not appear to absorb choline by a carrier-mediated process.

TABLE II

EFFECT OF INHIBITORS ON CHOLINE INFLUX

Control and experimental tissues were preincubated for identical times in the solution indicated. *n* is the number of influx determinations. Ouabain and 2,4-dinitrophenol were present at 0.1 mM. Sodium cyanide was present at 2.0 mM. Test solution contained 0.024 mM choline.

Preincubation solution (time)	Test solution	Choline influx ($nmol/cm^2$ per h ± S.E.)	*n*	*P*
Ringer	Ringer	2.4 ± 0.2	6	0.2
Ringer + dinitrophenol + cyanide (20 min)	Ringer + dinitrophenol + cyanide	2.3 ± 0.2	6	
Ringer	Ringer	2.7 ± 0.4	9	0.2
Ringer + ouabain (20 min)	Ringer plus ouabain	2.8 ± 0.3	9	

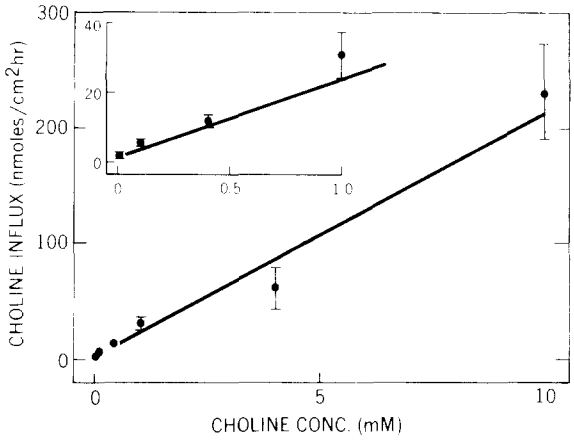

Fig. 4. Choline influx into colon from mucosal choline concentrations of 0.024–10 mM.

Discussion

Choline is present in the diet in low quantities and is required by the body in a variety of metabolic and structural roles. At physiological pH, choline is charged and is therefore not expected to be rapidly absorbed across intestinal tissue in the absence of a specific transport mechanism. Sanford and Smyth [1] interpreted the results of their experiments on everted sacs of hamster small intestine as indicating that choline is absorbed both by simple diffusion and by a saturable process. Transport was inhibited by anaerobic conditions and by dinitrophenol. However, since transport against a concentration gradient could not conclusively be demonstrated due to metabolism of [^{14}C]choline by intestinal cells, a clear demonstration of active transport was not made. Because the saturation kinetics of this earlier work were demonstrated with transmural flux studies, the proposed carrier mechanism could not be attributed specifically to either the mucosal or the serosal cell membrane.

Herzberg and Lerner [14] incubated segments of chick intestine in buffer and determined the absorption rate of [^{14}C]choline. Absorption followed saturation kinetics, was slightly dependent on Na^+ and was reduced by structural analogs but not by anaerobic conditions or ouabain. Because of the incubation technique used, however, the results of these experiments cannot be attributed specifically to transport events at either the mucosal or the serosal cell membranes; in addition, since muscle was apparently not removed from the tissue, the observed transport properties might apply to muscle cells rather than to the enterocytes. Other essential nutrients, including sugars, amino acids and water-soluble vitamins, that are absorbed into intestinal mucosa by specific carrier-mediated processes have been shown to be absorbed by transport mechanisms located in the brush border of absorptive cells. Thus, a model of choline transport by small bowel mucosa would be speculative in the absence of information on the mucosal influx process.

The present study provides information about the unidirectional influx of choline across the brush border of guinea pig jejunum and colon. In jejunum

this uptake appears to proceed primarily by a saturable, carrier-mediated mechanism at low lumenal choline concentrations and by a non-saturable, diffusional process at choline concentrations above 4 mM. Values of choline concentrations in the intestinal lumenal fluid are not available in the literature; the plasma concentration of choline has been reported to be approx. 1.0 μM [15].

The influx process in jejunum was further characterized to have little dependence on cellular metabolism or on the Na^+ concentration in either the mucosal bathing solution or the intracellular fluid. Influx of actively transported sugars, amino acids and ascorbic acid from low mucosal concentrations was reduced by 70—90% in the absence of mucosal Na^+. Thus, the favored mechanism of absorption for these substances involves simultaneous entry of Na^+ and substrate across the brush border membrane. With normal low intracellular Na^+ concentrations, active transport of 3-*O*-methyl glucose, alanine, and ascorbic acid into the cell has been demonstrated. In contrast, fructose movement across the brush border of rabbit ileum appears to proceed by a specific carrier-mediated process which is independent of mucosal Na^+ and does not appear to involve active transport. Thus, the properties of choline absorption in jejunum appear to resemble most closely those of the fructose transport process. In contrast, choline did not appear to enter colonic mucosa by a carrier-mediated process; its entry by diffusion proceeded at a rate somewhat greater than the rate of diffusional entry into jejunal tissue.

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