Biochimica et Biophysica Acta, 649 (1981) 759–768
Elsevier/North-Holland Biomedical Press

BBA 79507

AMINO ACID INFLUX ACROSS THE MUCOSAL BORDER OF THE RAT INTESTINE IN VIVO

J.J. HAJJAR [a] and H.P. SCHEDL [b]

[a] *Division of Gastroenterology, Department of Internal Medicine, Veterans Administration Medical Center, Newington, CT, University of Connecticut Health Center, Farmington, CT, and* [b] *Gastroenterology Research Laboratory, Department of Internal Medicine, University of Iowa, Iowa City, IA (U.S.A.)*

(Received April 15th, 1981)
(Revised manuscript received August 4th, 1981)

Key words: Amino acid influx; Na^+ dependence; Competitive inhibition; Saturation kinetics; (Rat intestinal epithelium)

The lack of an in vivo method for measuring influx of amino acid into the mucosa has prevented a systematic comparison of characteristics of amino acid influx in vivo with prior in vitro studies. We developed and validated a technique for measuring amino acid influx in vivo. The mucosa is exposed briefly to labelled amino acid perfused luminally at a rapid rate and tissue uptake is measured. The brief exposure period insures that amino acid is confined to the segment. The rapid perfusion rate minimizes concentration of endogenous Na^+ in the lumen and permits Na-dependency for α-aminoisobutyric acid influx to be demonstrated in vivo for the first time. We also demonstrated the inhibitory effect of K^+ and competition by glycine on α-aminoisobutyric acid influx in vivo. The saturation kinetics for L-leucine in vivo and in vitro were compared under varying perfusion rates and with and without stirring with air. Under optimal conditions of agitation (rapid perfusion and bubbling with air), the apparent Michaelis constant (K_t) is decreased to be almost equal to that determined under comparable influx conditions in vitro. These studies demonstrate no major difference between characteristics of amino acid transport under more physiologic in vivo conditions as compared with prior in vitro studies.

Introduction

Most investigators have utilized classical in vitro techniques [1–5] to characterize the mechanism of absorption of amino acids across the small intestine. There are studies, however, that suggest the presence of differences between the transport of amino acids in vivo and in vitro. Na-dependence of amino acid transport was found to be less prominent in vivo than in in vitro [6–9], and a difference was also noted with regard to the concurrent transport and accumulation of amino acids in intestinal cells. In vitro, the greatest accumulation occurred in the most rapidly transporting intestinal segments [10–12], while in vivo it was the least in segments with the greatest rates of absorption [13]. The accumulation in vitro was also found to be quite remarkable but in vivo it was minimal and practically nonexistent [8,9,14,15]. Furthermore, estimates of the apparent affinity constants of amino acid transport were generally described to be much higher in vivo than in vitro [16]. Although there are explanations that can be given to account for some, or all of the above differences, there is, however, very little information about the transport properties of the intestinal cell membranes in the more natural in vivo condition. The lack of a method for measuring influx of amino acid in vivo has prevented confirming the results of prior fundamental studies of amino acid influx in vitro [3,18] under more physiologic in vivo conditions. Availability of such a technique would enable in vitro findings on effect of Na^+ on influx, comparison of saturation kinetics and other parameters, to be evaluated. Validation of an in vivo method requires demonstrating that amino acid taken up from the lumen is confined to the segment studied and does not appear in portal

blood during the influx period, that extracellular fluid volume measurements are appropriate, and that essentially sodium-free conditions can be maintained intraluminally by the rapid perfusion rate. We devised such an in vivo method and describe its validation and application.

Methods

All experiments were performed on male Sprague-Dawley rats housed in stainless steel cages and fed chow (Ralston Purina Co., St. Louis, MO) until their weights were between 250 and 300 g when they were used in experiments done between 9 and 12:00 a.m. The rats were fed until the day of experimentation when they were anesthetized with intraperitoneal injection of sodium pentobarbital (55 mg/Kg) and were kept on a heating pad that maintained their rectal temperature at 37°C. The abdominal cavity was opened by a mid-line incision and the intestine was cannulated and perfused in situ or resected and incubated in vitro by methods that are described below. The perfusate or the incubation medium consisted of a Ringer solution containing 140 mM NaCl/10 mM $KHCO_3$/1.2 mM K_2HPO_4/0.2 mM KH_2PO_4/1.2 mM $MgCl_2$/1.2 mM $CaCl_2$ (the pH was maintained at 7.2 by bubbling with 95% O_2/5% CO_2). In some experiments, all the NaCl in the Ringer solution was replaced by either choline chloride, potassium chloride or lithium chloride. α-Aminoisobutyric acid, L-leucine (Sigma Chemical Co., St. Louis, MO), tracer quantities of their ^{14}C isotopes, [^{3}H]inulin and [^{3}H]-mannitol (New England Nuclear Corp., Boston, MA) were added from stock solutions.

Net absorption in vivo. Net absorption across the in situ intestine was measured by a method previously described [17]. Segments of jejunum, mid intestine and ileum measuring about 20–25 cm in length were cannulated by inlet and outlet cannulae. The segmenst were flushed with 10 ml Ringer solution and 20 ml air and were perfused simultaneously by a single-pass continuous perfusion technique. The perfusate consisted of Ringer solution containing phenol red (15 mg/l), an appropriate concentration of one of the amino acids and its ^{14}C-labelled isotope. The perfusion rate was 0.5 ml/min using a peristaltic pump (Manostat, New York, NY). Effluent solutions were collected 40, 60, 80 and 100 min after starting the perfusion and their ^{14}C contents were determined by liquid-scintillation spectrometry and their phenol red concentration by spectrophotometry. At the end of the experiments, the segments were resected, dried in an oven for 18 h at 90°C and their dry weights were determined. Absorption was calculated from the rate of disappearance of the amino acids from the perfusion solution taking into account water transport as measured by the change in phenol red concentration. The net fluxes measured during the final three 20-min periods were added to calculate net absorption per h and are presented per g dry weight of the perfused segment.

Influx measurement. Two adjacent segments of intestine (each about 6–8 cm in length) were isolated and cannulated (Fig. 1). The segments were flushed with 5 ml Ringer solution and 10 ml air. They were then replaced in the abdomen and perfused with Ringer solution at the rate of 0.5 ml/min for about 20 min using an infusion pump (Harvard Apparatus, Model 975, Millis, MA). At the end of this preincubation period, influx was measured across one of the segments in situ and across the other after resection and transfer to an in vitro lucite chamber.

The method for measuring influx in vivo is similar to an in vitro method described previously [18] but adapted to in vivo conditions. After preincubation, one of the intestinal segments was removed from the abdomen and placed on a Ringer-soaked gauze pad. A loose knot was placed around its blood vessels using a cotton thread. The segment was then perfused with Ringer solution for an additional 10 min at the end of which the perfusate was flushed out by injecting air through the three-way stopcock (Fig. 1B). A priming volume of 2.5 ml of test solution was then injected through the stopcock and the intestine was immediately perfused with the test solution at a relatively rapid rate (unless otherwise specified, 5 ml/min) for 45–50 s. The test solution was Ringer's containing an appropriate concentration of an amino acid, its ^{14}C-labelled isotope and [^{3}H]mannitol as an extracellular marker. At the end of the test period, the solution was rapidly flushed out by injecting air through the stopcock followed immediately by 6–8 ml ice-cold isotonic mannitol solution. The blood vessels of the segment were then occluded and the segment was immediately resected by cutting along its mesenteric attachment. It was then washed briefly

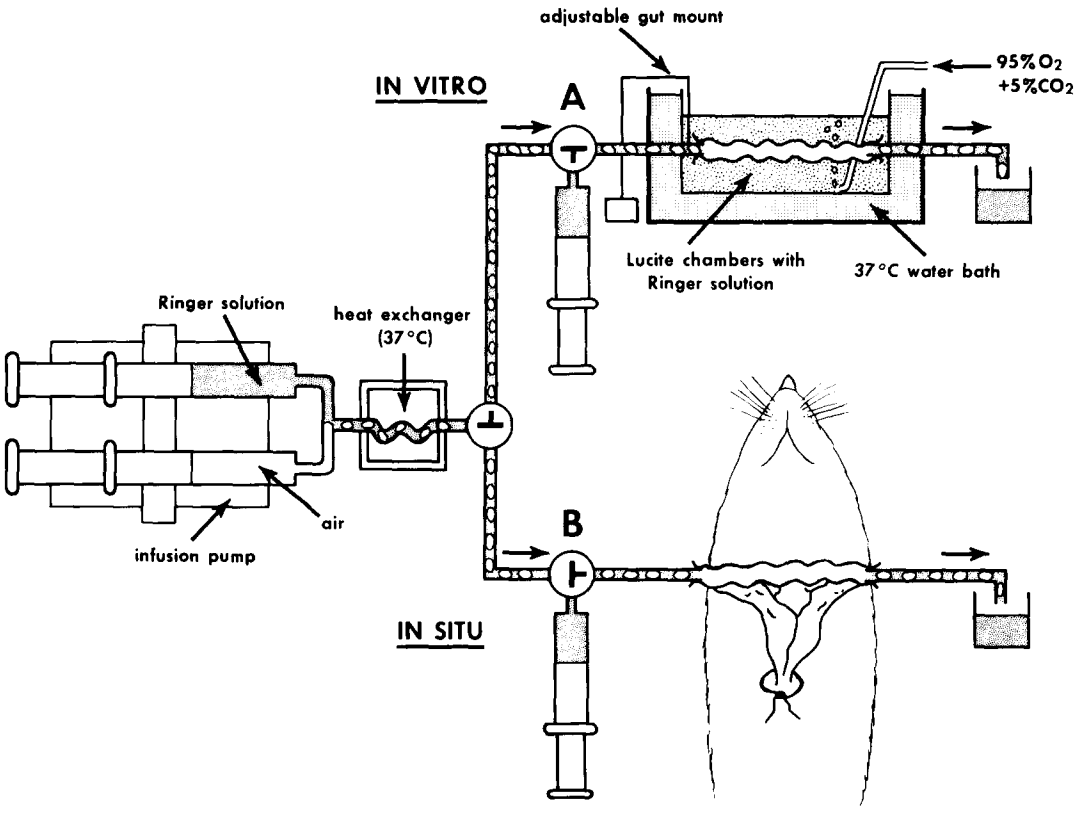

Fig. 1. Schematic representation of the apparatus for measurement of influx.

(3 s) with isotonic mannitol, blotted with Whatman No. 1 filter paper, then extracted with 0.1 N HNO_3 for 4 h. The time period between resection and extraction was 10–15 s; all studies by one of us (J.J.H.). Tissue residues after extraction were dried in an oven and their dry weights were determined. Aliquots of tissue extracts and test solutions were counted in a two-channel liquid-scintillation spectrometer (Nuclear Chicago, Isocap 300) and their ^{14}C and ^{3}H contents were determined using channel ratio values obtained with standards containing each of these isotopes alone. Influx was calculated from the amino acid content in tissue extracts after making a correction for the mannitol space. The time period of each influx determination was measured starting with the injection of the test solution and terminating with the injection of the isotonic mannitol solution. Influx is presented per h/g dry weight of the perfused segment (64 cm^2 serosal surface/g dry weight).

Influx in vitro was measured using the other intestinal segment after resecting and mounting that segment in the lucite chamber illustrated in Fig. 1. The segment maintained in the same orientation as in vivo was mounted to the inlet and outlet perfusion ports of the chamber and its length was adjusted to remain in line with the perfusion ports and to prevent it from being curved or twisted. The serosal surface of the segment was bathed with Ringer solution oxygenated with 95% O_2/5% CO_2 and maintained at 37°C. The luminal surface of the segment was perfused as for the in vivo influx experiments.

To reduce the effective unstirred layer thickness [19,20] air was perfused simultaneously with the Ringer solution for in vivo and in vitro influx studies. Air and the test solution were mixed in the inflow tubes (Fig. 1). In initial experiments, the circumference of the intestine was measured as described by Winne [20]. In six pairs of in vivo and in vitro

segments, the circumference was 1.78 ± 0.14 and 1.82 ± 0.16 cm, respectively. In some experiments, the Na concentration in the effluent solutions was determined by flame photometry (Instrumentation Laboratories Inc. Model 343).

Validation of the method for measuring in vivo influx. The rate of appearance of amino acids in portal and systemic blood after instilling the amino acid into the intestinal lumen was measured using α-aminoisobutyric acid. α-Amino[^{14}C]isobutyric acid (spec. act. 15 Ci/M) was perfused through a jejunal segment and after an appropriate period, the vessels of the segment were occluded and blood was immediately drawn by venipuncture from the portal and iliac veins. α-Amino[^{14}C]isobutyric acid was not detectable in blood samples obtained at 30 and 60 s (Fig. 2), but at 90 s ^{14}C increased significantly ($P < 0.01$) in the portal blood. At 120 s, the isotope was significantly increased in both portal ($P < 0.001$) and systemtic ($P < 0.001$) blood. The amount of tracer reaching the tissue during the first 60 s was considered to be equivalent to the entry of amino acid into the intestinal cell, i.e. influx.

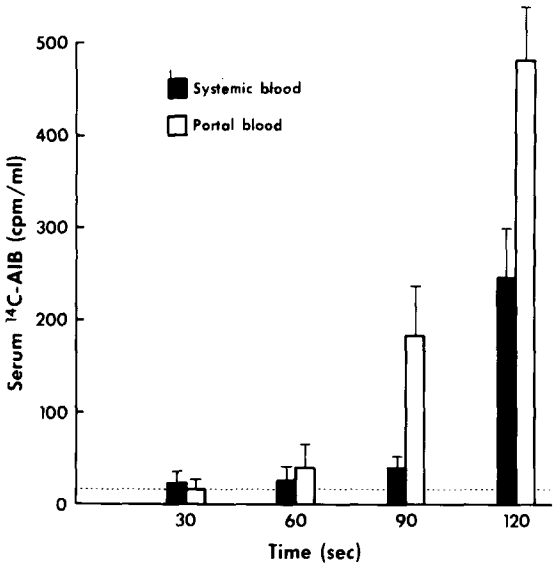

Fig. 2. The time course of appearance of α-amino[^{14}C]isobutyric acid ([^{14}C]AIB) in systemic (■) and portal blood (□) after it is perfused into the lumen of a segment of rat jejunum. The data are means ± S,E. of seven determinations. The dotted line represents the average ^{14}C background count.

Cumulative uptake of α-aminoisobutyric acid by the intestine was measured at 20, 40, 60 and 80 s using the in vivo influx technique as described above (Fig. 3). Uptake of ^{14}C was linear with time to 80 s. In all subsequent studies, influx was measured within the first 60 s of exposure of intestine to test solutions (usually 45 s). The fact that tissue uptake data (Fig. 3) extrapolate near the origin, suggests that binding of the amino acid to the luminal surface does not add significantly to the measurement of influx.

Accurate measurement of amino acid influx depends upon a reliable estimate of the extracellular volume of the test solution which remains adherent to the mucosal surface of the tissue after washing with mannitol. In preliminary experiments [^{3}H]inulin was assayed as a marker of this space since it does not permeate the intestinal cell. As shown in Table I, inulin spaces of both the jejunal and ileal segments were very small in vivo, and an extracellular marker of lower molecular weight was thought to be needed for a better estimate of this space in vivo. We evaluated [^{3}H]mannitol since it distributes into a larger space, and might be a better discriminator of the finer changes of the extracellular fluid that adheres to the mucosal surface of the absorbing epithelium. In Table I, the mannitol spaces, unlike inulin spaces, were larger in the jejunum than in the ileum, a finding that agrees with estimates of the extracellular spaces based on structural differences between the segments. The villi of the jejunum are longer and the intervillous spaces are thus larger than those of the ileum [21–23]. Accordingly, in all studies mannitol

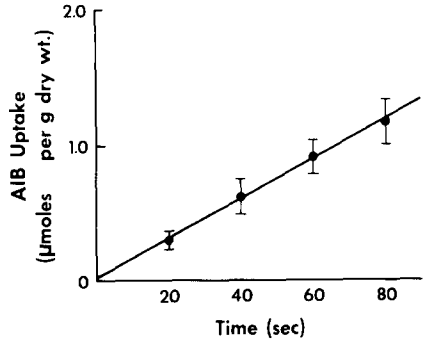

Fig. 3. The time course of uptake of α-amino[^{14}C]isobutyric acid (AIB) by rat jejunum segments. Each point is the mean ± S.E. fo eight determinations. The concentration of α-aminoisobutyric acid was 2 mM.

TABLE I

MUCOSAL INULIN AND MANNITOL SPACES AS DETERMINED IN INFLUX STUDIES OF COMPARABLE SEGMENTS OF RAT INTESTINE

Values are means ± S.E. of observations in 10 rats and are expressed as μl/100 mg dry weight. Four segments, two jejunal-ileal pair was studied in vivo, the other pair from adjacent sites was studied in vitro. The significance values in parenthesis after ileal data columns represent comparisons between jejunal and ileal segments, those below the column are in vivo and in vitro comparisons. The test solution in all the studies was perfused at the rate of 5 ml/min. Significance was measured using Student's *t*-test. (n.s., not significant).

	Jejunal space	Ileal space
[^{3}H]inulin		
In vivo	2.62 ± 0.23	2.31 ± 0.19 (n.s.)
In vitro	6.04 ± 0.72	5.00 ± 0.48 (n.s.)
Significance	$P < 0.001$	$P < 0.001$
[^{3}H]mannitol		
In vivo	7.48 ± 0.77	5.78 ± 0.46 ($P < 0.05$)
In vitro	22.82 ± 0.98	14.32 ± 0.83 ($P < 0.01$)
Significance	$P < 0.001$	$P < 0.001$

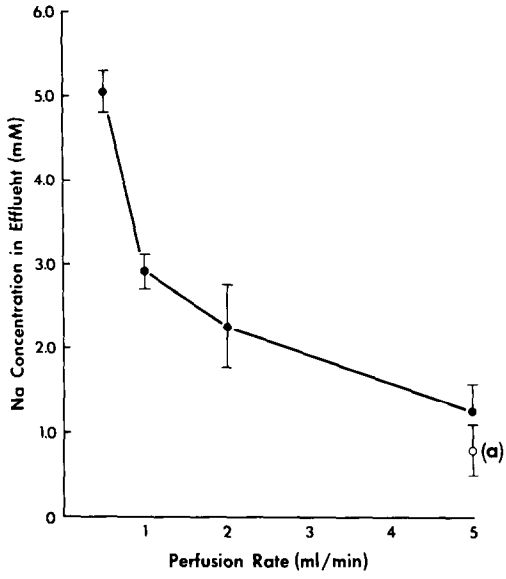

Fig. 4. The appearance of Na^+ in Na-free choline Ringer when perfused at varying rates into rat jejunal segments. Results are means ± S.E. of eight determinations. The value denoted (a) represents the Na^+ concentration where air was simultaneously perfused with the Ringer solution.

was used as an extracellular marker. A small amount of mannitol may be transported into the intestinal cells. This was considered to be insignificant since mannitol is transported into the intestinal cells by a diffusional process that very likely permits minimal accumulation during the 60-s period of experimentation. Also, Antonioli et al. [24] perfused rat intestine for 16 min with labelled mannitol and found that the radioactivity in the effluents did not change appreciably from that in the perfusion solution and the mannitol reaching the tissues during this time represented only 2% of the tissue water space.

Results

Effect of Na on amino acid influx in vivo

It is not possible to perfuse the intestine in vivo with a Na-free luminal solution. When the intestine is perfused with solutions devoid of Na^+, the cation enters the lumen rapidly. To measure Na entry rate (Fig. 4), a Na-free choline Ringer was perfused at varying rates into jejunal (10–12 cm) segments. Two successive 5 ml volumes were perfused at each given perfusion rate. The first 5 ml was considered a wash solution and discarded, the second was collected for Na determination. The Na^+ concentration in the effluent decreases with the increase in perfusion rate. At a rate of 5 ml/min. The mean Na^+ concentration was only 1.25 mM. This concentration was even lower though statistically not significantly when air was perfused simultaneously with the choline Ringer.

To test the effect of substituting other ions for Na^+ on α-aminoisobutyric acid influx in vivo, experiments were carried out where the intestinal segments were preincubated and tested with either normal sodium Ringer or with three Na-free media (Table II). The Na-free media were obtained by substituting the NaCl of normal Ringer with either choline chloride (choline Ringer), lithium chloride (lithium Ringer) or potassium chloride (potassium Ringer). Influx was significantly reduced by the absence of Na^+ and the presence of the three Na^+ substituents. The reduction was observed at the three regional sites of the intestine, but was most remarkable in the ileum. Of the three Na substituents, potassium substitution produced the greatest inhibition in α-aminoisobutyric acid influx.

TABLE II

EFFECT OF SODIUM SUBSTITUTION ON IN VIVO α-AMINOISOBUTYRIC ACID INFLUX

Average values ± S.E. are given for the number of experiments shown in parenthesis. Results are expressed in μmol/h per g dry weight of segment. Initial α-aminoisobutyric acid concentration was 2 mM in all solutions. Preincubation period was 30 min.

Preincubation and test solution	Jejunum	Mid-intesine	Ileum
Sodium Ringer	32.03 ± 1.31 (21)	37.07 ± 1.40 (23)	17.73 ± 1.27 (21)
Choline Ringer	16.33 ± 1.21 [a] (19)	16.60 ± 1.53 [a] (20)	6.04 ± 1.13 [a] (20)
Lithium Ringer	15.47 ± 1.19 [a] (9)	22.67 ± 1.51 [a] (8)	6.13 ± 0.53 [a] (9)
Potassium Ringer	12.74 ± 0.73 [b] (14)	12.40 ± 1.07 [b] (16)	5.13 ± 0.87 [b] (16)

[a] $P < 0.01$.

[b] $P < 0.001$ as compared to control fluxes in normal Ringer solution (Student's t-test).

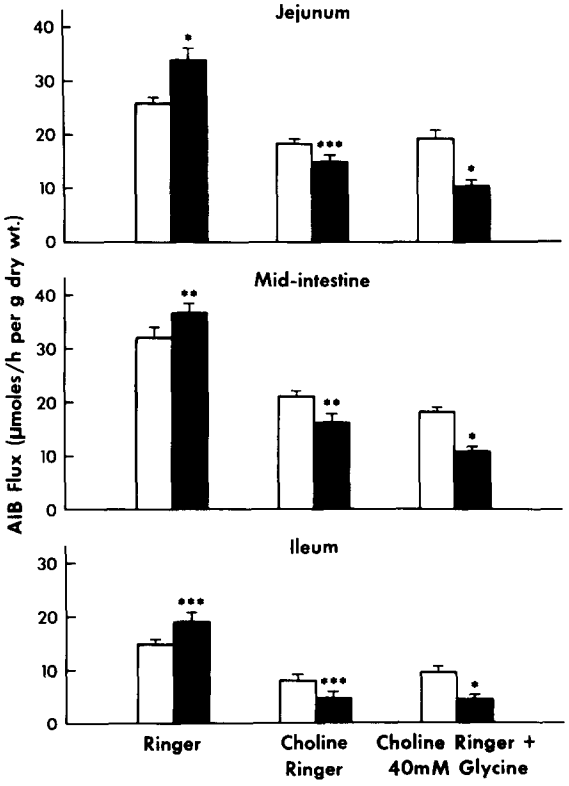

Fig. 5. Comparison between in vivo influx (■) and net absorption (□) of α-aminoisobutyric acid (AIB) (2 mM) across three regional sites of the rat intestine. Net absorption (n = 8) was measured simultaneously in three 30 cm segments: jejunal, 30 cm caudad to the ligament of Treitz; ileal, 30 cm orad to the ileocecal value; and mid-intestinal, a 30–40 cm segment in between the other two. Influx (n = 10) was measured in shorter segments (6–8 cm) at similar sites in another group of rats. Results are means ± S.E. P values are comparisons between net absorption and influx values. * $P < 0.001$; ** $P < 0.01$; *** $P < 0.05$.

Comparison between in vivo influx and net flux measurements

In vivo influx and net absorption of α-aminoisobutyric acid were measured under three experimental conditions: control, i.e., the normal sodium Ringer solution; Na-free conditions using choline Ringer; and choline Ringer containing glycine, a competitive inhibitor of α-aminoisobutyric acid transport. Influx and net absorption were tested across the jejunum, mid-intestine and ileum in two different groups of rats (Fig. 5). Under control conditions, α-aminoisobutyric acid influx was always larger than net absorption and the magnitudes of the two fluxes correlated fairly closely in the three regional sites of the intestine. When choline Ringer was used, both fluxes were reduced but influx was decreased more than net absorption, due to the lower concentrations of Na^+ in the influx than net absorption experiments. Less Na^+ enters the intestinal lumen at the higher perfusion rates that are utilized in the influx experiments (5 ml/min) in contrast to the perfusion rate of 0.5 ml/min used in net absorption experiments (see Fig. 4). The presence of glycine in the perfusates inhibits α-amino-

isobutyric acid influx more than it does net flux (Fig. 5).

Comparison of kinetic constants in different intestinal transport studies

To compare the in vivo influx measurement with other transport techniques, kinetic studies were carried out under four conditions that are summarized in Fig. 6. Because of difficulties in demonstrating rate-limiting kinetics with α-aminoisobutyric acid [25,26], L-leucine was used as a representative amino acid in all those studies.

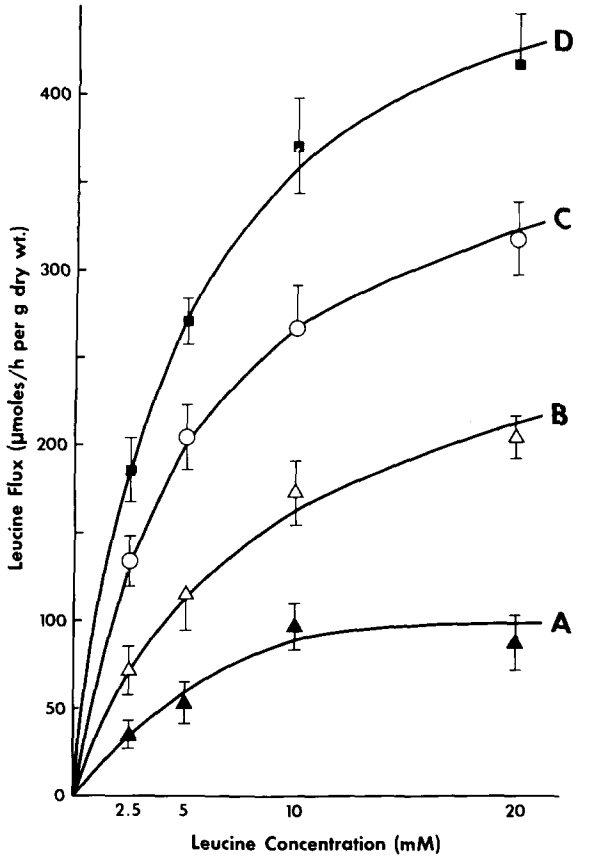

Fig. 6. Comparison of in vivo influx (A,B,C) and in vitro influx (D) of leucine across the rat jejunum. In A, influx was measured by perfusing the text solution at a rate of 0.5 ml/min, in B at 5 ml/min and in C by perfusing a mixture of air and test solutions at 5 ml/min. The perfusing system in D was similar to C but the segment was tested in vitro. Each point represents mean ± S.E. of 15 animals. The curves were drawn using the kinetic constant as determined in Fig. 7.

Using four leucine concentrations, influx was measured in vivo (Fig. 6A, B, C) and in vitro (Fig. 6D). Perfusion conditions were varied in the three in vivo influx experiments; the perfusion rate of the test solution was 0.5 ml/min for A, 5 ml/min for B, and 5 ml/min air for C. Influx in vitro (curve D) was measured under the same perfusion conditions as C. All experiments were done on upper jejunal segments measuring about 6–8 cm in length. The experiments were designed so that the different conditions and substrate concentrations constituted random blocks with different animals as the replicates. Flux rates plotted against the substrate concentrations are shown in Fig. 6. Linearization of the data with line-fitting by regression analysis, was done by using a double-reciprocal plot (Fig. 7). The kinetic constants

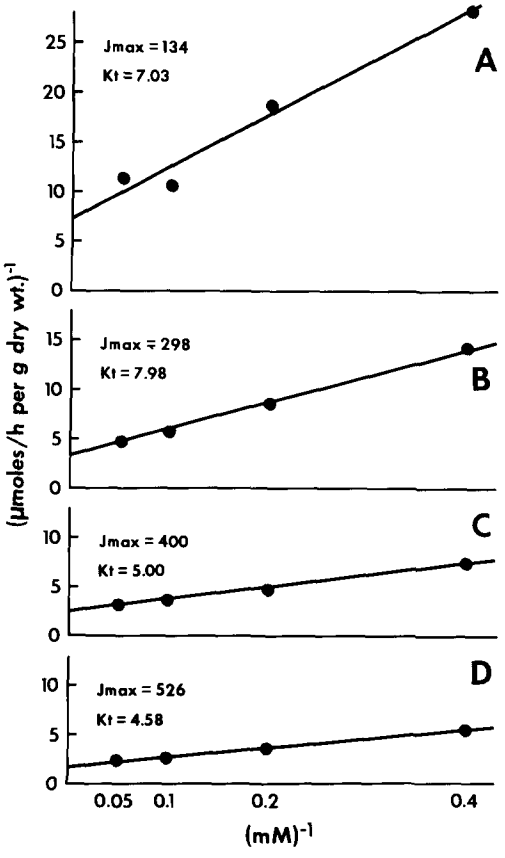

Fig. 7. Double-reciprocal plots of data in Fig. 6. The lines were fitted by regression analysis (least-squares fit) to derive the constants.

determined in Fig. 7 were used for plotting the curves shown in Fig. 6.

When influx is measured during slow perfusion (condition A) the Lineweaver-Burk plot in Fig. 7 shows a tendency to be curvilinear at the lower reciprocal values and the intercept of the plotted line is therefore inaccurate and cannot be used with reliability in calculating the J_{max} and K_t of this curve. The effect of stirring of the test solution during the influx determination is demonstrated best by considering the curves B and C (Fig. 7). The difference between these two conditions is that air is perfused with the test solution in C but not in B. The leucine influx rate was increased by the perfusion of air. This increase was observed in all the four substrate concentrations studies. As suggested by Winne [20] air-fluid perfusion reduces the thickness of the mucosal unstirred layer which may account for the enhanced influx rates and the reduction in K_t in conditions C as compared to B.

There are also some differences between the saturation curves C and D. Influx in these two conditions was measured using a similar perfusion technique but C was in vivo while D is in vitro. J_{max} and K_t values of C approached those of D but some, albeit small, differences seem to exist between the two conditions. One reason for this difference may relate to a possible increase in permeability of the intestinal epithelium in the in vitro condition. Evidence in favor of such an explanation is the fact that the mannitol space (see Table I) was found to be larger in vitro than in vivo. Although the space is routinely accounted for in the calculation of the leucine influx, the differences however between the molecular weights of the leucine and mannitol molecules and their permeation into the extracellular space become more critical when the space is large such as in vitro. Mannitol being a larger molecule than leucine, measures a smaller extracellular leucine space, which could then result in an overestimate of the intracellular leucine uptake. Such an error may not be of importance when the extracellular space is small but becomes clearly significant when the space is larger. It is possible therefore that the data in D may for that reason represent an overestimate of leucine influx.

Discussion

The absorption of amino acid across the intestinal epithelium involves transport across at least two membrane barriers, the mucosal brush border and the basolateral membranes of the intestinal cell. The transport properties of these barriers have been fairly well characterized in several direct in vitro studies of the isolated intestine [18,3,25–27]. There is however very little information about the function of these barriers in the intact in vivo condition. Since differences have been observed between net absorption of amino acids in vivo as compared to in vitro, this study was undertaken to test the nature of these differences and to determine whether dissimilarities exist between the unidirectional influx of amino acids across the mucosal border in vivo and in vitro. The results indicate that the in vivo influx process is basically similar to that in vitro and the observed differences in vivo are mainly related to variables that are inherent to the in vivo condition.

Na-dependence of amino acid absorption has been difficult to demonstrate in the classical in vivo perfusion studies [8,9] due to the rapid extrusion of Na^+ into intestinal perfusates that are devoid of Na^+. In the present studies the in vivo effect of sodium on transport was demonstrated by measuring influx. The Na-free media were perfused at a sufficiently rapid rate to maintain a low intraluminal Na^+ concentration (Fig. 4). Although the concentration of the luminal bulk phase may not represent the concentration in the nonstirred water layer at the brush border, our demonstration of a Na^+ effect on influx is strong evidence that extruded Na^+ in that region is reduced by rapid perfusion. We found that α-aminoisobutyric acid influx in vivo was markedly inhibited by the substitution of sodium by choline, lithium or potassium. The influx decreased by more than 50%, a value that is comparable with similar influx measurements across the rabbit ileum in vitro [18]. Also, in agreement with in vitro studies [18] is the finding that the replacement of sodium by potassium produced the greatest inhibition of α-aminoisobutyric acid influx. The potassium effect is though to be due to an added inhibitory effect of potassium on amino acid transport other than what can be attributed to Na^+ replacement. Finally, the time period of the influx measurement was brief in comparison with classical

perfusion experiments, and this also was of advantage since it avoids excessive interference with the intracellular ionic composition of the mucosal epithelial cells.

The in vivo α-aminoisobutyric acid influx measurement is a direct estimate of the entry of the amino acid into the cell and adds to the understanding of the mechanism of absorption of amino acids in the intact in vivo intestine. When influx was compared with net absorption, such as in the control conditions in Fig. 5 (Ringer), it was always higher than net absorption. It tended, like net absorption, to vary with the different regional sites of the intestine, being highest in the mid-intestine and the lowest in the ileum. Influx and net absorption were measured on two adjacent but separate segments of intestine, and these measurements can be used to estimate the efflux of amino acids from the in vivo intestine. Efflux is calculated by subtracting net absorption from influx. In Fig. 5 (Ringer), α-aminoisobutyric acid efflux is 7.8, 4.2 and 3.1 μmol/h per g dry weight in the jejunum, mid-intestine and ileum, respectively. The aboral decrease in amino acid efflux is not unexpected since the permeability of the intestine is known to decrease aborally.

Saturation kinetics can be estimated by the in vivo influx measurement technique described. As shown in Figs. 6 and 7, factors that normally complicate these measurements in vivo [16] are, partly at least, controlled by this method. When the kinetic constants are measured using classical in vivo perfusion techniques, a large unstirred mucosal layer exists and it overestimates the affinity constant [28,29]. In Fig. 7, a gradual decrease in K_t was observed with the increase in stirring conditions. This agrees with what has been described to occur in other in vitro transport systems [28–32]. When the nonstirred water layer was reduced by perfusing air with the Ringer solution, leucine influx was generally increased and K_t decreased to values approaching those that are observed in vitro. Although the kinetic measurements in vivo may not approximate the conditions prevailing during physiological absorption and the measurements in vitro may not reflect the intrinsic properties of the leucine transport system (relatively limited stirring of uneverted intestine) yet these measurements coincide, suggesting a basic similarity between in vivo and in vitro leucine influx. With increased stirring there was also an increase in J_{max} from condition A through B and C (Fig. 7). This may be due to an increase in the absorptive surface area due to distention during rapid perfusion. We have reason to believe that this is an unlikely explanation since our measurements of the external diameters of the intestinal segments were found to be unaffected by changes in perfusion rate. Also, the perfusion rates were similar in conditions C and D but J_{max} was different. Other factors [31] may account for the increase in J_{max} with increased stirring, such as an increase in passive permeability of leucine or that J_{max} is underestimated during slow perfusion due to the effect of a high resistance diffusion pathway [31, 32].

Using the in vivo influx method also provided a way for comparing in vivo and in vitro leucine transport on adjacent intestinal segements of the same animal. A significant finding that was apparent from this comparison is the presence of an increased mucosal mannitol space and a relatively larger leucine influx rate in vitro as compared to in vivo. These findings suggest the presence of an increased permeability of the intestine in vitro. Further studies are, however, needed to test this possibility.

Acknowledgement

The authors greatly acknowledge the technical assistance of Mrs. Tatjana Tomicic and the secretarial help of Mrs. Michelle Toucey in preparation of the manuscript. Supported by the Medical Research Service of the Veterans Administration; VA Medical Centrer, Newington, CT and VA Medical Center, Iowa City, IA.

References

1 Agar, W.T., Hird, F.J.R. and Sidhu, G.S. (1954) Biochim. Biophys. Acta 14, 80–84
2 Wiseman, G. (1953) J. Physiol. Lond. 120, 63–72
3 Curran, P.F., Schultz, S.G., Chez, R.A. and Fuisz, R.E. (1967) J. Gen. Physiol. 50, 1261–1286
4 Csaky, T.Z. (1961) Am. J. Physiol. 201, 999–1001
5 Newey, H., Rampone, A.J. and Smyth, D.H. (1970) J. Physiol. Lond. 211, 539–549
6 Fleshler, B., Butt, J.H. and Wismar, J.D. (1966) J. Clin. Invest. 45, 1433–1441
7 Annegers, J.H. (1964) Proc. Soc. Exp. Biol. Med. 116, 933–938

8 Fleshler, B. and Nelson, R.A. (1970) Gut 11, 240–244
9 Adibi, S.A. (1970) J. Appl. Physiol. 28, 753–757
10 McConnell, K.P. and Cho, G.J. (1967) Am. J. Physiol. 213, 150–156
11 Ramaswany, K. and Radhakrishnan (1966) Ind. J. Biochem. 3, 138–143
12 Samiy, A.H. and Spencer, R.P. (1961) Am. J. Physiol. 200, 505–507
13 Schedl, H.P., Pierce, C.E., Rider, A. and Clifton, J.A. (1968) J. Clin. Invest. 47, 417–425
14 Boyd, C.A.Z., Cheesman, C. and Parsons, D. (1975) J. Physiol. Lond. 250, 409–429
15 Schedl, H.P. and Wilson, H.O. (1971) Am. J. Physiol. 220, 1739–1745
16 Curran, P.F. (1972) Arch. Int. Med. 129, 258–269
17 Hajjar, J.J., Khuri, R.N. and Bikhazi, A.B. (1975) Am. J. Physiol. 229, 518–523
18 Schultz, S.G., Curran, P.F., Fuisz, R.E. and Chez, R.A. (1967) J. Gen. Physiol. 50, 1241–1260
19 Fisher, R.B. and Gardner, M.L.G. (1974) J. Physiol. Lond. 241, 211–234
20 Winne, D. (1978) Naunym-Schmeideberg's Arch. Pharmacol. 304, 175–181
21 Warren, R. (1939) Anat. Rec. 75, 427–437
22 Wood, H.O. (1944) J. Anat. 78, 103–105
23 Fisher, R.B. and Parson, D.S. (1950) J. Anat. 84, 272–282
24 Antonioli, J.A., Joseph, C. and Robinson, J.W.L. (1978) Biochem. Biophys. Acta 512, 172–191
25 Hajjar, J.J., Lamont, A.S. and Curran, P.F. (1970) J. Chem. Physiol. 55, 277–296
26 Hajjar, J.J., Khuri, R.N. and Curran, P.F. (1972) J. Gen. Physiol. 60, 720–734
27 Nassar, C.F., Khuri, R.N. and Hajjar, J.J. (1980) Biochim. Biophys. Acta 595, 121–125
28 Dietschy, J.M., Sallee, V.L. and Wilson, F.A. (1971) Gastroenterology 61, 932–943
29 Winne, D. (1973) Biochim. Biophys. Acta 298, 27–31
30 Wilson, F.A. and Dietschy, J.M. (1974) Biochim. Biophys. Acta 363, 112–126
31 Thomson, A.B.R. and Dietschy, J.M. (1977) J. Theor. Biol. 64, 277–294
32 Thomson, A.B.R. and Dietschy, J.M. (1980) J. Membrane Biol. 54, 211–229