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MECHANISMS OF INHIBITION OF GLYCYLGLYCINE TRANSPORT BY GLYCYL-L-LEUCINE AND L-LEUCINE IN GUINEA-PIG SMALL INTESTINE

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Inhibition of glycylglycine (Gly-Gly) transport by glycyl-L-leucine (Gly-Leu) and L-leucine was studied in isolated guinea-pig ileum. Kinetically, inhibition by Gly-Leu of Gly-Gly influx across the mucosal border was found to be mixed type, whereas the inhibition by leucine was 'pseudo-competitive'. Kinetics of Gly-Leu inhibition, which could be defined as linear mixed type, suggests that Gly-Gly transport carrier has a high-affinity binding site for Gly-Leu besides the site for Gly-Gly, and that binding of Gly-Leu to its own site is inhibitory to Gly-Gly transport but independent of Gly-Leu transfer. Gly-Leu hydrolases at the brush border membrane had a high-affinity component, but this did not seem to be related to the inhibitory binding of Gly-Leu, since bestatin (0.3 mM) completely suppressed the high-affinity component of the enzymes but little affected Gly-Gly transport. The 'pseudo-competitive' type of inhibition by leucine suggests that the Gly-Gly carrier has another binding site to which leucine can bind, that leucine binding to this site is inhibitory to Gly-Gly transport and that leucine is partly transported by the Gly-Gly carrier system.

Introduction

A number of dipeptides and tripeptides are known to be transported as such across the intestinal brush border membrane either by an Na^+-dependent or an Na^+-independent carrier mechanism [1,2]. Attempts to classify the carrier(s) specific for groups of dipeptides and/or tripeptides have been made by many authors using various animals. Adibi and Soleimanpour [3] first demonstrated that Gly-Gly was competitively inhibited by Gly-Leu in human jejunum, and Das and Radhakrishnan [4] obtained results with monkey intestine showing the existence of a carrier system with an extremely broad specificity. Furthermore, Taylor et al. [5] showed that glycylsarcosine, L-glutamyl-L-glutamate and L-lysyl-L-lysine were all transported by a common carrier in hamster jejunum. On the other hand, Gupta and Edward [6] postulated the existence of at least three separate systems for neutral dipeptides in rat intestine. Thus, the classification of carrier(s) for intact peptide transport has not yet been clearly established.

One of the difficulties in defining separate or common carrier(s) for peptides by competition experiments seems to be due to the presence of non- or uncompetitive types of inhibition among peptides. Himukai and Hoshi [7] showed that Gly-Gly transport was strongly inhibited by Gly-Leu, but Gly-Gly did not inhibit Gly-Leu transport at all although K_t values for these two dipeptides were not so different. They also showed that the inhibition by Gly-Leu was not competitive but mixed type. From these results, they postulated that the sites of binding for these two dipeptides were separated but closely located so that the binding of Gly-Leu to its own binding site caused

interference with Gly-Gly binding to the transport carrier site, probably in an allosteric manner. As this sort of interaction was thought to be important among di- and tripeptides, an attempt has been made to analyze in more detail the mechanism of inhibition of Gly-Gly transport by Gly-Leu by performing more rigorous kinetic studies. Particularly, the nature of the Gly-Leu binding site which is effective in the transport was investigated in detail. With regard to this problem, the effect of bestatin, a strong competitive inhibitor of aminopeptidase B and leucine-aminopeptidase [8], was studied in order to discover whether the inhibitory binding of Gly-Leu was related to Gly-Leu hydrolysis, since the K_i for Gly-Leu and K_m of the high-affinity component of the brush border membrane hydrolases for Gly-Leu were quite similar.

Methods

Preparations and uptake experiments

Everted preparations of guinea-pig ileum were used in all experiments. The surgical operations and fixation of the everted intestine on a polyethylene tube have been described in detail in our previous paper [7]. As previously described, a device was made to obtain a constant serosal surface area for every preparation by fixing the everted intestine over a calculated area of the surface of a polyethylene tube. The uptake of Gly-Gly by each preparations was examined by incubating the preparations for 1 min at 37°C in a medium containing Gly-Gly at various concentrations and [1-^{14}C]Gly-Gly (Amersham International, custom-made) at 0.2 μCi/ml. Previous observations [9] revealed that the uptake of this dipeptide from the mucosal solution containing the peptide at a sufficiently high concentration increased linearly with time up to 2 min; thereafter the increase gradually slowed down. Therefore, 1 min incubation was thought to be safe for obtaining the initial influxes. After the end of incubation, the preparations were rapidly rinsed in ice-cold isotonic mannitol solution for 5 s, blotted on filter paper, and extracted in 2 ml of 3% trichloroacetic acid. The radioactivities of the extraction fluids and the test media were counted in a liquid scintillation counter (ALOKA-LSC 703, Tokyo). The data of uptake obtained from several different animals are presented as means ± S.E. (nmol/min per cm^{-2} serosal surface area) unless otherwise noted.

From the obtained uptake data, kinetic parameters of transport were determined in the following way. As previously reported [9], the initial influxes (J) of Gly-Gly across the mucosal border of guinea-pig intestine can be expressed by the following equation:

$$J = \frac{J_{max} \cdot [\text{Gly-Gly}]}{K_t + [\text{Gly-Gly}]} + K_D \cdot [\text{Gly-Gly}] \quad (1)$$

where J is the initial influx, J_{max} the maximum influx through a carrier-mediated process, K_D the coefficient of passive non-mediated uptake. In most of previous studies [7,9,10], the passive non-mediated uptake component was calculated from the value of simultaneously measured mannitol or inulin space. However, it has been pointed out that mannitol and inulin are not ideal ECF markers for the study of uptake of amino acids or peptides [11,12]. Theoretically, the substance identical with a test substance should be used for determination of the non-mediated uptake component. Matthews et al. [11] determined the value of K_D for glycylsarcosine (Gly-Sar) by the self-inhibition of transport; namely, by inhibiting competitively the isotope influx of Gly-Sar by adding unlabelled Gly-Sar into the influx medium at various concentrations. They demonstrated that an inadequately estimated value of K_D (for example a value determined with inulin) led to a misinterpretation that there were double-mediated processes. Another reasonable way of estimating all kinetic parameters included in Eqn. 1 is that proposed by Robinson and Alvarado [12]. Their method is to obtain a set of values of the parameters involved in Eqn. 1 which gives the best fit of calculated values to the data actually obtained over a wide range of substrate concentration. In this method, systematic iteration of values of J_{max}, K_t and K_D is made to minimize the χ^2 residues as defined by the following equation:

$$\chi^2 = \Sigma \left(\frac{J_o - J_c}{\text{S.E.}} \right)^2 \quad (2)$$

where J_o is the mean value of observed influxes at a given substrate concentration, and S.E. is the standard error of J_o. J_c is the uptake value calcu-

lated according to Eqn. 1. In the present study, the latter way of estimation was adopted. In this study, J_o was obtained at six different concentrations and J_c at each concentration was calculated by introducing various values for J_{max}, K_t and K_D into Eqn. 1. A combination of these parameters which gave the minimum χ^2 residue was finally obtained. A desk computer (Olivetti, P 6060) was used for this calculation.

Enzyme assays with brush border membrane

Brush border membranes were prepared from guinea-pig small intestine by the method of Kessler et al. [13]. Briefly, scraped mucosal surface materials were homogenized in a hypotonic 100 mM mannitol solution containing 5 mM Hepes-Tris (pH 7.4) by the use of a Waring Blender (18000 rev./min 3 min). Then $CaCl_2$ was added to the homogenate to the final concentration of 10 mM, and the homogenate was centrifuged at 6000 $\times g$ for 10 min. The supernatant was centrifuged at 38000 $\times g$ for 30 min to separate soluble proteins and a particulate fraction. The pellet (particulate fraction) was resuspended in the same buffer as that used for homogenization. This suspension was used as a crude brush border membrane preparation. This preparation had sucrase and alkaline phosphatase activities enriched by a factor of 13 as compared to the original homogenate, and $(Na^+ + K^+)$-ATPase about 2-fold enriched. Small but significant contamination of the basolateral membrane did not interfere with the present investigation. Lactic dehydrogenase activity was not detected in this fraction. Activities of Gly-Leu hydrolase of the membrane fraction and cytosol were determined by the one-step L-amino acid oxidase method [14].

Chemicals

All chemicals used were of reagent grade. Bestatin, ((2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutanoyl)-L-leucine, was kindly supplied by Dr. T. Aoyagi, Institute for Microbial Chemistry, Tokyo.

Results

Kinetic parameters of Gly-Gly and Gly-Leu transport

The kinetic parameters of Gly-Gly and Gly-Leu transport were reinvestigated by using Eqn. 1 and the way of estimation described by Robinson and Alvarado [12]. The obtained values for J_{max}, K_t and K_D for both Gly-Gly and Gly-Leu are listed in Table I. Under control conditions (in the presence of Na^+ at 100 mM), the values of J_{max} and K_D for Gly-Gly averaged 36.2 nmol $\cdot$ min^{-1} $\cdot$ cm^{-2}, and 1.93 nmol $\cdot$ min^{-1} $\cdot$ cm^{-2} $\cdot$ mM^{-1}, respectively. In the absence of Na^+, the average values of J_{max} and K_D were 36.5 nmol $\cdot$ min^{-1} $\cdot$ cm^{-2} and 1.99 nmol $\cdot$ min^{-1} $\cdot$ cm^{-2} $\cdot$ mM^{-1}. The values were not so different from control values. The value of K_t appeared to increase slightly when Na^+ in the incubation medium was completely replaced with mannitol. But further paired studies indicated that

TABLE I

CALCULATED KINETIC PARAMETERS OF Gly-Gly AND [^{14}C]-Gly-Leu TRANSPORT

[^{14}C]Gly-Leu transport (transport of glycine residue) represents the influx component of the intact transport of Gly-Leu. Standard medium contained 100 mM Na^+. Na^+ was replaced by D-mannitol without changing the osmolality of the solutions. Calculations as per Methods section.

	Gly-Gly transport				[^{14}C]Gly-Leu transport Na^+ present	
	Na^+ present		Na^+ absent			
	average	range	average	range	average	range
J_{max} (nmol $\cdot$ min^{-1} $\cdot$ cm^{-2})	36.2	(35.7 –36.6)	36.5	(35.9 –37.0)	49.7	(49.5 –49.9)
K_t (mM)	1.66	(1.63– 1.69)	2.03	(2.00– 2.06)	1.24	(1.23– 1.25)
K_D (nmol $\cdot$ min^{-1} $\cdot$ cm^{-2} $\cdot$ mM^{-1})	1.93	(1.90– 1.96)	1.99	(1.97– 2.01)	1.90	(1.88– 1.91)
χ^2	1.18		0.32		1.64	

the difference was statistically insignificant (unpublished data).

Thus, Gly-Gly influx is completely independent of the presence of Na^+, this being strikingly different from free glycine influx which reveals absolute Na^+ dependence as demonstrated in our previous study [9]. If Gly-Gly influx has a component of influx of free glycine liberated by superficial membrane hydrolysis, the value of J_{max} measured in the presence of Na^+ would be much greater than that obtained in the absence of Na^+.

To obtain the carrier mediated component of Gly-Gly influx, all data were corrected with K_D value (1.93 in the presence of Na^+ and 1.99 nmol · $min^{-1} \cdot cm^{-2} \cdot mM^{-1}$ in the absence of Na^+).

The parameters for Gly-Leu transport were determined by using the data obtained in a previous study [7] with glycine-labelled tracer ([1-^{14}C]Gly-Leu, purchased from Amersham International) and by the present method of determination. As demonstrated in our previous study [7], Gly-Leu is transported in two different ways; one is intact peptide transport and the other is transport of free amino acids (leucine) after superficial membrane hydrolysis. But [1-^{14}C]Gly-Leu influx represents the influx component of intact Gly-Leu transport since glycine transport after hydrolysis is negligibly small. Transport of Gly-Leu in intact peptide form was Na^+-independent [7]. The values of kinetic parameters for intact Gly-Leu transport are also shown in Table I. The value of K_D were not so different between two dipeptides tested and between two different experimental conditions (the presence or absence of Na^+).

Inhibitory action of Gly-Leu on Gly-Gly transport

Our previous study [7] showed that inhibition of Gly-Gly transport by Gly-Leu in the presence of 100 mM Na^+ was mixed type when the data were plotted according to the Lineweaver-Burk method. In the present study, the inhibitory action of Gly-Leu was reinvestigated in the Na^+-free medium and by correcting uptake data with K_D value obtained by the method of Robinson and Alvarado [12]. The reason for the use of Na^+-free conditions are to see whether the mixed-type inhibition is dependent on the presence of Na^+, and to minimize the effect of involvement of influxes of free amino acids liberated, which are strongly

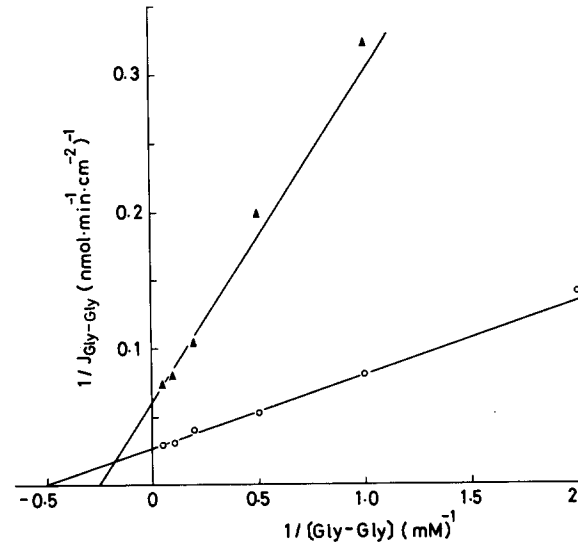

Fig. 1. Double-reciprocal plots of Gly-Gly influxes measured in the absence (○) and presence (▲) of 1.5 mM Gly-Leu. The experiments were carried out in the absence of medium Na^+. Each value is the mean of seven observations from different animals. All values were corrected for a diffusive component of entry with a K_D of 1.99 nmol · $min^{-1} \cdot cm^{-2} \cdot mM^{-1}$.

strongly dependent on Na^+.

The results are summarized in Fig. 1 which shows again the inhibition is mixed type, confirming the previous observation. J_{max} for Gly-Gly decreased from 37.4 ± 1.50 to 17.7 ± 0.90 ($P < 0.001$) nmol · $min^{-1} \cdot cm^{-2}$, whereas K_t for Gly-Gly increased from 2.12 ± 0.09 to 4.75 ± 0.24 mM ($P < 0.001$) when Gly-Leu was added to the medium to a final concentration of 1.5 mM. The mixed-type inhibition as defined by Lineweaver-Burk plot can arise from some special situations. In cases of enzyme reactions, Segal [15] devided this form of inhibition into two types, a linear mixed type and a hyperbolic mixed type, on the basis of behavior on both Lineweaver-Burk and Dixon plots. The former is defined as a type of inhibition in which the influx of substrate becomes virtually zero when inhibitor concentration is greatly elevated (a linear relationship in the Dixon plot). In the case of the latter, on the other hand, the inhibition is not complete but usually partial, even when the concentration of inhibitor is greatly elevated (a hyperbolic relationship in the Dixon plot). Segel postulated molecular mechanisms of these two types

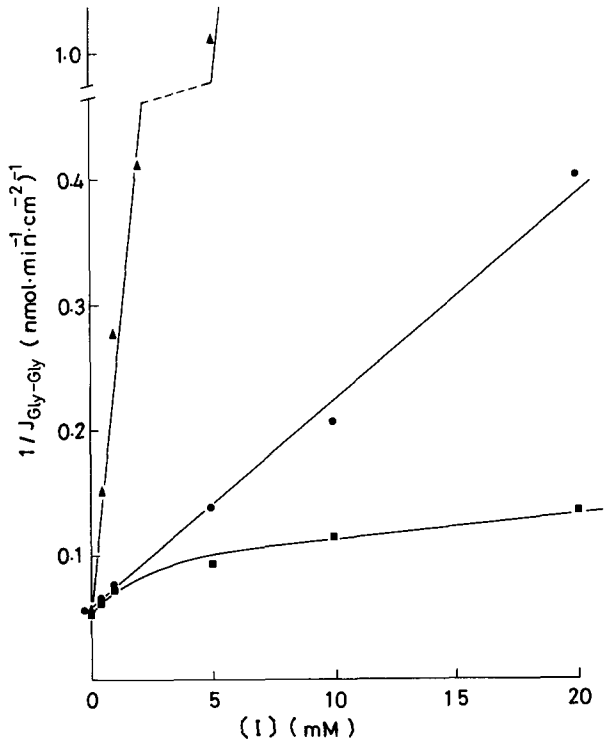

Fig. 2. Inhibition of Gly-Gly influxes by Gly-Leu (▲), Gly-Gly-Gly (●) and leucine (■). Dixon plots. Each value is the mean of six observations from different animals in the presence of Na^+ (100 mM) in the medium. All values were corrected for a diffusive component of entry with a K_D of 1.93 nmol·min^{-1}·cm^{-2}·mM^{-1}.

of inhibition. Therefore, we examined further the inhibition by Gly-Leu by plotting the data using Dixon's method (Fig. 2). The reciprocal of Gly-Gly influx was found to be of linear mixed type. The value of inhibitor constant (K_i) was calculated to be 0.4 mM.

Comparison of the inhibitory mechanism of Gly-Gly transport by leucine and Gly-Gly-Gly

It is now established that many di- and tripeptides are transported across the mucosal membrane by mediated process(es) distinct from amino acid transport systems [1,2]. However, a weak but significant interaction of transport between dipeptides and amino acids has been reported by many authors; for example, Gly-Pro transport was inhibited by proline in newborn rabbit jejunum [16], leucine inhibited Gly-Gly transport in monkey intestine [17], and carnosine transport was also inhibited by leucine in frog intestine [18]. It is unlikely that the observed inhibition is due to the inhibition of influx component transferred as amino acid which is liberated by membrane hydrolysis of peptides, because all the above peptides have been demonstrated to be transported in intact peptides forms.

Radhakrishnan [17] reported that Gly-Gly transport was competitively inhibited by leucine in monkey intestine, and he suggested that leucine and Gly-Gly shared a common carrier. However, there are at least two cases in the competitive inhibition defined by Lineweaver-Burk plot; one is purely competitive, and the other 'pseudo-competitive' as defined by Robinson and Alvarado [12]. The discrimination of the two cases can be made by Dixon plot of data of inhibition experiments. Namely, the reciprocal of the substrate influx ($1/J_s$) and the concentration of inhibitor [I] is linear in the case of purely competitive, whereas the relation is hyperbolic in the case of 'pseudo-competitive' [12]. In the present study, we compared two different substances, Gly-Gly-Gly and leucine in regard to their inhibitory action on Gly-Gly transport. The inhibition by these two substances was competitive when a Lineweaver-Burk plot was made as shown in Figs. 3 and 4. The mean values of J_{max}, K_t, in the absence and pres-

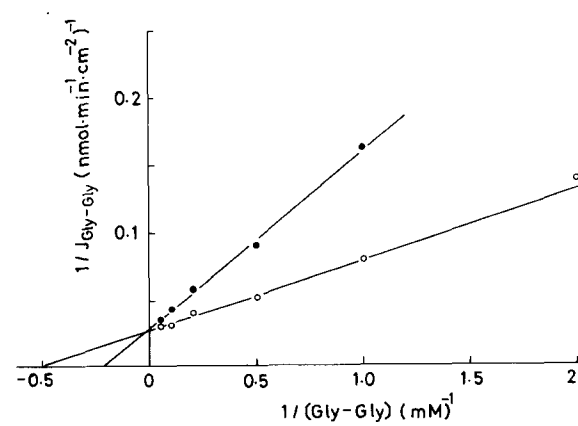

Fig. 3. Double-reciprocal plots of Gly-Gly influxes measured in the absence (○) and presence (●) of 5 mM Gly-Gly-Gly. The experiments were carried out in the absence of medium Na^+. Each value is the mean of seven observations from different animals. All values were corrected for a diffusive component of entry with $K_D = 1.99$ nmol·min^{-1}·cm^{-2}·mM^{-1}.

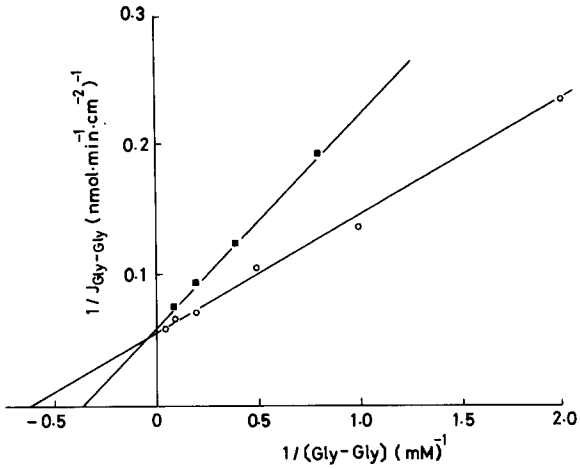

Fig. 4. Double reciprocal plots of Gly-Gly influxes measured in the absence (○) and presence (■) of 1.5 mM Leu. The experiments were carried out in the presence of Na^+ (100 mM) because the K_t of leucine transport is much higher in Na^+ free medium than in 100 mM Na^+ medium. Each value is the mean of seven observations from different animals. All values were corrected for a diffusive component of entry with $K_D = 1.93$ nmol·min^{-1}·cm^{-2}·mM^{-1}.

ence of 5 mM Gly-Gly-Gly were 37.4 ± 1.50 and 33.4 ± 1.70 (n.s.) nmol·min^{-1}·cm^{-2}, 2.12 ± 0.09 and 4.39 ± 0.22 mM ($P < 0.001$), respectively. In the case of leucine inhibition, the mean values of J_{max}, K_t for Gly-Gly in the absence and presence of 1.5 mM leucine were 35.7 ± 1.50 and 34.4 ± 0.40 (n.s.) nmol·min^{-1}·cm^{-2}, 1.58 ± 0.07 and 2.83 ± 0.03 mM ($P < 0.001$), respectively. However, the Dixon plot revealed that the inhibition of these two substances is of different nature. The reciprocal of Gly-Gly influx vs. triglycine concentration was linear (Fig. 2); thus, the inhibition can be defined as pure competitive. K_i was calculated to be 4 mM. In the case of leucine, the Dixon plot was hyperbolic, thus the inhibition is defined as 'pseudo-competitive'.

Gly-Leu hydrolase at the brush border membrane and its relation to the Gly-Gly transport carrier

The kinetic behavior of Gly-Leu inhibition which can be defined as linear mixed type would suggest that there may be a high-affinity binding site for Gly-Leu in Gly-Gly transport system which is separated from the binding site for Gly-Gly itself, but that Gly-Leu bound to such a binding site may not be transported. One of the possibilities for such an inhibitory binding site is that part of Gly-Leu hydrolase is located in the vicinity of Gly-Gly transport site and binding of Gly-Leu to its hydrolase may interfere with Gly-Gly binding and transport. In order to examine this possibility, Gly-Leu hydrolysis at the brush border membrane was first examined in an attempt to discover whether such a high affinity system ($K_i = 0.4$ mM) was present in the Gly-Leu hydrolase or not. Fig. 5 shows a Hofstee plot of the data obtained, showing that there were two components in Gly-Leu hydrolysis; one (system 1) with a higher-affinity constant ($K_m = 0.1$ mM) and a low maximum velocity ($V_{max} = 0.29$ μmol/min per mg protein) and the other (system 2) with a higher K_t value (1.5 mM) and a higher maximum velocity ($V_{max} = 1.79$ μmol/min per mg protein). Bestatin, a strong competitive inhibitor of aminopeptidase B and leucine-aminopeptidase [8], completely inhibited the hydrolyzing activity of the system 1 even at a very low concentration of the drug (0.3 mM). This agent also partly inhibited system 2, in a noncompetitive manner; the values of K_m and V_{max} changed from 1.5 to 1.6 mM and from 1.79 to 0.78 μmol/min per mg protein, respectively. On the other hand, the influx of Gly-Gly was only slightly inhibited (by about 22%) by 0.3 mM bestatin (Table IIa). From these results, it seems unlikely that the high-affinity binding site for Gly-Leu on

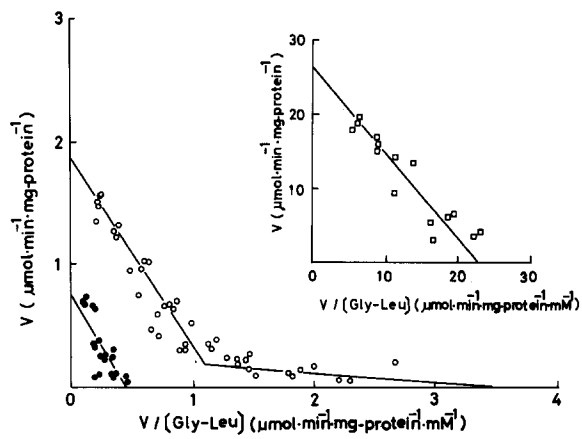

Fig. 5. Hofstee plots (V vs. $V/[S]$) if Gly-Leu hydrolysis by brush border membrane in the absence (○) and presence (●) of 0.3 mM bestatin. The inset shows Gly-Leu hydrolysis by the soluble fraction.

TABLE II

EFFECT OF BESTATIN ON THE INFLUX OF Gly-Gly

Incubation medium contained 100 mM Na^+. The data are given as mean ± S.E. The influxes are corrected for a diffusive component of entry with $K_D = 1.93$ nmol·min^{-1}·cm^{-2}·mM^{-1}. n, the number of observations from different animals.

Substrate (mM)	Inhibitor (mM)	n	Influx (nmol·min^{-1}·cm^{-2})	P (paired t-test)
a Gly-Gly (1)	—	6	13.0 ± 1.92	
Gly-Gly (1)	bestatin (0.3)	6	10.2 ± 1.07	$P<0.05$
b Gly-Gly (0.5)	—	6	6.48 ± 0.41	
Gly-Gly (0.5)	bestatin (10)	6	0.34 ± 0.02	$P<0.001$

the hydrolase is related to inhibition of Gly-Gly transport. For the sake of comparison, Gly-Leu hydrolase in the soluble fraction was also examined and the results are illustrated in the inset of Fig. 5. The value of K_m and V_{max} in the absence of bestatin were 1.2 mM and 27.0 μmol/min per mg protein, respectively. And the activity was completely inhibited by bestatin at 0.3 mM.

Nature of effect of bestatin on Gly-Gly transport

As bestatin was found to have a weak but significant inhibitory action on Gly-Gly transport (Table IIa), further experiments were carried out to investigate its inhibitory mechanism. Measurements of Gly-Gly influxes were carried out in the presence or absence of 0.5 mM bestatin, and the data are summarized in Fig. 6. Bestatin caused an increase in K_t without changing J_{max}. The inhibition by bestatin was found to be competitive when observed on double-reciprocal plot. Moreover, a high concentration of bestatin (10 mM) inhibited Gly-Gly transport almost completely (Table IIb), suggesting that bestatin can share the common binding site with Gly-Gly. The K_i for bestatin was 1.1 mM. The present study did not determine whether bestatin was transported across the membrane, but Yasumoto and Sugiyama [19] showed that, in isolated epithelial cells of rat intestine, intracellular hydrolysis of Gly-Leu was inhibited in the presence of bestatin, suggesting that this substance was transported across the brush border membrane and able to enter the cells.

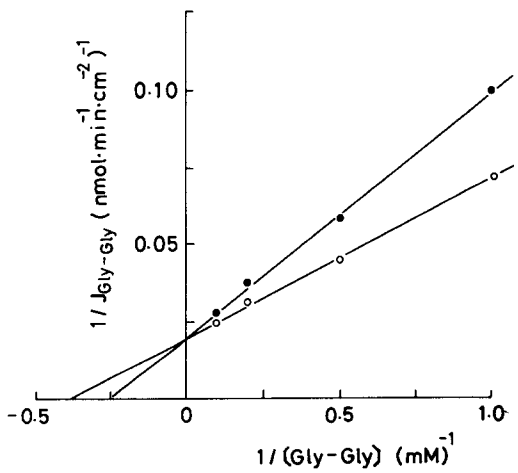

Fig. 6. Double-reciprocal plots of Gly-Gly influxes measured in the absence (○) and presence (●) of 0.5 mM bestatin. The experiments were carried out in the presence of 100 mM Na^+ in the medium. Each value is the mean of six observations from different animals. All values were corrected for a diffusive component of entry with $K_D = 1.93$ nmol·min^{-1}·cm^{-2}·mM^{-1}.

Discussion

The results of the present study confirm our previous finding that inhibition of Gly-Gly transport by Gly-Leu was not competitive. The additional data indicate that the inhibition is linear mixed type. As previously reported, Gly-Gly did not inhibit Gly-Leu transport even though K_t values for these two dipeptides are not so different (1.7 mM for Gly-Gly and 1.2 mM for Gly-Leu). Such a non-mutual inhibition, together with mixed-type inhibition kinetics cannot be explained on the basis of simple competition for a common carrier binding site. According to Segel [15], inhibition of linear mixed type can be explained

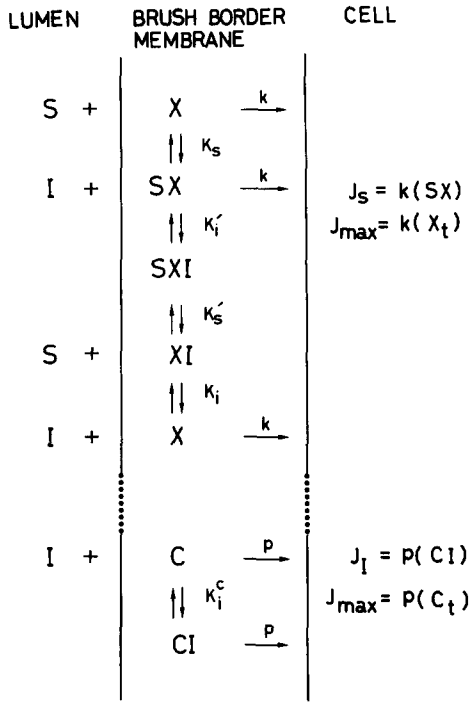

Fig. 7. Kinetic model which explains the interaction between a transported substrate S (Gly-Gly) and an inhibitor I (Gly-Leu). The complexes, XI and SXI, are assumed not to be transported. X and C are kinetically different carriers. $K_s(K_s')$, $K_i(K_i')$ and K_i^c are dissociation constants for S and I, respectively. k and p, translocation rate constants; X_t and C_t, local carrier density.

when a kinetic model as shown in Fig. 7 is assumed. In this model, the carrier is assumed to have two distinct binding sites for S (Gly-Gly in the present case) and I (Gly-Leu), and the dissociation constants for S (K_s) and I (K_i) change to K_s' and K_i' when S and I bind to their own binding sites. It is also assumed that I bound to the carrier is not transported, i.e., the formation of the complex (SXI) is assumed to be 'dead end' in regard to transport. In this case, the influx of S in the presence of I can be described as

$$J_s = \frac{\frac{K_i'}{K_i'+[I]} \cdot J_{max} \cdot [S]}{K_s \cdot \left(\frac{K_i+[I]}{K_i}\right)\left(\frac{K_i'}{K_i'+[I]}\right)+[S]} \qquad (3)$$

Eqn. 3 can be rewritten for Dixon plot in the following form:

$$\frac{1}{J_s} = \frac{1}{J_{max}}\left\{1+\frac{K_s}{[S]}+\frac{K_s'+[S]}{K_i' \cdot [S]} \cdot [I]\right\} \qquad (4)$$

Eqn. 4 means that $1/J_s$ vs. [I] is linear on the Dixon plot. Although we speculated that the dead-end complex may be the complex of Gly-Leu with Gly-Leu hydrolase on the brush border membrane, the results of the present kinetic study of hydrolase activity and the effect of bestatin on the enzyme indicate that this possibility is unlikely. Therefore, the special function of Gly-Leu binding site in the Gly-Gly transport system is unknown at present. Since Gly-Leu bound to such an inhibitory site is considered not to be transported, the concept of a polyfunctional carrier as postulated by Alvarado [20] cannot be applied to the inhibition by Gly-Leu.

The inhibition of Gly-Gly transport by leucine could be defined as 'pseudo-competitive' from both Lineweaver-Burk and Dixon plots. Such a type of kinetic behavior can be explained if we assume a kinetic model as shown in Fig. 8. This model is very similar to that for the hyperbolic mixed type. But in the case of hyperbolic mixed type, it is assumed that the rate constants for [SX] and [SXI] are different. In Fig. 8, the rate constants are as-

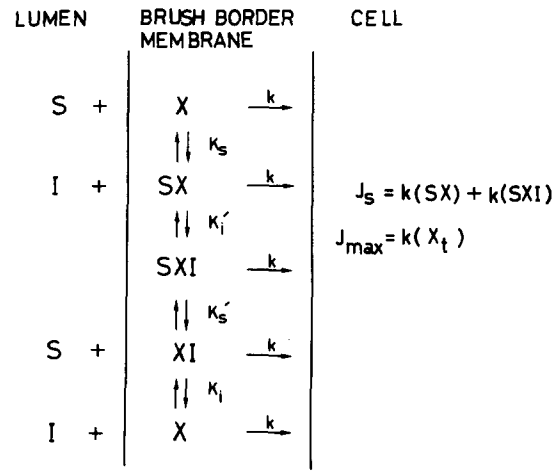

Fig. 8. Kinetic model for the 'pseudo-competitive' inhibition of the transport of a substrate S (Gly-Gly) by an inhibitor I (leucine). $K_s(K_s')$ and $K_i(K_i')$ are dissociation constants for S and I, respectively. $K_s/K_s' = K_i/K_i'$, by diffinition. k, translocation rate constant; X_t, local carrier density.

sumed to the same. In this model, a substrate, S (Gly-Gly in this case) and an inhibitor I (leucine in this case) are assumed to be transported by a common carrier which has two different binding sites for S and I. In such a case, the presence of I gives rise to so-called 'pseudo-competitive' inhibition [12]. Also in this case, the dissociation constants for I (K_i) and S (K_s) are assumed to change to K_i' and K_s' when the other ligand binds to its own carrier site. On the basis of such assumptions, the influx of S (J_s) can be given by:

$$J_s = \frac{J_{max} \cdot [S]}{K_s' \cdot \left(\frac{[I] + K_i}{[I] + K_i'} \right) + [S]} \quad (5)$$

Eqn. 5 indicates that the presence of I increases K_t without changing J_{max}. However, Dixon plot of $1/J_s$ vs. [I] is not linear but hyperbolic. Eqn. 5 can be rewritten as:

$$\frac{1}{J_s} = \frac{1}{J_{max}} \cdot \left\{ 1 + \frac{K_s'}{[S]} + \frac{K_s'}{[S]} \cdot \frac{K_i' - K_i}{[I] + K_i} \right\} \quad (6)$$

The inhibition by leucine is in accord with this type of interaction kinetically. However, this is not to say that all leucine is transported by the same carrier as that employed for Gly-Gly, since as previously reported [7], the value of J_{max} for leucine is about 3-times greater than that for Gly-Gly in guinea-pig ileum. Accordingly, about one-third of total leucine influx may be carried by the Gly-Gly transport system. In this regard, it can be said that Gly-Gly carrier has a feature of polyfunctional carrier as originally suggested by Alvarado [20].

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