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A DISTINCT, Na^+-DEPENDENT GLYCINE TRANSPORT SYSTEM IN AVIAN SMALL INTESTINE*

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SUMMARY

The intestinal absorption of glycine in the chicken has been studied by a tissue-accumulation procedure. Glycine entry is dependent upon Na^+ and is inhibited by anaerobiosis. By a kinetic test it has been shown that glycine is absorbed at a site different from the neutral (methionine) transport site previously characterized in this species. The glycine site has highest affinity for glycine, alanine, and other neutral amino acids, in that order. The order of affinities for the methionine site is: nonpolar, neutral amino acids>alanine>>glycine. For the glycine site, the affinities of neutral amino acids are about one order of magnitude smaller than for the methionine site. However, like the methionine site preference for L-amino acids is seen, and acidic and basic amino acids have little or no affinity. Additionally, requirements are noted for an α-hydrogen atom and for a free α-amino group; substrates such as α-aminoisobutyric acid, proline, hydroxyproline, sarcosine and β-alanine do not interfere with glycine entry. Glycine, when used as a test inhibitor, has little influence on the absorptions of glutamic acid, lysine, alanine or proline. The shapes of the time courses of glycine and methionine uptake are similar, and both amino acids have Q_{10} values of about 1.7.

INTRODUCTION

The transport of glycine has been characterized in numerous as well as diverse types of tissues and single cells[1–27]. Many studies indicate that glycine can be taken up by one or more discrete systems and that it occupies a unique position in transport of neutral amino acids. In this regard VIDAVER *et al.*[1,2] have shown glycine mediation in pigeon red blood cells to occur by way of an entry route highly specific for that substrate and its *N*-methyl and *N*-ethyl derivatives, and HILLMAN *et al.*[3] have identified three distinct agencies in rabbit renal tubules which have been termed the 'alanine-shared,' the 'proline-shared,' and the 'unshared' systems. In avian small intestine the so-called 'methionine' transport system, which has high specificity

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for bulky, lipophilic L-amino acids (but little affinity for D- or charged side-chain species), has been noted by LERNER AND TAYLOR[28] not to serve as a primary receptor for glycine as evidenced by the very low affinity of glycine for this agency. The work to be reported below deals with the characteristics of glycine entry into this tissue and with delineation of the glycine route from the previously documented methionine system.

The results of this study indicate that distinctions exist between glycine transport and that of other neutral amino acids in avian small intestine. A kinetic treatment has been employed to describe a distinct glycine mediator which has low affinity for many neutral amino acids, but which serves primarily in the entry process of glycine.

MATERIALS AND METHODS

Golden Comet female chickens, 7–14 weeks old, were fasted 24 h prior to sacrifice and then killed by cervical dislocation. A portion of small intestine 15 cm on either side of the yolk stalk was excised and immersed in previously gassed (O_2–CO_2, 95:5, by vol.) physiological saline enriched with 0.3 % glucose. This solution was maintained at 37°, or in the case of temperature studies, at the temperature of the incubation solution. The intestine was cut into 8 or 10 segments, and these were cut lengthwise and blotted dry. Even-numbered or odd-numbered segments of tissue were segregated and incubated at 37° (or at temperatures described in RESULTS AND DISCUSSION) with shaking in gassed (O_2–CO_2, 95:5, by vol.) 25-ml portions of Krebs–Henseleit buffer containing the test [^{14}C]amino acid. Except as where noted, 0.3 % glucose was added to the incubation solutions. In most studies the incubation period was five min. Afterwards, the segments were blotted, weighed and then shaken overnight in a portion of aqueous–ethanol solution (95 % ethanol–water, 1:1, v/v) using 10 ml of this solution per g of tissue[28]. The extracts were centrifuged at 12 100 × g for 10 min, and the [^{14}C]amino acid was assayed by counting a 0.2 ml aliquot of the clarified extract which was added to 20 ml of BRAY'S[29] scintillation solution. Radioactivity was determined in a Packard Tri-Carb liquid scintillation spectrometer, and counts were corrected for quench by the channels-ratio method. The uptake of [^{14}C]amino acid was expressed in nmoles accumulated per ml of extract, this representing a constant fraction of the concentration accumulated[28]. In the inhibition studies, percent inhibition of initial uptake was calculated from the ratio v'/v, where v' = substrate accumulated per min by even-numbered segments incubated in the presence of inhibitor; v = substrate accumulated per min by odd-numbered segments incubated without inhibitor.

Except for compounds noted below, all amino acids were purchased from the Sigma Chemical Co. Sarcosine, glycylglycine, taurine, β-alanine, and α-aminoisobutyric acid were obtained from the Nutritional Biochemicals Corp. L-[*Me*-^{14}C]Methionine, [2-^{14}C]glycine, L-[2-^{14}C]alanine and L-[$^{14}C_5$]proline were purchased from the New England Nuclear Corp. and L-[$^{14}C_6$]leucine, L-[$^{14}C_5$]glutamic acid and L-[$^{14}C_6$]lysine from the International Chemical and Nuclear Corp. The compounds were of the highest purity offered.

RESULTS AND DISCUSSION

In Fig. 1 are shown the progress curves for the accumulations of 1 mM glycine and methionine. The data indicate that the uptakes of both amino acids are linear through the first five min of incubation; in subsequent experiments the initial rates of entry have been determined from uptake values at that time. The results also show that methionine attains a steady-state level about three times higher than that of glycine and has a much faster initial rate of entry. Note, however, that these influx data hold only for initial velocities observed with substrate concentrations below about 12 mM because methionine has a smaller v_{max} than glycine (see Fig. 2). Moreover, both compounds reach their respective steady states after approximately 40 min and have curves of similar shape.

The initial rates of 1 mM glycine and methionine uptake are further characterized as being dependent upon oxidative metabolism as evidenced by their reduction (73 % inhibition of methionine; 42 % inhibition of glycine) when incubation takes place in an atmosphere of (N_2–CO_2, 95:5, by vol.) as opposed to an oxygenated atmosphere, and upon the presence of Na^+ as indicated by their diminution (90 % inhibition of methionine; 79 % inhibition of glycine) in a K^+-substituted incubation medium compared with Krebs–Henseleit buffer (see Table I). Entry rates observed when incubation takes place under anaerobic conditions are significantly greater than those obtained with incubation in the K^+-modified buffer. A possible explanation for these faster rates is that glycolysis (involving endogenous substrates) may contribute energy for transport, or the build-up of lactate during incubation may increase

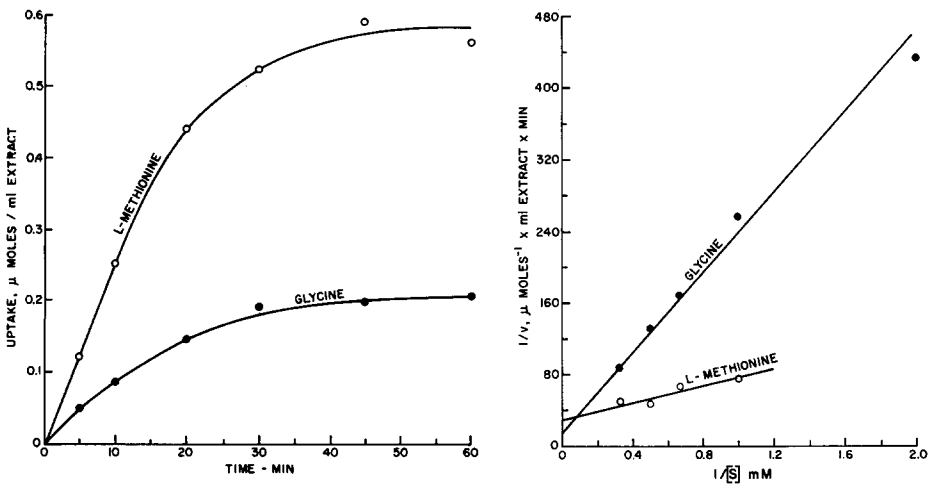

Fig. 1. Time course of glycine and methionine accumulation. Labeled amino acids were incubated in Krebs–Henseleit buffer as described in the text. Each graphed value for the uptake of 1 mM glycine or methionine represents the mean of four determinations on a total of 16–20 intestinal segments from two animals.

Fig. 2 Lineweaver–Burk plots for the initial (5 min) uptakes of glycine and methionine. Rates have been corrected for the Na^+-independent component (see text). Incubation of labeled amino acids was in Krebs–Henseleit buffer as described in the text. Each graphed value represents the mean of 8–18 determinations on a total of 32–90 intestinal segments. Generally, four to nine animals were used to obtain each data point.

TABLE I

EFFECT OF ANAEROBIOSIS AND OF REMOVING MUCOSAL Na^+

Anaerobiosis experiment. Segments of intestine were prepared for incubation in a bathing medium of previously gassed (N_2–CO_2, 95:5, by vol.) physiological saline. The segments were pre-incubated in Krebs–Henseleit buffer in an atmosphere of N_2: CO_2 for 15 min (ref. 30), then rapidly blotted and incubated 5 min with [^{14}C]amino acid as described in the text, except in the nitrogen atmosphere. None of these solutions contained glucose. *Na^+-replacement experiment.* Segments were prepared for incubation as described in the text, except that the bathing medium contained KCl (iso-osmotic with physiological saline) and 0.3% glucose. The segments were then pre-incubated 15 min under O_2 in a KCl and $KHCO_3$- substituted Krebs–Henseleit buffer enriched with 0.3% glucose. After blotting the tissues were incubated 5 min as described in the text with [^{14}C]amino acid in the K^+-modified Krebs–Henseleit buffer. *Control.* Segments were prepared for incubation as described in the text and then pre-incubated 15 min in previously gassed (O_2–CO_2, 95:5, by vol.), glucose-enriched Krebs–Henseleit buffer. The segments were blotted and incubated 5 min with [^{14}C]amino acid as described before. Transport is based upon the uptake by 4 or 5 alternate sections of tissue from one animal. The number of animals used is given in parentheses. Variability is represented by S. E. Probability (P) is based on Student's t distribution by the small sample method.

Amino acid (*1 mM*)	*Control*	*Initial velocity (nmoles/ml extract per min)*		
		N_2	K^+ *buffer*	
L-Methionine	13.3 ± 0.5 (4)	3.6 ± 0.7 (4) $P<0.001$	1.3 ± 0.2 (8) $p<0.001$	(N_2 *vs.* K^+ buffer) $P<0.02$
Glycine	4.8 ± 0.1 (4)	2.8 ± 0.2 (4) $P<0.001$	1.0 ± 0.1 (8) $P<0.001$	(N_2 *vs.* K^+ buffer) $P<0.001$

cell membrane permeability by virtue of a pH effect. TEWS AND HARPER[30] found that anoxia (N_2 atmosphere; preincubation 15 min; incubation 60 min) inhibits the uptake of 1 mM α-aminoisobutyric acid into rat liver slices by less than 50%; they have suggested that glycolysis provides energy in this system and observed that glucose is ineffective in increasing uptake of α-aminoisobutyric acid. They have concluded that energy for transport of α-aminoisobutyric acid arises from endogenous substrates. Additionally, these authors have reported that there is a marked decrease in α-aminoisobutyric acid transport (about 77%) caused by Na^+ replacement with iso-osmolar K^+ (ref. 30). In reference to Na^+-depleted systems BIHLER AND CRANE[31], who have studied sugar transport in rodent small intestine, first proposed that K^+ competes with Na^+ for a cation binding site on the sugar carrier. With regard to amino acid carrier systems EDDY AND HOGG[32] have recently demonstrated that glycine entry into mouse Ascites cells in the absence of Na^+ is independent of K^+ concentration and is about 3% of the rate of 1 mM glycine uptake in the presence of 150 mequiv/l Na^+. They showed by means of kinetic analysis that K^+ competitively inhibits the action of Na^+ (ref. 32).

Lineweaver–Burk plots for the initial uptakes of methionine and glycine are presented in Fig. 2. In determining kinetic constants, substrate concentrations employed ranged from 0.5 to 3.0 mM, and the total accumulations were corrected for Na^+-independent transport according to the method of SCHAFER AND JACQUEZ[33]. The apparent Michaelis constants for glycine and methionine are 15 mM and 1.8 mM, respectively; v_{max} values are 67 nmoles glycine/ml extract per min and 34 nmoles methionine/ml extract per min. In a previous investigation which involved a study of L-methionine absorption by avian small intestine, LERNER AND TAYLOR[28] measured

the affinity of this amino acid to be 4.1 mM (K value) and 4.5 mM (K_i on D-methionine, an analog transported by the same site) as analyzed by steady-state kinetic treatment. Because of the different experimental procedure used to obtain these values, no attempt is made to compare them statistically with the estimated constant found from initial rates. Other investigators, however, have noted apparent Michaelis constants to compare favorably when measured either after 5 min or at steady state[34–36]. Further validation of equilibrium values comes from observations which show that preloading tissue with unlabeled amino acid does not change the steady-state level nor the initial influx of amino acid[37,38]. Moreover, ROBINSON[36] has recently considered some of the mechanistic concepts which might have bearing upon the validity of initial *versus* steady-state comparisons.

In terms of comparative biochemistry it is noted that SCHEDL *et al.*[39] have reported an apparent Michaelis constant of 2.0–5.7 mM for transport of L-methionine in the distil, human small intestine. STEINER *et al.*[40] measured a K value of 4.6 $\pm$ 3.3 mM for valine uptake also in human intestine; this can be compared with the affinity constant of 5.8 mM recorded for valine in chicken intestine[28]. With reference to glycine transport, others have indeed observed typically high v_{max} values and large affinity constants in intestinal tissue. For example, MATTHEWS AND LASTER[4,5] have discovered a relationship between K, v_{max} and hydrocarbon side-chain length among neutral amino acids which they studied in rat small intestine. At one extreme they found glycine to have an apparent Michaelis constant of 43.2 mM and a v_{max} of 2570 μmoles/g per h, whereas, at the other, leucine gave values of 1.55 mM and

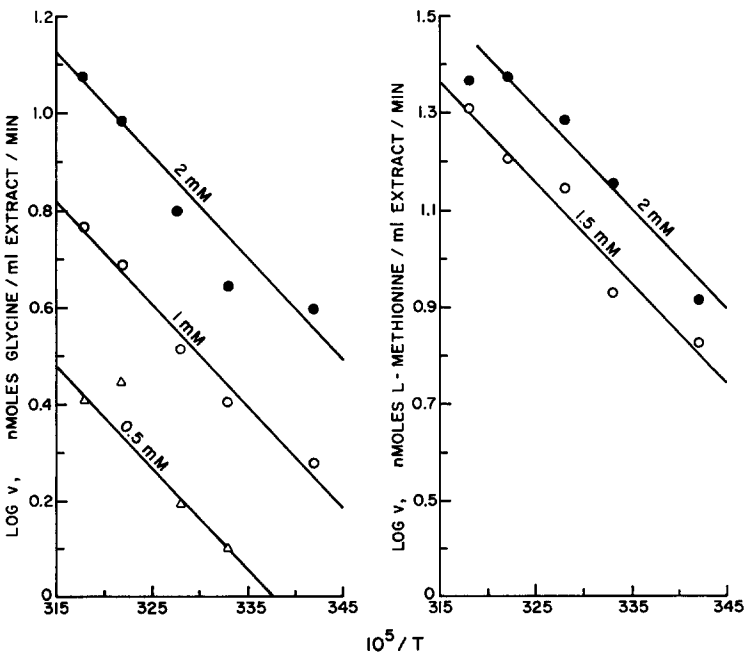

Fig. 3. Arrhenius plots for the influence of temperature on initial (5 min) uptakes of glycine and methionine. Tissues were prepared and incubated as indicated in the text, except that pre-incubations were done at the temperatures of the respective experiments. Each graphed value represents the mean of 8–18 determinations on a total of 32–90 intestinal segments. Generally, 4–9 animals were used to obtain each data point.

490 μmoles/g per h (refs. 4, 5). These results corroborate earlier findings of FINCH AND HIRD[23] who showed glycine to have a K value of 27 mM, but alanine, valine and leucine gave values of 5 mM, 2.1 mM and 0.65 mM, in that order. LIN *et al.*[7] have emphasized the direct relationship between increasing alkyl side-chain length (and ethanol–water solubility ratios) and increasing affinities for a series of amino acids. Lastly, in rabbit renal tubules a K of 35 mM has been noted for a glycine transport system which operates over a concentration range from 1 mM to 150 mM (ref. 3).

In Fig. 3 are illustrated Arrhenius plots of the influence of temperature in the range of 19–42° on the initial rates of glycine and methionine uptake. Both compounds have Q_{10} values of about 1.7. This value is equivalent to an apparent activation energy (E_a) of 9.6 kcal/degree·mole. In this regard SEGAL AND CRAWHALL[41] have found Q_{10} values of 2 and 1.5 for the accumulations of cystine and cysteine, respectively, in rat kidney cortex slices. They calculated E_a values of 13.5 kcal/degree·mole for cystine entry and 7.25 kcal/degree·mole for cysteine[41]. In preliminary experiments we studied the initial uptake of methionine at temperatures from 19–52° and found from Lineweaver–Burk plots that methionine entry is mediated over this temperature range and has the following kinetic constants: v_{max} = 16, 31, 37, 43 and 71 nmoles/ml extract per min and K = 1.8, 1.4, 1.6, 1.4, and 12 mM for 19, 32, 37, 42, and 52°, respectively. K is thus independent of temperature over the range employed, except that the system appears to be partially denatured by 52°, as indicated by the decrease in affinity. The temperature effect seems, then, to be upon the rate-limiting step in transport (v_{max}). When v_{max} values for methionine were used in making an Arrhenius plot, the Q_{10} was found to be 1.6 and the E_a equal to 8.5 kcal/degree·mole.

The influence of amino acids on the initial uptake of glycine is reported in Table II. Many amino acids cause little or no inhibition of glycine transport, and those which do are only moderately effective at best. The latter observation is particularly relevant in view of the high inhibitor to substrate concentration ratio (40:1). The greatest affinities are noted for neutral, nonpolar as well as neutral, polar L-amino acids; among these L-alanine is the best inhibitor, being significantly more effective than methionine. Moreover, the acidic and basic amino acids, in addition to the imino acids, have relatively little or no influence on glycine accumulation; this is also true for those substances bearing the D-configuration. In addition, a requirement is seen for a free α-amino group, as evidenced by the lack of interaction of β-alanine and sarcosine, and for an α-hydrogen, by the absence of effect with α-aminoisobutyric acid. Inspection of the K_i values listed in Table II will show that of all the test inhibitors, none possesses an affinity as high as glycine for this transport system. Lastly, we state that no inhibitory response is seen with glycylglycine; this finding corroborates that of FERN *et al.*[42] who recently showed that this dipeptide has only a small effect on glycine accumulation in rat jejunum and that it is also taken up by the tissue in unchanged form.

In Table III are summarized the minimal effects that glycine has on the transports of other amino acids. These findings indicate the relatively small overlap which glycine has with other transport systems. Thus, it is noteworthy that inhibition of alanine uptake is small; this implies that the major component of alanine accumulation enters the tissue *via* a site having little or no affinity for glycine. The small degree of alanine inhibition probably arises from interaction with glycine at the presumed glycine receptor site. The exclusion of glycine as a substrate from the

TABLE II

PERCENT INHIBITION AND K_i VALUES FOR INHIBITORS OF GLYCINE UPTAKE

Experimental conditions are given in the text. Values for percent inhibition of glycine uptake were found from the fraction uptake v'/v (see text) using 4 or 5 pairs of segments from one animal. The number of animals used is given in parentheses. Inhibitor concentration 20 mM. Glycine concentration 0.5 mM. K_i values were determined as reported previously[28].

Inhibitor	*Inhibition of glycine uptake* (% ± S. E.)	K_i (*mM*)
L-Alanine (I)	43.5 ± 1.4 (4)	25 (I) *vs.* (II) $P < 0.01$
L-Methionine (II)	33.0 ± 1.7 (4)	39
L-Isoleucine	31.0 ± 3.3 (4)	43
L-Serine	30.5 ± 2.4 (4)	44
L-Glutamine	28.7 ± 5.4 (4)	48
L-Phenylalanine	28.0 ± 6.3 (3)	49
L-Asparagine	27.8 ± 6.6 (4)	52
L-Valine	26.6 ± 2.3 (3)	53
L-Threonine	23.0 ± 5.9 (4)	64
L-Aspartic acid	10.8 ± 5.1 (4)	>100
L-Glutamic acid	0.7 ± 6.1 (3)	>100
L-Proline	9.0 ± 5.5 (3)	>100
L-Hydroxyproline	8.0 ± 7.0 (3)	>100
Sarcosine	5.0 ± 4.8 (4)	>100
L-Arginine	6.0 ± 5.5 (3)	>100
L-Lysine	2.5 ± 3.6 (4)	>100
D-Phenylalanine	6.5 ± 4.2 (4)	>100
D-Serine	4.2 ± 4.3 (4)	>100
D-Alanine	2.8 ± 2.9 (4)	>100
D-Methionine	2.0 ± 6.6 (4)	>100
D-Lysine	−13.0 ± 6.6 (4)	>100
Glycylglycine	− 4.5 ± 4.5 (4)	
Taurine	− 5.5 ± 3.6 (4)	
β-Alanine	− 8.3 ± 2.9 (4)	
α-Aminoisobutyric acid	− 9.3 ± 7.9 (4)	

TABLE III

EFFECT OF GLYCINE ON AMINO ACID ENTRY

Experimental conditions are given in the text and in Table II. Glycine concentration 20 mM. Substrate concentration 0.5 mM. The number of animals used is given in parentheses. Four or five pairs of tissue segments were used per animal.

Substrate	*Inhibition of uptake* (% ± S.E.)
L-Alanine	18.0 ± 6.3 (4)
L-Proline	15.0 ± 4.4 (4)
L-Glutamic acid	7.7 ± 3.9 (4)
L-Lysine	3.5 ± 3.3 (4)

so-called methionine transport system is considered next by way of the following kinetic treatment.

The affinity of glycine for transport ($K = 15$ mM) is much greater than its affinity when tested as an inhibitor of L-methionine transport ($K_i = 71$ mM) (see

Table IV). Also, methionine has relatively high affinity for transport (K = 1.8 mM) but gives a K_i value of 39 mM when used as an inhibitor of glycine uptake. Since the apparent Michaelis constant for the transport of glycine (methionine) does not equal its K_i when acting as an inhibitor of methionine (glycine), two distinct sites can be postulated to function in their respective absorptions. Furthermore, L-alanine gives K_i values of 25 mM (range = 24–27 mM) and 12 mM (range = 10–14 mM) on the accumulations of glycine and methionine, respectively. These values are significantly different ($P<0.01$). On the other hand, if a common site were present for the uptakes of both glycine and methionine, then a third amino acid would have the same K_i when used as an inhibitor of either substrate. Additionally, the non-polar amino acid leucine has a K_i value of 4.6 mM when measured on methionine uptake, but its structural analogs, valine and isoleucine, have affinity constants for the inhibition of glycine one order of magnitude larger than this value (see Table II). While alanine, the most similar structural analog of glycine, has the highest affinity for the glycine site with the exception of glycine itself, affinity for the methionine site increases with increasing amino acid side-chain length (Table IV). Moreover, other long-chain, nonpolar amino acids have been shown to possess approximately the same affinity as methionine for the methionine agency in avian intestine[28]. Previous work in this species has also indicated that D-methionine, in contrast to other D-amino acids, has relatively high affinity for the latter system[28]. In the present study, however, D-methionine is no more effective in inhibiting glycine than any other D-isomer tested (Table II) and, in fact, has virtually no affinity for the glycine system. Lastly, the following order of substrate affinities for these two neutral systems holds: glycine site: glycine>alanine>methionine and other neutral amino acids; methionine site: methionine and other neutral amino acids (primarily nonpolar species)>alanine>>glycine.

The results of this report point to the distinctions between glycine transport and uptake of other neutral amino acids. The glycine site is a low affinity receptor which, as opposed to the methionine site, has only about 1/10 the tolerance for substrates with neutral side chains; other substrates with charged groups, imino acids,

TABLE IV

AFFINITY CONSTANTS

Values for K for methionine and glycine were determined from Lineweaver–Burk plots of initial (5 min) uptake (Fig. 2). K_i values were calculated as reported previously[28]. The range of the K_i values is given in parentheses. The number of animals used in each experiment is reported in either Fig. 2 or Table II. Inhibitor concentration in methionine experiments 20 mM. L-Methionine concentration 0.5 mM.

Substrate or inhibitor	*K (mM)*	*K_i on glycine transport (mM)*	*K_i on L-methionine transport (mM)*
Glycine	15		71 (61–85)*
L-Methionine	1.8	39 (36–42)	
L-Alanine		25 (24–27)	12 (10–14)**
L-Leucine			4.6***

* Inhibition of methionine uptake (% ± S.E.) = 18.0 ± 2.4 (n = 4).
** (% ± S.E.) = 56.0 ± 4.0 (n = 4).
*** (% ± S.E.) = 77.0 ± 0.7 (n = 4).

or D-amino acids are even less reactive than the neutral substances. Glycine, furthermore has little influence on the transports of other classes of amino acids. It is of interest to contrast our findings with those on glycine transport in other tissues and single cells. In this respect, VIDAVER *et al.*[1,2] have described a system for glycine transport in pigeon red blood cells in which no amino acids other than *N*-methyl- and *N*-ethylglycine greatly inhibit glycine entry. They also found alanine influx not to be greatly influenced by glycine. Nevertheless, minor routes for glycine entry do exist in these cells because many amino acids cause a small amount of inhibition of glycine uptake[1,2]. Moreover, EAVENSON AND CHRISTENSEN[25] showed that at high glycine concentrations (and low Na^+ concentrations) an appreciable portion of glycine entry into pigeon erythrocytes occurs by way of the main alanine transport system, which is distinct from the main glycine route.

The imino acids and glycine are thought to share common transport systems in mammalian kidney. In man their transports are achieved by an agency with high capacity and low affinity as well as by two or more mediators each with relatively low capacity, but high affinity[26]. Thus SCRIVER AND WILSON[26] have discovered that at high imino acid and glycine concentrations a common mediator is shared, whereas at low concentrations glycine is transported by a system different from the agency serving the imino acids. In distinction to these results, glycine transport in the avian small intestine is influenced to a minor extent by proline, hydroxyproline or sarcosine (N-methylglycine). These findings, however, do not preclude the possibility that glycine can interact with secondary amino acids under other concentration conditions in the avian. Indeed, HILLMAN *et al.*[3] have found several distinct transport systems for glycine in isolated renal tubule segments which can be distinguished kinetically partly on the basis of judicious selection of substrate level. Hence, they have reported a transport system shared by alanine and glycine which is active at low glycine concentrations; this has high affinity for glycine, but a low transport capacity. A second system which they found is shared by proline and glycine; this has low affinity, high capacity, and is somewhat active at low glycine concentrations. Lastly, a system unshared with either of these acids has been discovered which has about the same capacity as the proline-shared system but has a still lower affinity and is effective only at high glycine concentrations[3].

The well-documented transport of neutral amino acids in the Ehrlich cell is partitioned into the so-called 'A' and 'L' systems[9,27]. The A system has high specificity for glycine, alanine, α-aminoisobutyric acid and methionine but discriminates against branched-chain amino acids of 5 or 6 carbons; the L system on the other hand has greater affinity for substrates with appreciable side-chain bulk and length such as valine and leucine; it includes methionine within its specificity limits[9,27]. Both systems, in analogy to the glycine and methionine mediators in the avian, are stereospecific, having preference for L-isomers. Furthermore the glycine agency in the chicken has affinity for glycine, alanine and methionine, but not for α-aminoisobutyric acid as evidenced by inhibition data given in this report. The avian methionine mediator appears to be akin to the L system and has somewhat higher affinity for alanine than does the glycine transport system.

The uptake of neutral amino acids in rat small intestine appears to involve the function of two carriers designated the 'methionine' and 'sarcosine' transport systems[14,16,24]. Certain features of these agencies bear resemblance to the avian

systems which we have described, whereas some interesting departures can be noted. Thus, NEWEY AND SMYTH[24] found that methionine, leucine, alanine, glycine and proline are handled by the methionine site, while the other handles glycine and proline in addition to sarcosine and β-alanine, although it excludes methionine and leucine. DANIELS *et al.*[14] have reported for the rat that methionine transport is progressively inhibited as a function of increasing chain length in the series glycine, alanine, and norvaline (or norleucine); thus, the longer the carbon chain the greater the inhibition. These findings concur with our results on the specificity requirements of the avian methionine transport site. The stereospecificity for L-isomers exhibited by the rat system also parallels the optical preference seen in the avian methionine system[28,43]. The sarcosine mediator in one respect resembles the avian glycine transport system in that glycine and alanine have much higher affinities for this agency than long-chain hydrophobic species such as norvaline and norleucine[14]. However, other aspects of the chemical specificities of these systems differ between species. Our system, for example, shows a marked requirement for a free α-amino group; hence, proline, β-alanine and sarcosine are excluded. Likewise we have shown a lack of affinity among the D-enantiomers, but DANIELS *et al.*[43] have obtained evidence which indicates that D- and L-amino acids inhibit sarcosine nearly equally.

The evidence presented in this paper establishes a distinct glycine entry route in avian small intestine and lends support to the fact that, like the complex metabolic fates of glycine, the transport of this amino acid into cells is also a complex process[3].

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