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AMYLASE TRANSPORT ACROSS ILEAL EPITHELIUM IN VITRO

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Summary

Amylase transport was measured across the rabbit ileum in vitro employing a modified Ussing chamber. Amylase was moved preferentially in the mucosal to serosal direction. Its rate of transfer was 2—3 orders of magnitude greater than that for inulin. Mucosal to serosal transport of exogenous amylase was completely inhibited in the absence of oxygen. There was also a constant release of endogenous amylase from intestinal tissue into both mucosal and serosal compartments in the absence of an exogenous source. An estimate of the rate of amylase absorption indicates that it may be of sufficient magnitude to account for the enteropancreatic circulation of amylase secreted by the pancreas during augmented secretion.

Recent experiments from our laboratory have suggested an enteropancreatic circulation of digestive enzyme in which secreted enzyme is absorbed across the intestinal epithelium into blood and subsequently secreted again by the pancreas [1—6]. Two key steps in the circulation process are transpancreatic transport and intestinal absorption. We report here our attempt to measure the latter for pancreatic amylase across the ileum in a defined in vitro system.

Methods

The studies were performed in vitro with a modified Ussing-Zehran [7] apparatus. Male New Zealand White rabbits (2—3 kg) were anesthetized after an overnight fast (18—22 h) with 0.7 ml/kg body weight of a mixture of 0.1 g allobarbital per ml/0.4 g urethan per ml/0.4 g monoethylurea per ml, injected intraperitoneally. Pieces of terminal ileum were removed, rinsed with saline, and blotted gently on filter paper. They were then placed in a beaker containing 100 ml of a Krebs-Henseleit bicarbonate-buffered solution [8], pH 7.4,

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containing 11 mM glucose and 1 mg/ml bovine albumin (Type V, Calbiochem) for 3 min. This rinse was to help remove adsorbed amylase from the intestinal surface. The tissue was then mounted between two round plexiglas half-chambers of 0.49 cm^2 surface area and bathed on both sides by the medium described above. Each compartment contained 15 ml of solution. Temperature was kept constant at 37° C. Solutions were gassed with 95% O_2 plus 5% CO_2. In specified experiments, 95% N_2 plus 5% CO_2 was used instead. To remove any residual amylase from the surface of the tissue the bathing solution was then changed on both sides every 3 min for a total of 12 min and the contents of both serosal and mucosal media analyzed for amylase. Thereafter, the experiments were performed in two ways: (1) controls: The medium on both sides was changed at 5 and 10 min and then at 10-min intervals for a total of 90 min. (2) Experimental: α-amylase (1 mg/ml) (Type VIa, Sigma) was added to either the mucosal or serosal medium and the bathing solution alone added to the opposite half-chamber. 50 μCi [^{14}C]inulin (Amersham Searle) was added to the amylase-containing compartment. Both bathing solutions (mucosal and serosal) were removed at the time of sampling. The amylase-inulin-containing solution was added back to the same half-chamber, while a fresh aliquot of medium was added to the other half-chamber. Sampling intervals were the same as for the control experiments. The transfer of amylase and inulin was measured by the appearance of both compounds on the opposite side as determined by enzyme activity for amylase and ^{14}C for inulin.

When [^{14}C]inulin was used, a 1.5 ml aliquot of medium was set aside for counting. The rest of the 15 ml sample was concentrated about 15-fold with high-pressure ultrafiltration (Diaflo membranes UM10, Amicon).

Amylase activity was measured by a modification of the method of Rinderknecht et al. [9]. In this assay, hydrolysis of insoluble starch covalently labeled with Remazol brilliant blue R (Amylose Azure, Calbiochem Grade B) is measured. The substrate mixture was 4 g of substrate in 100 ml 0.02 M sodium phosphate buffer (pH 7.0) containing 0.05 M NaCl. 0.5 ml samples were added to 2.25 ml of the substrate mixture and incubated for 15 min in a shaking water bath at 37° C. The reaction was stopped with 1 ml 0.2 M acetic acid. After filtration (No. 1 Whatman filter paper), the filtrate was read at 595 nm.

The albumin in the salt solution had some amylolytic activity and all values have been corrected for it.

Samples to be used for liquid scintillation counting were dissolved in 4.5 ml BBS3 (Beckman Instruments Co.) and 15 min later toluene and a mixture of fluors (Flouralloy, TLA, Beckman) were added. Counting efficiency for ^{14}C was between 85 and 90%. All samples were counted twice to within 5% error.

Results

Mucosal-serosal transfer of amylase across the ileum. When amylase was added to the mucosal fluid, amylase activity on the serosal side rose rapidly to some 4—5 times that of time-paired controls and remained at that level for the remainder of the experiment (Fig. 1). Since the bathing medium was completely changed after each sampling period, the constant level indicates that the flux was relatively constant from period to period. However, transport was not

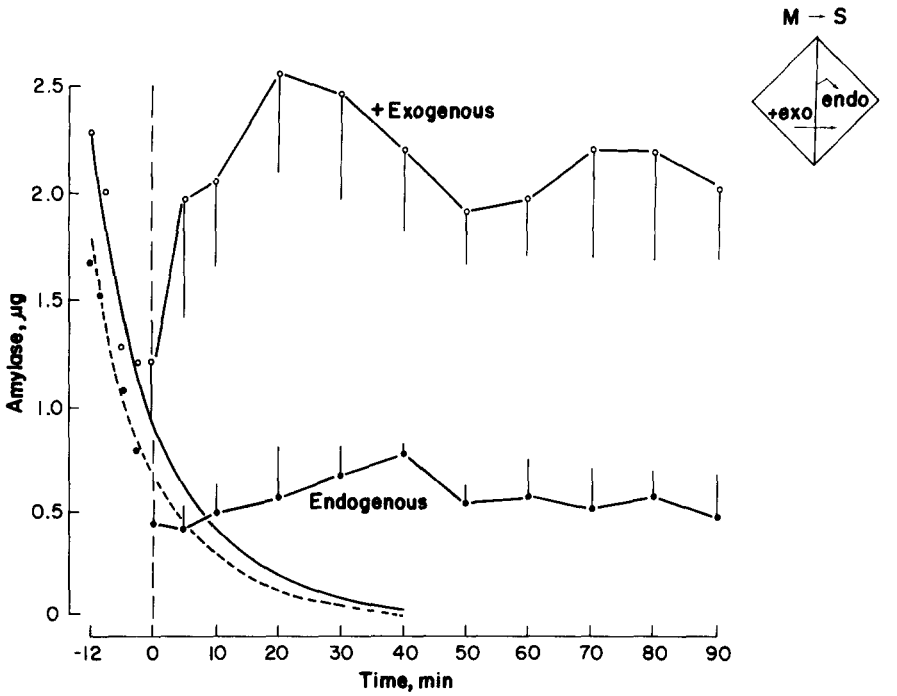

Fig. 1. Mucosal-serosal (M → S) transfer of amylase across the rabbit ileum in vitro over time. Amylase release into the serosal medium was determined after addition of exogenous amylase (+ exogenous) to the mucosal surface at time 0 (n = 6) (open circles) and in the absence of exogenously added amylase ("endogenous" controls, n = 6) (closed circles). The two exponential functions (broken line, control group; solid line, experimental group) were calculated from four sequential washout periods (—12 to 0) which preceded the experiments.

at the initial rate, because doubling the collection period from 5 to 10 min only increased the amount transfered by about 25% (see Fig. 1 and compare period 10 min to period 20 min).

The release of endogenous amylase. In designing these experiments we were concerned that endogenous amylase either of pancreatic, salivary or intestinal origin might be adsorbed to the intestinal surface and confound our measurements unless it was removed. We attempted to do this by a preincubation wash in a relatively large volume followed by four sequential washes after the tissue was mounted in the chamber. A washout of amylase was seen with the sequential washes (see Figs. 1 and 2 for serosal and mucosal curves), but it did not conform to the expected exponential function reaching an assymptote at zero amylase (predicted exponential functions shown in Figs. 1 and 2). Rather, both mucosal and serosal curves fell sharply initially, but to a value significantly above zero. After the initial fall-off, the rate of amylase appearance in both mucosal and serosal medium remained relatively constant over time, despite repeated bath changes (Figs. 1 and 2). Mucosal release actually increased toward the end of the experiment (Fig. 2).

Amylase vs. inulin transfer. We added [^{14}C]inulin (M_r = approx 5—7000), along with amylase (M_r = 45—48 000) to the mucosal side. Very little inulin crossed the epithelium at early times as compared to amylase (some 10^2—10^3

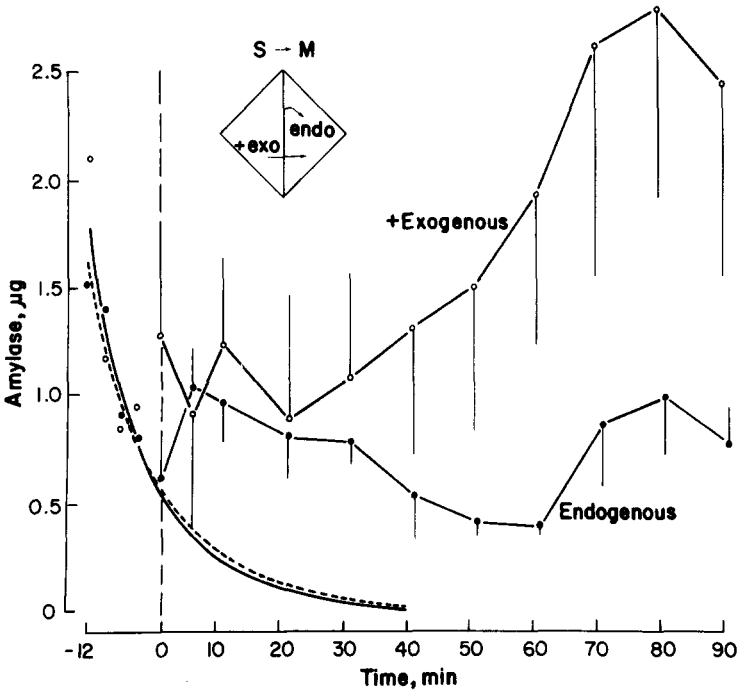

Fig. 2. Serosal-mucosal (S → M) transfer of amylase in vitro over time. Amylase release into the mucosal medium was measured in the presence (+ exogenous, open circles) and the absence (endogenous, closed circles) of exogenously added amylase (n = 6 in each group). The two exponential functions (broken line, controls; solid line, experimental group) were calculated from the amount of amylase released into the mucosal medium in four sequential 3-min washout periods prior to the experiments.

times less; Fig. 3 and Table I). The amount of inulin that was transfered increased with time and approached the rate of amylase transport by 80 min (Fig. 3). This could have been the result of an increased leakiness of the tissue with time or a rather slow approch of inulin movement across the epithelium to its steady-state transport rate. The latter explanation is suggested by the fact that amylase permeability remained relatively constant over the course of the experiment.

The serosal to mucosal flux. In a parallel series of experiments amylase was added to the serosal surface and its appearance followed in the mucosal medium (Fig. 2). During the first 30 min, the addition of exogenous amylase did not increase mucosal amylase above controls (endogenous amylase) (Fig. 2). However, by 50 min, a significant flux of amylase had developed which reached a peak at 80 min and was by that time of about the same magnitude as the mucosal to serosal (MS) flux (Figs. 1 and 2). As with inulin, this may indicate that amylase transport in the serosal to mucosal (SM) direction reaches a steady-state rate more slowly than in the MS direction, rather than being due to a time-dependent increase in the leakiness of the tissue since MS amylase flux was not increased over this time period (Fig. 1). Even though we could not statistically distinguish the transfer of exogenous amylase from controls at early times, a comparison of the amylase and inulin transfer rates (SM) for those early periods showed a relatively large difference between them, i.e., amylase

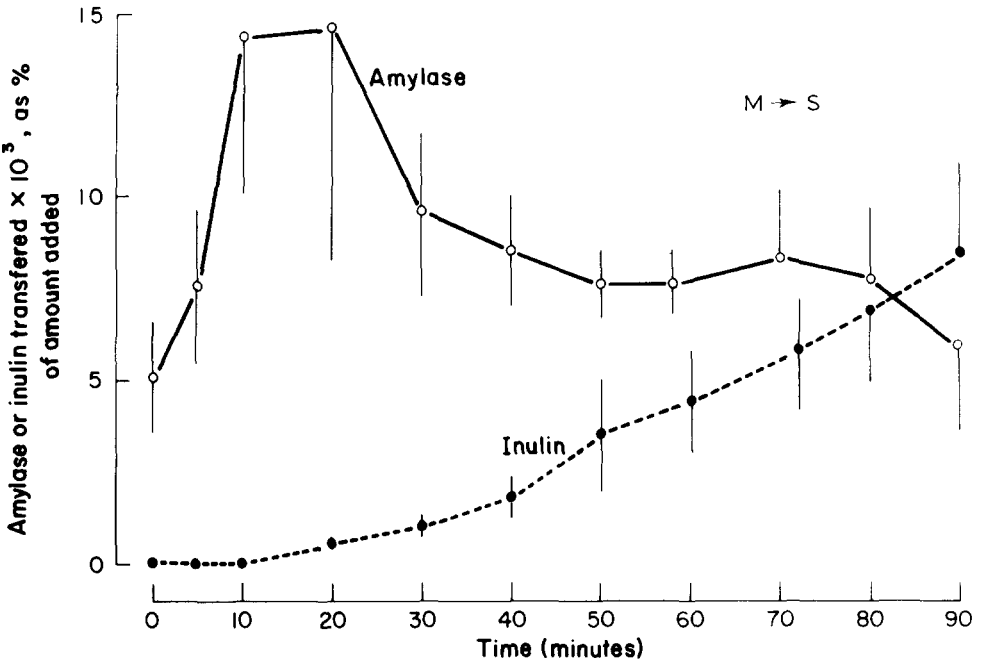

Fig. 3. Relative amounts of amylase and [^{14}C]inulin appearing in the serosal medium following their simultaneous addition to the mucosal surface. Amylase data were corrected for endogenous release (n = 6).

transfer was over an order of magnitude greater than inulin (0.0015% transfered vs. 0.00008% transfered for inulin, n = 6).

The metabolic inhibition of amylase flux. The MS flux of amylase was measured in the absence of oxygen to determine if transfer was dependent on aerobic metabolism. When N_2 replaced O_2 in the gas mixture, exogenous amylase transport was completely inhibited (Fig. 4). Moreover, at least for the 30- and 40-min periods, the appearance of amylase on the serosal side was significantly less in the absence of oxygen than when amylase from endogenous sources alone was available in its presence (controls labeled "endogenous", Fig. 1) ($P < 0.05$ for 30 min and $P < 0.005$ for 40 min).

TABLE I

RELATIVE RATES OF TRANSFER OF AMYLASE AND INULIN IN THE MS DIRECTION

Time (min)	% Transferred MS	
	Amylase (activity)	Inulin (cpm)
5	0.0075 (0.002) *	0.000013 (0.000013)
10	0.014 (0.0035)	0.00007 (0.000025)
20	0.015 (0.006)	0.00049 (0.00013)

* $\bar{x}$ (S.E.M.); n = 6.

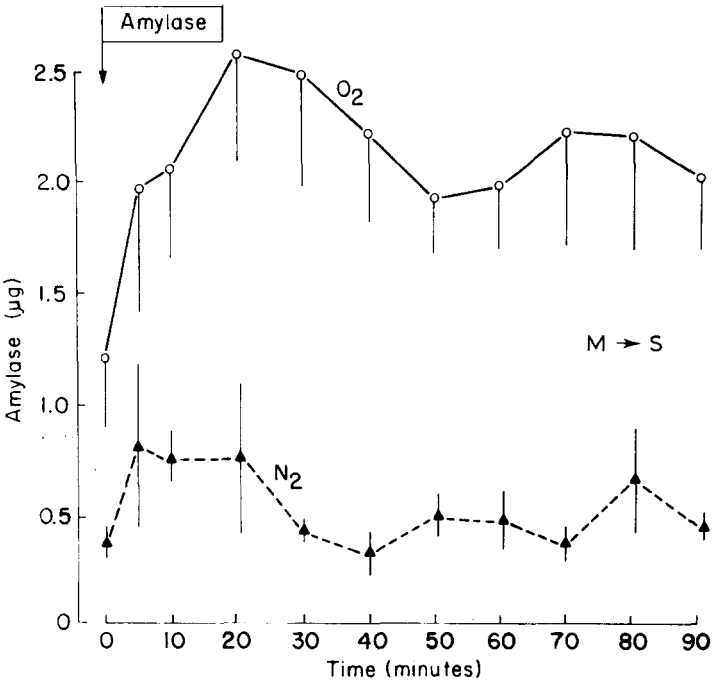

Fig. 4. The effect of metabolic inhibition on mucosal to serosal amylase transport in vitro. The release of amylase into the serosal medium after the addition of amylase at time 0 was determined in the presence of an N_2/CO_2 gas mixture (triangles, $n = 6$) and compared to the results obtained with O_2/CO_2 (open circles, $n = 6$).

Discussion

Amylase was transported at substantial rates across the rabbit ileal epithelium in vitro preferentially in the mucosal to serosal direction. Initially, the rate of MS transfer was 2-3 orders of magnitude greater than that of inulin added simultaneously to the muscosal surface. Since we did not remove muscle layers from our preparation, our in vitro estimate may underestimate the in vivo permeability of the rabbit ileum to amylase. MS transport of exogenous amylase was also metabolically dependent and was completely inhibited in the absence of oxygen.

In control experiments in which no exogenous amylase was added to either the mucosal or serosal side, continuous amylase release was still observed from the tissue. Release of amylase from the mucosal surface was not surprising since pancreatic amylase might well have been adsorbed to it, but a release of similar magnitude from the serosal surface was unexpected. Neither serosal nor mucosal release decreased with the repetitive replacement of bathing medium as it would have if it were simply adsorbed to the surface of the tissue (see calculated functions in Figs. 1 and 2). Similarly, Alpers and Solin [10] found it difficult to remove amylase from the mucosal surface of rat small intestine with repeated washings and yet recovered most of it in supernatant fractions of intestinal homogenates. Moreover, they found that most of the amylase asso-

ciated with the intestine was pancreatic amylase. This could be explained if pancreatic amylase is normally contained in and released from intestinal cells.

Central to the enteropancreatic circulation hypothesis is an intestinal permeability to digestive enzyme. Others have reported such a permeability in the past for several molecules in a variety of in situ systems [11—15]. The present study demonstrates an intestinal permeability to amylase in a well defined in vitro system. However, intestinal permeability to digestive enzyme is a necessary but not a sufficient criterion for a bulk circulation process. The intestine must not only be able to transport enzyme, but do so in sufficient amounts to account for circulation. Since in situ experiments in rabbits by the present authors provide indirect evidence that bulk circulation occurs, and indeed, under certain circumstances appears to approch unity (the observed increase in enzyme secretion being about equal to the input of enzyme) [2]; we were interested in evaluating the rabbit intestine's quantitative capacity for amylase transport and its relation to the magnitude of pancreatic amylase secretion, i.e., what percentage of amylase secretion could be circulated at the steady state. If the circulation hypothesis is correct, then the capacity of the absorptive system must be equal to or larger than the amount of enzyme circulated under steady-state conditions [2]. If, for purposes of estimation, we assume that (1) the specific activity of secreted rabbit amylase and the pig amylase transported across the intestine are roughly the same; (2) amylase absorption in vitro is equal to that in situ; (3) no duodenal, jejunal, or colonic absorption occurs; and (4) absorptive transfer measured in the present experiments represents the maximal rate of amylase transfer across the intestine for this in vitro system, then the calculated absorptive capacity is 50—65% of the circulated fraction of amylase. This is probably a minimal estimate and if some of our simplifying assumptions are incorrect, e.g. that absorption does not occur elsewhere in the bowel, then the percentage would, of course, be higher. In any event, as an order of magnitude approximation, the intestine seems able to account for the amount of enzyme absorption required by the enteropancreatic circulation hypothesis.

Acknowledgement

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