BBA 75563

PATTERNS OF NEUTRAL AMINO ACID UPTAKE ALONG RAT SMALL INTESTINE

R. DAVID BAKER AND MARY JEAN GEORGE
Department of Physiology, University of Texas Medical Branch, Galveston, Texas (U.S.A.)
(Received August 3rd, 1970)

SUMMARY

Uptake of seven neutral amino acids by rat small intestine during 60 min incubations is presented as a function of position along intestine. There was considerable variation from one amino acid to another in the shape of the uptake curves. The two extremes were the uptake patterns for L-methionine and betaine. These two patterns were sufficiently different from each other to provide new and independent evidence for the existence of two systems for the transport of neutral amino acids. These transport systems are here called N_1 and N_2; they are apparently distributed somewhat differently along rat intestine.

Uptake patterns for the other amino acids studied (L-leucine, L-alanine, L-proline, glycine and α-aminoisobutyric acid) were between those for methionine and betaine. This situation would be expected if leucine, alanine, proline, glycine and α-aminoisobutyric acid were transported by both N_1 and N_2.

By competitively excluding proline from N_1 by adding methionine, it was possible to shift the uptake pattern of proline toward that of betaine. This result adds further support to the idea that proline is transported by both N_1 and N_2. The converse experiment — shifting the uptake pattern of proline toward that of methionine by adding betaine — was not successful, probably because betaine had a stimulatory effect on proline uptake.

INTRODUCTION

The existence of three major transport systems for L-amino acids in mammalian small intestine is generally accepted[1–3]. The principal role of one of these is the absorption of basic amino acids and probably cystine. The other two systems transport principally the neutral amino acids. In this report these two neutral amino acid transport systems will be called N_1 and N_2. The N_1 system has high affinity for methionine and other neutral amino acids with non-polar side chains, while the N_2 system has more affinity for the imino acids, proline and hydroxyproline, and the N-substituted glycine derivatives such as betaine and sarcosine[3]. Proline, hydroxyproline, glycine, alanine, and leucine probably share both the N_1 and N_2 systems[4–6]. Active transport of acidic amino acids by mammalian small intestine has not been demonstrated.

The evidence leading to this classification of transport systems is derived mainly from two types of investigation: (1) studies of competitive inhibition among various

transported amino acids[4–13], and (2) studies of inherited disorders of amino acid transport [14,55]. The present investigation is an attempt to provide another approach to the functional classification of amino acids. This approach is based on the possibility that different amino acid transport systems might have different longitudinal distributions of activity along the small intestine. If this is the case, then the pattern along the intestine for transport ability will depend upon which amino acid is being tested.

The data presented below show that different transport patterns are observed for several neutral amino acids and help us to rank these amino acids according to their relative participation in the N_1 or N_2 systems. By competitive exclusion of proline from the N_1 system it has been possible to shift its uptake pattern toward that of the N_2 system.

MATERIALS AND METHODS

The technique was an *in vitro* tissue accumulation method[16] similar to those used by SPENCER *et al.*[17] and NEAME[18]. Each rat (male, Holtzman, 165–385 g) was deprived of food for 20–24 h, then anesthetized with ether and its small intestine rinsed with ice-cold Krebs–Ringer bicarbonate solution[19]. The entire small intestine was removed by cutting at the radix; the mesentery was stripped off, and the small intestine was slit open along its entire length. Spontaneous eversion always occurred upon slitting. Ligatures were placed on each end of the gut to facilitate handling. The gut was then transferred to the incubation medium. The time elapsing from interruption of blood supply until transfer to incubation medium was 3–4 min. During this time the intestine was kept immersed in ice-cold Krebs-Ringer bicarbonate gassed with 5 % CO_2 in O_2.

The incubation medium was 50 ml of Krebs–Ringer bicarbonate solution containing D-glucose (11.1 mM) and a ^{14}C-labeled amino acid, usually at a concentration of 10.0 mM (0.1 μc/ml). Incubation was carried out at 37° for 1 h in a 250 ml Erlenmeyer flask. The flask was shaken in a Dubnoff incubator at 60–70 oscillations per min and its contents were continuously gassed with humidified 5 % CO_2 in O_2. After incubation the intestine was removed, quickly rinsed in fresh Krebs Ringer bicarbonate, blotted on filter paper, and rapidly divided into 8 segments of nearly equal length. Each segment was placed in a tared tube and weighed.

Free amino acids were extracted from each segment by soaking at 0–3° in 10.0 ml of 70 % ethanol for at least 48 h. Twenty-one tests with 7 different amino acids showed this procedure to recover from 92 % to 101 % (mean = 97 %) of the radioactivity that could be recovered by homogenization. After centrifugation of the extracts 0.5 ml samples of supernatant were dried on aluminum planchets in duplicate and counted in a thin-window, glas-flow, Geiger–Müller counter. Since the ethanol precipitated most of the proteins, the dried samples were thin enough that no correction was necessary for self-absorption. Samples (1.0 ml) of the incubation medium were taken both before and after the incubation period. These samples were diluted with 10.0 ml of 70 % ethanol and 0.5 ml aliquots were dried on planchets and counted. The sample of incubation medium taken before adding the intestine served as a standard from which the μmoles of amino acid in other samples were calculated from their radioactivity. Unfortunately, the rats had a wide range of body weights, undoubtedly contributing to the variability in results.[20]

Uptake of the following ^{14}C-labeled amino acids was measured: L-methionine, L-leucine, L-alanine, L-proline, glycine, α-aminoisobutyric acid, and the betaine of glycine. All labeled amino acids were obtained from New England Nuclear Corp. Methionine and betaine were methyl labeled; α-aminoisobutyric acid was labeled at carbon 1; all others were uniformly labeled. Unlabeled methionine and glycine were obtained from Nutritional Biochemicals Corp.; all other unlabeled amino acids were from Calbiochem. All optically active amino acids were in the L-form.

Results were expressed as μmoles of amino acid accumulated per ml of tissue water. The amount of tissue water was determined in preliminary experiments by drying intestines to constant weight at 120° after 1 h incubation with 11.1 mM D-glucose and 10.0 mM methionine. Tissue water averaged 86 % of total wet weight. This value was assumed to be true with other amino acids as well and was used in all calculations. The volume of tissue water per g wet weight was found to be essentially the same at all eight levels of intestine studied (also see ref. 21).

Chromatography. To test for radiochemical purity, samples of incubation media and intestinal homogenates were chromatographed on Whatman No. 1 paper strips (descending) using phenol–water–8-hydroxyquinoline (100:39:0.04, w/v/%) as solvent. After development the strips were either sprayed with ninhydrin or were scanned for location of radioactivity. Samples of incubation media were diluted 1:11 with 70 % ethanol before spotting. Intestinal homogenates were prepared by blending the entire small intestine, following the usual 1 h incubation, with 100 ml of 70 % ethanol.

Statistical analysis. We tested the following hypothesis with a standard groups-by-treatments, repeated-measurement analysis of variance: the ratio between uptake of one amino acid and that of another does not depend on the level of intestine. This null hypothesis was tested on all possible pairings of the seven amino acids studied. When it can be rejected we conclude that the two amino acids in question exhibit significantly different uptake patterns along the small intestine. The null hypothesis was rejected if its change of being correct was 5.0 % or less.

RESULTS

Preliminary studies

Test for radiochemical purity. Each amino acid tested was radio-chemically pure as indicated by paper chromatography of the initial incubation media (samples taken prior to adding intestine). In each case all radioactivity was located in a single spot with R_F corresponding to the ninhydrin spot. The same was true with the final incubation media (samples taken after incubation of intestine) and with intestinal homogenates, except for alanine. Therefore, in experiments with methionine, leucine, proline, glycine, α-aminoisobutyric acid, and betaine accumulation of radioactivity can be considered equal to accumulation of amino acid. Alanine was metabolized; a second spot containing about 20–25 % of the total radioactivity appeared on the chromatograms of final incubation medium and tissue homogenate. The chemical identity of this metabolite was not determined; it was not ninhydrin-positive and it had an R_F of 0.35 (compared to 0.57 for alanine). Consequently, interpretation of the alanine data must be regarded with some scepticism.

The relative metabolic inertness of these amino acids (except alanine) during incubation with rat intestine has been reported previously[7,9]. Metabolic change of

L-alanine during incubation with rat intestine has also been reported[9,22]. This apparently does not occur to as large an extent with hamster[23,24] or rabbit[25] intestine, and was not found to this degree by FINCH AND HIRD[7] in rat intestine.

Time course of amino acid uptake. Several experiments were performed with glycine and with methionine in which serial samples of incubation medium were taken at 10 min intervals. Uptake was essentially complete by 30 min; there was no appreciable difference between the concentration in the medium at 30 min and that at 60 min for either of these amino acids. We conclude that at 60 min, the time chosen for subsequent experiments, these amino acids were distributed in approximately a steady state between tissue and medium. We assume the same to be true for the other amino acids studied because of the above results with methionine and glycine, and because similar results have been obtained with a variety of amino acids by several investigators[7,16,26–28].

Patterns for uptake of neutral amino acids

The principal results of this study are presented in Table I. The segments are numbered 1 through 8. Segment 1 corresponds roughly to the duodenum, Segments 2–4 to jejunum, and Segments 5–8 to ileum. All amino acids studied were actively transported as indicated by tissue:medium concentration ratios greater than 1.0.

TABLE I

UPTAKE OF SEVEN NEUTRAL L-AMINO ACIDS BY EIGHT LEVELS OF RAT SMALL INTESTINE

Uptake is expressed as μmoles of amino acid accumulated during the 1 h incubation period per ml of tissue water $\pm$ the standard error. Values in parentheses are mean final tissue:medium concentration ratios.

Amino acid *	*Number of rats*	*Segment*		
		1	*2*	*3*
Methionine	9	10.1 $\pm$ 0.23 (1.22)	9.8 $\pm$ 0.22 (1.17)	10.3 $\pm$ 0.23 (1.23)
Leucine	6	11.3 $\pm$ 0.38 (1.50)	11.2 $\pm$ 0.39 (1.49)	11.8 $\pm$ 0.45 (1.57)
Alanine	6	14.4 $\pm$ 0.44 (2.15)	15.5 $\pm$ 0.25 (2.33)	16.6 $\pm$ 0.30 (2.48)
Proline	10	10.2 $\pm$ 0.99 (1.32)	11.8 $\pm$ 0.60 (1.55)	13.6 $\pm$ 0.58 (1.79)
Glycine	6	9.3 $\pm$ 1.01 (1.29)	12.3 $\pm$ 1.09 (1.70)	13.1 $\pm$ 1.01 (1.81)
α-Aminoisobutyric acid	6	5.9 $\pm$ 0.37 (0.72)	8.4 $\pm$ 0.58 (1.04)	9.8 $\pm$ 0.55 (1.22)
Betaine	6	6.4 $\pm$ 0.61 (0.66)	12.1 $\pm$ 0.87 (1.23)	13.9 $\pm$ 0.91 (1.42)
Proline** (+ methionine)	6	5.2 $\pm$ 0.51 (0.61)	8.7 $\pm$ 0.75 (1.01)	9.9 $\pm$ 0.73 (1.15)
Methionine (1.0 mM)	3	2.8 $\pm$ 0.31 (6.09)	2.8 $\pm$ 0.11 (6.11)	2.9 $\pm$ 0.17 (6.30)
Proline (1.0 mM)	4	2.0 $\pm$ 0.29 (3.41)	2.7 $\pm$ 0.28 (4.65)	2.7 $\pm$ 0.23 (4.60)

* The initial concentration of amino acid in the incubation medium was 10.0 mM except where indicated.

** Proline uptake was measured. Methionine was present as an inhibitor at an inititial concentration of 10.0 mM.

However, in the case of α-amino isobutyric acid and betaine, concentrative uptake occurred only in the mid-portions of small intestine. Ratios were higher when the concentration in initial medium was only 1.0 mM than when it was 10.0 mM for both methionine and proline. Similar observations have previously been made for various amino acids (*e.g.* see refs. 24, 26, 29, 30). At a concentration of 1.0 mM, uptake of methionine was greater than that of proline, but at 10.0 mM this order was reversed, confirming previous theoretical[31] and experimental[7, 24] work. At most levels of intestine the relative uptakes of alanine, leucine, glycine and α-aminoisobutyric acid were in the same order as found by MATTHEWS AND LASTER[24] for transport across hamster ileum from an initial concentration of 10.0 mM (*i.e.* alanine > leucine > glycine > α-aminoisobutyric acid).

Patterns along the intestine for uptake of all seven neutral amino acids were similar in that the mid-intestine transported more of each amino acid than did either end. However, certain differences in the shape of these patterns were apparent. In fact, in some cases uptake of one amino acid was greater than that of another at one level of intestine but less at another level. For example, uptake of betaine in Segment 4 was greater than that of any other amino acid except alanine; however, in Segment 8 uptake of betaine was less than that of all other amino acids studied. When these

					Mean
4	*5*	*6*	*7*	*8*	
11.6 ± 0.44	12.8 ± 0.47	13.1 ± 0.34	12.1 ± 0.70	12.5 ± 0.67	11.5 ± 0.31
(1.39)	(1.53)	(1.57)	(1.46)	(1.50)	(1.38)
14.6 ± 0.55	16.0 ± 0.51	15.0 ± 0.60	13.3 ± 0.80	13.6 ± 0.47	13.4 ± 0.39
(1.95)	(2.13)	(1.99)	(1.78)	(1.82)	(1.78)
19.6 ± 0.63	20.1 ± 0.53	19.1 ± 0.46	15.9 ± 0.91	15.8 ± 0.45	17.2 ± 0.21
(2.94)	(3.01)	(2.86)	(2.39)	(2.37)	(2.57)
14.6 ± 0.76	14.7 ± 0.69	13.9 ± 0.61	11.7 ± 0.67	12.1 ± 0.94	12.8 ± 0.54
(1.91)	(1.93)	(1.82)	(1.54)	(1.59)	(1.68)
15.0 ± 1.24	15.7 ± 1.32	13.7 ± 1.44	10.8 ± 0.77	10.3 ± 0.64	12.5 ± 0.99
(2.07)	(2.17)	(1.90)	(1.49)	(1.42)	(1.73)
10.6 ± 0.37	10.8 ± 0.44	8.4 ± 0.39	6.0 ± 0.31	6.1 ± 0.23	8.3 ± 0.30
(1.31)	(1.34)	(1.04)	(0.74)	(0.76)	(1.02)
15.6 ± 0.62	14.4 ± 0.77	10.3 ± 0.67	5.0 ± 0.62	3.8 ± 0.44	10.2 ± 0.42
(1.59)	(1.47)	(1.05)	(0.51)	(0.39)	(1.04)
10.5 ± 0.76	9.2 ± 0.49	6.3 ± 0.45	3.8 ± 0.27	3.3 ± 0.20	7.1 ± 0.43
(1.22)	(1.07)	(0.73)	(0.44)	(0.38)	(0.83)
3.2 ± 0.13	3.7 ± 0.38	3.6 ± 0.54	4.2 ± 0.28	3.8 ± 0.39	3.4 ± 0.22
(7.06)	(7.98)	(7.83)	(9.11)	(8.15)	(7.33)
2.9 ± 0.23	2.9 ± 0.36	2.8 ± 0.34	2.3 ± 0.14	1.7 ± 0.25	2.5 ± 0.24
(5.03)	(5.07)	(4.81)	(3.95)	(2.95)	(4.31)

patterns were compared to each other by analysis of variance, many statistically significant differences in shape were detected. The pattern for methionine was significantly different from that for each of the other amino acids studied; the same was true for leucine and betaine. Among the other amino acids (alanine, proline, glycine and α-amino isobutyric acid) the only difference in pattern which was statistically significant was that for alanine *versus* α-aminoisobutyric acid.

To facilitate comparison of these patterns, the graphical presentation of Fig. 1 has been adopted. For each amino acid, the uptake by each segment is expressed as per cent of the mean uptake by all eight segments. This conversion does not change the patterns but does move results for all amino acids to about the same vertical position on the graph, making comparison much easier. The uptake patterns which differed the most from each other were those of methionine and betaine. For methionine, maximal uptake occurred in Segment 6; uptake at no segment differed from the mean by more than 15 %. Nearly maximal uptake was maintained throughout the lower half of the small intestine. For betaine, maximal uptake occurred in Segment 4 and was 153 % of the mean, while uptakes at the upper and lower ends of the intestine were quite deficient (63 and 38 % of the mean, respectively). The other amino acids had patterns which were intermediate in shape between those of methionine and betaine. Arranged in order of decreasing similarity to the methionine pattern these amino acids followed the sequence: methionine, leucine, alanine, proline, glycine, α-aminoisobutyric acid and betaine.

Uptake of methionine and of proline were also studied at an initial concentration of 1.0 mM in the incubation medium. The results are compared to those at 10.0 mM in Fig. 2. The pattern for methionine was significantly different from that for proline when concentrations of either 1.0 mM or 10.0 mM were used. No significant difference was detected between the pattern at 1.0 mM and that at 10.0 mM for either of these amino acids. These results tend to increase our confidence in the value of these patterns as a fairly reproducible characteristic for each amino acid.

The difference between the methionine and betaine patterns is considerable; it is

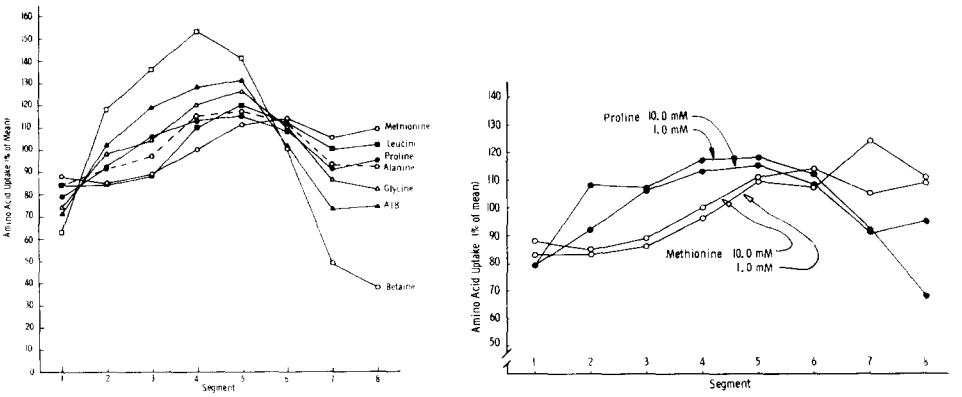

Fig. 1. Uptake patterns for seven neutral amino acids along rat small intestine. For each amino acid the data are expressed as percent of the mean uptake for all eight segments. Each point is the mean from at least six rats. Segment 1 is duodenum, Segment 8 is terminal ileum. AIB, α-aminoisobyturic acid.

Fig. 2. Uptake patterns for proline and methionine at two different initial concentrations.

extreme enough to indicate strongly the participation of methionine and betaine in two different transport systems (N_1 and N_2) which are distributed differently along the intestine. The intermediate shape of the other uptake patterns would be expected if these amino acids were transported by both the N_1 and N_2 systems as competition data have previously indicated[4–6].

Effect of methionine on the uptake pattern for proline

If the above interpretation is correct, then it might be possible to shift the uptake pattern for one of these intermediate amino acids toward either the methionine or betaine pattern by competitively inhibiting its entry into either N_2 or N_1, respectively. An attempt to cause such a shift in pattern has been made with proline.

Proline uptake was measured at an initial concentration of 10.0 mM in the presence of 10.0 mM methionine. At all levels of intestine proline uptake was significantly less (by '*t*' test) in the presence of methionine than in its absence. Averaged for all eight segments of intestine, proline uptake was depressed 44.4 % by equimolar methionine. However, the percentage inhibition was not equal at different sites along the intestine as it would have been if only one transport system were used by proline.

Since degree of inhibition depended on level of intestine, the shape of the uptake curve was altered. Uptake patterns are shown in Fig. 3. For comparison, the patterns for methionine and betaine are also shown. In the presence of methionine the uptake pattern for proline was shifted toward that for betaine, in fact, the two were barely distinguishable. Participation of proline in N_1 was apparently blocked or substantially reduced, revealing residual proline uptake to be distributed like betaine uptake.

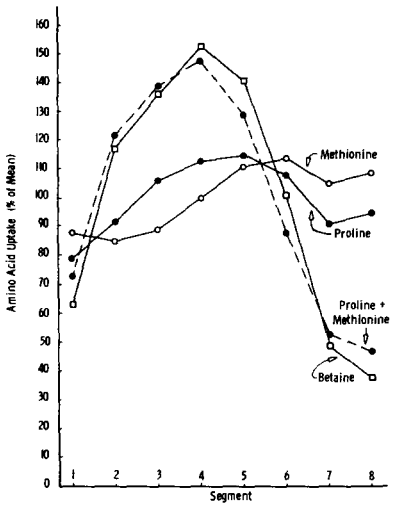

Fig. 3. Shift in the uptake pattern for proline in the presence of equimolar methionine. Uptake patterns for proline, methionine, and betaine are included for comparison. Each point is the mean from all animals studied; n = 9 for methionine, 10 for proline, 6 for betaine, and 6 for proline in the presence of equimolar methionine.

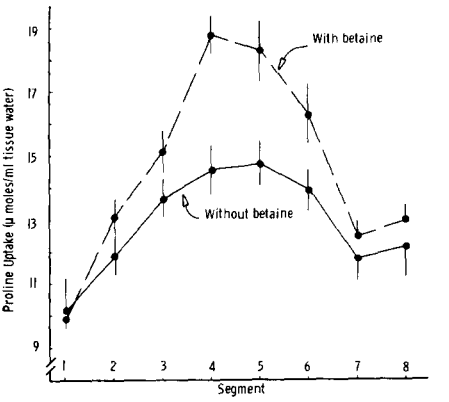

Fig. 4. Uptake of proline in the presence and absence of betaine at equimolar concentration (10.0 mM). The means ± S. E. are shown for seven animals with betaine and ten animals without betaine.

Effect of betaine on uptake of proline

Proline uptake was measured at an initial concentration of 10.0 mM in the presence of 10.0 mM betaine, with the hope of converting the proline pattern into a methionine-like pattern. But, surprisingly, uptake of proline was stimulated by equimolar betaine. Results averaged for all eights segments showed 12 % stimulation. Segments-by-segment data are in Fig. 4. Stimulation was greatest in the region of greatest betaine transport and was absent or slight in those segments (1,7 and 8) having poor betaine uptake.

The ratio of betaine to proline was increased in an effort to achieve inhibition of N_2; proline uptake was measured from an initial concentration of 1.0 mM in the presence of 10.0 mM betaine. When the average for all eight segments of intestine was calculated, there was 29 % inhibition of proline uptake. There was definite inhibition at some levels of intestine as shown in Fig. 5, but in Segment 8, where betaine transport is very poor, inhibition was essentially zero.

There was not much difference in the shapes of the proline uptake curves in Fig. 5. Thus we failed to shift the proline pattern toward the methionine pattern and attributed this failure to the complication of a stimulatory effect.

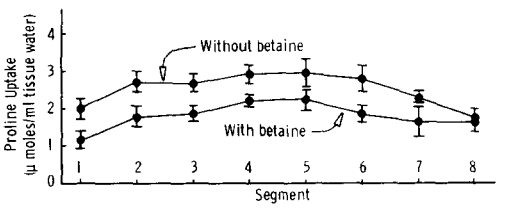

Fig. 5. Uptake of proline in the presence of betaine at 10 times the proline concentration. Proline was present at an initial concentration of 1.0 mM. The means ± S.E. are shown for four animals with betaine and four animals without betaine.

DISCUSSION

Steady state accumulation of an amino acid is a complex function of whatever active and passive amino acid transport processes occur within the tissue, and, of course, does not measure the density of transport sites on any particular membrane. It is influenced by a variety of factors besides the properties of the transport sites themselves, some of which are discussed below. However, under a given set of conditions, steady state accumulation does provide a convenient index of relative overall transport activity and has been used as such in many previous studies *in vitro*. Part of this accumulation is in the epithelial cells[32], but a good deal is apparently located in the subepithelial layers[33]. Subepithelial accumulation represents a balance between transepithelial transport and subepithelial diffusion into the serosal solution. The fact that the longitudinal distribution patterns for methionine and proline uptake were not changed by a ten-fold change in initial concentration (see Fig. 2) indicates that merely changing the rate of transepithelial transport and, therefore, the amount of epithelial and subepithelial accumulation does not change the patterns as long as the same transport system(s) is used.

Uptake of all seven neutral amino acids studied was greater in the midportions of rat small intestine than at either end. Previous mapping experiments utilizing *in*

vitro techniques have usually revealed a similar pattern along both rat and hamster small intestine[9,24,27,29,34–44], although a few conflicting reports are also recorded[11,26,37,45,46]. In two studies using *in vivo* techniques, ability to absorb amino acids declined in an aboral direction along the small intestine[40,47], but in a more recent study *in vivo* adsorption of α-aminoisobutyric acid was maximal in the mid-portions of rat small intestine[48].

Although the general pattern was similar for all amino acids studied, we were able to detect statistically significant differences in shape. These differences have not been detected previously in rat[9,46] or in hamster[2,4] intestine, but GREER AND LAWRENCE[49] have found different sites for maximal transport of neutral an basic amino acids in the posterior intestine of a mollusk.

The two most dissimilar patterns were those for methionine and betaine. Maximum uptake was at different locations (Segment 6 for methionine, Segment 4 for betaine). Segmental changes in betaine uptake were often not accompanied by corresponding changes in methionine uptake. For example, uptake of betaine nearly doubled from Segment 1 to Segment 2, while uptake of methionine did not change appreciably; uptake of betaine decreased by 76 % from Segment 4 to Segment 8, while uptake of methionine increased slightly. We conclude that these two amino acids are not transported by identical transport systems.

Thus, the concept of two transport systems for neutral amino acids, referred to here as N_1 and N_2, is supported by an independent method. This concept was developed mainly on the basis of competition experiments[4–13], and studies on patients with inherited disorders of amino acid transport[14,15]; it has previously been supported by studies on the development of amino acid transport systems in fetal intestine[50,51], by studies on the effect of glucose on amino acid transport under anaerobic conditions[52], and by kinetic data for leucine transport explicable in terms of two parallel transport pathways[53].

The uptake patterns for the seven neutral amino acids formed a gradual transition from one extreme (the methionine pattern) to the other extreme (the betaine pattern). This relationship would be expected if leucine, alanine, proline, glycine and α-aminoisobutyric acid are transported by both N_1 and N_2, as previously indicated by competition experiments[4–6,54], but have different relative affinities for these two systems. The order of similarity to the methionine pattern was leucine > alanine ~ proline > glycine > α-aminoisobutyric acid > betaine. This progression corresponds with that found by MATTHEWS AND LASTER[24] for the affinity of leucine, alanine, glycine and α-aminoisobutyric acid for the transport system in hamster midileum, and the progression from methionine to glycine corresponds with the length of the nonpolar chain. Increasing length of the chain from glycine to methionine apparently favors increasing utilization of N_1, while additional substitutions on the α-carbon atom, as in α-aminoisobutyric acid, or on the amino group, as in betaine, apparently interfere with utilization of N_1.

When proline was blocked from N_1 by methionine, its residual uptake pattern closely resembled that for betaine. This finding strengthens the interpretation that proline is transported by both systems.

An attempt to shift the proline pattern toward the methionine pattern by blocking N_2 with betaine was confounded by a stimulatory effect of betaine on proline transport. Stimulation of the transport of one amino acid by other amino acids has

been observed before in small intestine[28,53,55–58], and has recently been discussed at length by MUNCK AND SCHULTZ[53].

In seeking an explanation for the distributions of transport activity along the intestine, we must consider: (1) distribution of passive permeability properties; (2) distribution of energy supply; (3) distribution of the sensitivity to exogenous factors in the incubation medium, such as glucose and Na^+; and (4) distribution of membrane transport sites (carriers). A pattern of passive permeability would not be likely to have one shape for methionine and another for betaine. The fact that diffusion of L-methionine down its concentration gradient is about equal across all levels of rat small intestine in the presence of 2,4-dinitrophenol[34] also provides some evidence against this explanation, although it cannot be entirely dismissed as a possible contributory factor. The finding of different patterns for different amino acids argues against the suggestion that the distribution of energy supply is the entire explanation for the uptake patterns (although it could be contributory). Furthermore, the patterns for rate of oxygen consumption[59], rate of glycolysis[59], glycose utilization[60], ATP content[61,62], and ATPase activity[61] do not resemble those for amino acid uptake. NUNN AND ELLERT[21] have found the mucosal concentrations of Na^+ and K^+ to be essentially constant along rat small intestine. Therefore, even though Na^+ is involved in amino acid transport by rat intestine[63], its longitudinal distribution does not account for the shape of the amino acid transport patterns. Glucose was included in the incubation medium as a substrate; it probably induced water flow more at some levels of intestine than at others[60], and its effect an amino acid transport was probably not longitudinally uniform[64]. If this influence on the uptake pattern varied from one amino acid to another, it could have been a factor in producing the results.

Since the uptake pattern for proline depends upon the presence or absence of methionine, and since the competitive interaction between amino acids is generally regarded as being located at binding sites on membrane carriers, it follows that the pattern for proline uptake is at least partly dependent upon the distribution of membrane carriers. Specific longitudinal distributions of membrane carriers seem the most likely explanation for specific distributions of transport activity.

These results demonstrate the desirability of using more than one level of intestine in transport studies. Not only absolute transport activity but also relative activity for various amino acids depends upon intestinal level. It is especially important to realize that competitive effects among amino acids depend on which part of the small intestine is studied.

ACKNOWLEDGEMENTS

This work was supported by U.S. Public Health Service Grant No. AM-05778. Mrs. Jo Llu Long assisted with some of the experiments. During the performance of this work M. J. George was supported by U. S. Public Health Service Training Grant No. 5TI-GM-772.

REFERENCES

1 J. A. BENSON, JR. AND A. J. RAMPONE, *Ann. Rev. Physiol.*, 28 (1966) 201.
2 S. J. SAUNDERS AND K. J. ISSELBACHER, *Gastroenterology*, 50 (1966) 586.
3 T. H. WILSON, *Intestinal Absorption*, Saunders, Philadelphia, 1962.
4 H. HAGIHIRA, T. H. WILSON AND E. C. C. LIN, *Am. J. Physiol.*, 203 (1962) 637.

5 B. G. MUNCK, *Biochim. Biophys. Acta*, 120 (1966) 97.
6 H. NEWEY AND D. H. SMYTH, *J. Physiol.*, 170 (1964) 328.
7 L. R. FINCH AND F. J. R. HIRD, *Biochim. Biophys. Acta*, 43 (1960) 278.
8 H. HAGIHIRA, E. C. C. LIN, A. H. SAMIY AND T. H. WILSON, *Biochem. Biophys. Res. Commun.*, 4 (1961) 478.
9 P. R. LARSEN, J. E. ROSS AND D. F. TAPLEY, *Biochim. Biophys. Acta*, 88 (1964) 570.
10 D. M. MATTHEWS AND L. LASTER, *Am. J. Physiol.*, 208 (1965) 601.
11 D. NATHANS, D. F. TAPLEY AND J. E. ROSS, *Biochim. Biophys. Acta*, 41 (1960) 271.
12 V. G. DANIELS, A.G. DAWSON, H. NEWEY AND D. H. SMITH, *Biochim. Biophys. Acta* 173 (1969) 575.
13 J. H. ANNEGERS, *Am. J. Physiol.*, 216 (1969) 1.
14 M. D. MILNE, *Brit. Med. Bull.*, 23 (1967) 279.
15 S. O. THIER AND D. H. ALPERS, *Am. J. Diseases Children*, 117 (1969) 13.
16 W. T. AGAR, F. J. R. HIRD, AND G. S. SIDHU, *Biochim. Biophys. Acta*, 14 (1954) 80.
17 R. P. SPENCER, J. WEINSTEIN, A. SUSSMAN, T. M. BOW AND M. A. MARKULIS, *Am. J. Physiol.*, 203 (1962) 634.
18 K. D. NEAME, *J. Physiol.*, 162 (1962) 1.
19 W. W. UMBREIT, R. H. BURRIS AND J. F. STAUFFER, *Manometric Techniques*, Burgess, Minneapolis, p. 149.
20 M. NING, S. REISER AND P. A. CHRISTIANSEN, *Proc. Soc. Exptl. Biol. Med.*, 129 (1968) 799.
21 A. S. NUNN, Jr. AND M. S. ELLERT, *Am. J. Physiol.*, 212 (1967) 711.
22 B. C. WHALER, *J. Physiol.*, 130 (1955) 278.
23 E. C. C. LIN, H. HAGIHIRA AND T. H. WILSON, *Am. J. Physiol.*, 202 (1962) 919.
24 D. M. MATTHEWS AND L. LASTER, *Am. J. Physiol.*, 208 (1965) 593.
25 S. G. SCHULTZ, R. E. FUISZ AND P. F. CURRAN, *J. Gen. Physiol.*, 49 (1966) 849.
26 S. REISER AND P. A. CHRISTIANSEN, *Am. J. Physiol.*, 208 (1965) 914.
27 A. H. E. SAMIY AND R. P. SPENCER, *Am. J. Physiol.*, 200 (1961) 505.
28 J. LERNER AND M. W. TAYLOR, *Biochim. Biophys. Acta*, 135 (1967) 991.
29 L. L. COHEN AND K. C. HUANG, *Am. J. Physiol.*, 206 (1964) 647.
30 S. SZMELCMAN AND K. GUGGENHEIM, *Biochem. J.*, 100 (1966) 7.
31 W. WILBRANDT, *J. Cellular Comp. Physiol.*, 47 (1956) 137.
32 W. B. KINTER AND T. H. WILSON, *J. Cell Biol.*, 25 (1965) 19.
33 D. SCHACHTER, S. KOWARSKI, J. D. FINKELSTEIN AND RONG-INE WANG MA, *Am. J. Physiol.*, 211 (1966) 1131.
34 R. D. BAKER AND D. B. COPP, *Experientia*, 21 (1965) 510.
35 T. CHANG, J. LEWIS AND A. J. GLAZKO, *Biochim. Biophys. Acta*, 135 (1967) 244.
36 J. M. DIETSCHY AND N. W. CARTER, *Science*, 150 (1965) 1294.
37 J. T. HINDMARSH, D. KILBY, B. ROSS AND G. WISEMAN, *J. Physiol.*, 188 (1967) 207.
38 E. C. C. LIN AND T. H. WILSON, *Am. J. Physiol.*, 199 (1960) 127.
39 K. P. MCCONNELL AND G. J. CHO, *Am. J. Physiol.*, 213 (1967) 150.
40 M. W. NEIL, *Biochem. J.*, 71 (1959) 118.
41 R. P. SPENCER AND K. R. BRODY, *Biochim. Biophys. Acta*, 88 (1964) 400.
42 R. P. SPENCER, K. R. BRODY AND F. VISHNO, *Life Sci.*, 5 (1966) 1163.
43 R. P. SPENCER AND A. H. E. SAMIY, *Am. J. Physiol.*, 199 (1960) 1033.
44 R. P. SPENCER AND A. H. E. SAMIY, *Am. J. Physiol.*, 200 (1961) 501.
45 K. C. HUANG, *J. Pharmacol. Exptl. Therapy*, 136 (1962) 361.
46 K. RAMASWAMY AND A. N. RADHAKRISHNAN, *Indian J. Biochem.*, 3 (1966) 138.
47 H. P. SCHEDL, C. E. PIERCE, A. RIDER AND J. A. CLIFTON, *J. Clin. Invest.*, 47 (1968) 417.
48 H. P. SCHEDL, D. L. MILLER, H. D. WILSON AND P. FLORES, *Am. J. Physiol.*, 216 (1969) 1131.
49 M. L. GREER AND A. L. LAWRENCE, *Comp. Biochem. Physiol.*, 22 (1967) 665.
50 J. J. DEREN, E. W. STRAUSS AND T. H. WILSON, *Develop. Biol.*, 12 (1965) 467.
51 J. H. BUTT II AND T. H. WILSON, *Am. J. Physiol.*, 215 (1968) 1468.
52 J. DE LA NOÜE, *Biochim. Biophys. Acta*, 203 (1970) 360.
53 B. G. MUNCK AND S. G. SCHULTZ, *Biochim. Biophys, Acta.* 183 (1969) 182.
54 H. AKEDO AND H. N. CHRISTENSEN, *J. Biol. Chem.*, 237 (1962) 113.
55 J. W. L. ROBINSON AND J.-P. FELBER, *Gastroenterologia*, 101 (1964)330.
56 J. W. L. ROBINSON AND J.-P. FELBER, *Biochem. Z.*, 343 (1965) 1.
57 B. G. MUNCK, *Biochim Biophys, Acta.* 120 (1966) 282.
58 B. G. MUNCK, *Biochim. Biophys, Acta*, 126 (1966) 299.
59 T. H. WILSON AND G. WISEMAN, *J. Physiol.*, 123 (1954) 126.
60 R. D. BAKER, *Relative Ability of Various Portions of Rat Small Intestine to Absorb Glucose and Sorbose*, Doctoral Dissertation, State University of Iowa, Iowa City, Iowa, 1959.
61 O. HANNINEN, K. HARTIALA AND V. NURMIKKO, *Acta Chem. Scand.*, 18 (1964) 937.
62 R. P. SPENCER, K. R. BRODY AND T. M. BOW, *Gastroenterology*, 44 (1963) 282.
63 I. H. ROSENBERG, A. L. COLEMAN AND L. E. ROSENBERG, *Biochim. Biophys. Acta*, 102 (1965) 161.
64 S. REISER AND P. A. CHRISTIANSEN, *Am. J. Physiol.*, 216 (1969) 915.