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INTERACTION OF GLYCYLGLYCINE AND Na^+ AT THE MUCOSAL BORDER OF GUINEA-PIG SMALL INTESTINE

A NON-MUTUAL STIMULATION OF TRANSPORT

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Sodium-dependence of glycylglycine (Gly-Gly) influx and stimulation of Na^+ transport by Gly-Gly were studied in everted sacs, sheet preparations and brush-border membrane vesicles isolated from guinea-pig ileum. Gly-Gly influx was found to be independent of the presence of Na^+, while Na^+ transport was stimulated by Gly-Gly as evidenced by increases in transmural potential difference (PD_t), short-circuit current (I_{sc}) and Na^+ influx. The change in PD_t (ΔPD_t) induced by Gly-Gly was a saturable function of Gly-Gly concentration, showing a Michaelis-Menten type relationship. The half-saturation concentration for Gly-Gly estimated from the electrical data was nearly identical with that estimated from influx data. At a constant Gly-Gly concentration the relationship between I_{sc} and Na^+ concentration was sigmoid, and the Hill coefficient was 1.5. Kinetic analysis according to Garay and Garrahan indicates that each Gly-Gly carrier has two equivalent non-interacting binding sites for Na^+, and that translocation of Na^+ occurs when the two Na^+ sites on the carrier loaded with Gly-Gly are occupied by Na^+. However, our results indicate that the resultant Na^+ flow is not capable of stimulating Gly-Gly translocation.

Introduction

Since carrier-mediated transport of intact dipeptides or tripeptides was demonstrated in the small intestine, many investigators have tried to characterize the transport system(s) for the oligopeptides [1,2]. However, several important problems have still remained unsolved; for example, how many transport systems exist and how does Na^+ interact with the peptides at the carriers?

Early investigations [3–6] suggested that transport of some poorly hydrolyzed di- or tripeptides, such as glycylsarcosine or glycylsarcosylsarcosine, involved cotransport with Na^+, since Na^+-dependent uphill transport was found for these peptides. However, most recent investigators, who examined additional dipeptides, failed to demonstrate the Na^+-dependent nature of transport [7–12]. Some of these investigators have concluded that the Na^+-independence is rather common property of intact-peptide transport.

Our previous studies with everted intestines of guinea pig showed that transport of either intact glycyl-L-leucine (Gly-Leu) or Gly-Gly was not dependent on the presence of Na^+ [13,14]. However, it was seen in those studies that Gly-Gly which was transported in the intact form [15,16] evoked concentration-dependent changes in the transmural potential. Furthermore, the change in the transmural potential obeyed Michaelis-Menten kinetics as is the case for changes in the transmural poten-

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tial induced by D-glucose or amino acids which are cotransported with Na^+. These findings suggest that the carrier of Gly-Gly may mediate Na^+ transport when loaded with dipeptide, but Gly-Gly transport itself is not accelerated by the induced Na^+ flow through the same carrier.

The present study has been undertaken to characterize in more detail the interaction of Gly-Gly and Na^+ at the mucosal border of the small intestine. For this purpose, both the Na^+-dependence of Gly-Gly transport and the stimulation of Na^+ transport by Gly-Gly were reinvestigated in various preparations of guinea-pig ileum. In particular, characteristics of the Gly-Gly-induced increases in the transmural potential, short-circuit current and Na^+ influx across the mucosal border were studied in detail in the present study.

Materials and Methods

Preparation of everted intestine and influx measurements

The preparation of everted intestine and the methods of influx measurements were the same as those described in our previous paper [13]. Briefly, short segments, 2–3 cm long, of the ileum were excised from guinea-pigs anesthetized with urethane (1 g/kg body weight). The isolated segments were everted and fixed on a polyethylene tube of 5 mm outer diameter. Each tube had been marked with two lines to obtain a constant serosal surface area of 2 cm^2, and the everted intestine was fixed just over this area. The preparations were incubated in buffer solutions containing Gly-Gly or glycine at various concentrations for 1 min at 37°C. [1-^{14}C]Gly-Gly and [1-^{14}C]glycine were used as the tracers. These radioactive compounds were added to the incubation media at 0.2 μCi/ml. D-[1-^{3}H]Mannitol (2 μCi/ml) was also added simultaneously to estimate the volume of the extracellular fluid adhering to the mucosal surface. After completion of the incubation, the preparations were rinsed with ice-cold isotonic mannitol solution for 5 s, blotted on filter paper and extracted in 2 ml of 3% trichloroacetic acid. Na^+ influx was also measured under similar incubation conditions by using $^{24}Na^+$, which was added to the incubation medium at 2–3 μCi/ml. The radioactivities of ^{14}C, ^{3}H and ^{24}Na were counted in a liquid scintillation counter (ALOKA, LSC-703). [1-^{14}C]Gly-Gly (custom made), [1-^{14}C]glycine and [1-^{3}H]mannitol were purchased from Amersham International. ^{24}Na was supplied form the National Institute of Atomic Energy, Tokaimura, Japan. All data of uptake experiments were presented as means ±S.E. ($nmol \cdot min^{-1} \cdot cm^{-2}$ of the serosal surface area).

The standard buffer solution used in the present study had the following composition (mM): Na_2SO_4, 50; D-mannitol, 160; $KHCO_3$, 2.5; KH_2PO_4, 0.25; $CaSO_4$, 1.5; $MgSO_4$, 1.0; Tris-H_2SO_4 (pH 7.4), 20 (SO_4^{2-}-Ringer's solution). The solutions of lower Na^+ concentrations were prepared by replacing Na_2SO_4 with osmotically equivalent amounts of D-mannitol, Tris-sulfate or choline chloride. The reason for the use of the SO_4^{2-}-Ringer's solution was to create the same ionic conditions as those employed in electrical measurements. In electrical measurements, the use of the SO_4^{2-}-Ringer's solution had various advantages as described below. The replacement of Cl^- with SO_4^{2-} has no effect on glycine influx [16] and Gly-Gly influx (Himukai, M., unpublished data) across the mucosal border of guinea-pig small intestine.

Recording of Gly-Gly- and glycine-induced increases in the PD_t and I_{sc} from sheet preparations

In this series of experiments, the isolated ileal segments were opened along the mesenteric border and the sheet preparations were fixed between the Ussing-type half-chambers. Both the PD_t and I_{sc} were measured at 37°C and in the SO_4^{2-}-Ringer's solution above described, since the electrical potential changes induced by Gly-Gly or glycine were much augmented in the SO_4^{2-}-Ringer's solution because of reduction of the paracellular electrical conductance. Therefore, more precise measurements were possible in the SO_4^{2-}-Ringer's solution than in the Cl^--Ringer's solution. Also, the reproducibility of the electrical changes was maintained longer in the SO_4^{2-}-Ringer's solution than in the Cl^--Ringer's solution as noticed in a previous paper [13]. In the measurements of I_{sc}, non-polarizable electrodes consisting of Zn|$ZnSO_4$ cells were used for passing DC current across the preparations. Polyethylene tube bridges filled with 2% agar/1 M Tris-H_2SO_4 were used to connect

between the cells and the chambers. To record the PD_t, another pair of polyethylene tube bridges filled with 2% agar/1 M KCl were used. The tips of the bridges were placed in the vicinity of the mucosal and serosal surfaces of the preparations. The fluid resistance between the PD-recording electrodes was determined in the absence of the tissue, and the *IR* drop across this resistance was taken into consideration in the determination of I_{sc}. Gly-Gly or glycine was added to the mucosal side at various concentrations. When it was desired to eliminate any osmotic effect, the substance was added to the both sides at the same time. The ionic composition of the both sides was always identical.

Uptake experiments in brush-border membrane vesicles

Brush-border membrane vesicles were prepared by the Ca^{2+} precipitation method of Kessler et al. [17]. Briefly, the scraped materials of the intestinal mucosal surface were homogenized in a Waring blender after adding 100-fold volume of a hypotonic mannitol (100 mM) solution containing 5 mM Hepes-Tris (pH 7.4). After adding $CaCl_2$ to the final concentration of 10 mM, the homogenate was centrifuged at $6000 \times g$ for 10 min and the supernatant was subjected to differential centrifugation at $38\,000 \times g$ for 30 min. The pellet was resuspended in the same solution as that used for homogenization at the concentration of about 1 mg protein per ml. The specific activities of sucrase and alkaline phosphatase of the final preparations were found to have been enriched by a factor of 13 as compared with the original homogenate. ($Na^+ + K^+$)-ATPase was enriched about 2-fold, while lactate dehydrogenase activity was not detected.

Uptake of Gly-Gly and glycine by the membrane vesicles was measured by the rapid filtration method [18] at 25°C. The composition of the incubation medium was as follows (mM): D-mannitol, 100; Hepes-Tris (pH 7.4), 1; NaSCN or KSCN, 100; 0.1 Gly-Gly plus 1 μCi/ml [1-^{14}C]Gly-Gly or 0.1 glycine plus 1 μCi/ml [1-^{14}C]glycine.

Results

Effect of Na^+ on Gly-Gly influx in everted preparations

Our previous study [16] showed that Gly-Gly influx across the mucosal border of guinea-pig small intestine was poorly dependent on Na^+, but the difference in uptake in the presence of 100 mM Na^+ and absence of Na^+ was statistically significant. In that study, the everted preparations were incubated with Gly-Gly for 2 min, since the tissue uptake of Gly-Gly in the standard solution was found to increase linearly with time up to 2 or 3 min. However, it was noticed in the later observations that the linear uptake of Gly-Gly in the absence of Na^+ did not continue up to 2 min after the start of incubation. This led us to reexamination of the Na^+-dependence of Gly-Gly uptake by shortening the incubation period to 1 min.

The results of the reinvestigation are shown in Table I. As shown in the table, the influx of Gly-Gly was found to be unaffected entirely by the total replacement of Na^+ by either D-mannitol, Tris or choline. In contrast, the influx of glycine was markedly reduced or nearly abolished by the replacement of Na^+ as reported previously [16].

Effect of Na^+ on Gly-Gly uptake by brush-border membrane vesicles

In order to ascertain further the Na^+-independent nature of Gly-Gly transport, the effect of Na^+ on Gly-Gly transport was examined in isolated brush-border membrane vesicles. Fig. 1 shows a comparison of the time-course of uptake between glycine and Gly-Gly in the presence and absence of a Na^+ gradient. In the case of glycine, an overshoot uptake was seen when a Na^+ gradient was present. In contrast, there was no difference in the time-course of Gly-Gly uptake between the two different conditions, i.e., in the presence and the absence of Na^+ gradient, indicating the lack of any specific effect of Na^+ on Gly-Gly transport.

Effect of Gly-Gly on Na^+ influx in everted sac preparations

In contrast to the observed Na^+-independence of Gly-Gly influx, Na^+ influx across the mucosal border was significantly enhanced by Gly-Gly ad-

TABLE I
EFFECT OF TOTAL REPLACEMENT OF Na^+ ON GLY-GLY AND GLYCINE INFLUXES

Na^+ was totally replaced by D-mannitol, Tris-sulfate or choline chloride without changing the osmolality of the solutions. All data were obtained from six different animals. Influx data are given as means ± S.E.

Substrate (mM)	Incubation medium	Influx ($nmol \cdot min^{-1} \cdot cm^{-2}$)	Percentage of control	P (paired t-test)
Gly-Gly (1)	Standard solution (100 mM Na^+)	16.6 ± 1.34		
	Mannitol-substituted solution	15.7 ± 1.40	95	n.s.
	Tris-substituted solution	17.4 ± 1.62	105	n.s.
	Choline-substituted solution	15.9 ± 1.57	96	n.s.
Glycine (10)	Standard solution (100 mM Na^+)	75.4 ± 4.44		
	Tris-substituted solution	3.5 ± 1.12	5	$P < 0.001$

ded to the mucosal solution. As shown in Table II, 10 mM Gly-Gly increased Na^+ influx by about 47 $nmol \cdot min^{-1} \cdot cm^{-2}$, which corresponded to about 70% of the increase caused by 10 mM glycine. Such a large increase in Na^+ influx induced by Gly-Gly cannot be accounted for by the glycine liberated by the surface membrane hydrolysis of added Gly-Gly, since the hydrolysis of Gly-Gly is very scanty [15], and the K_t value for glycine transport by guinea-pig intestine is very high (about 30 mM) [16].

Effect of Gly-Gly on the transmural potential

When Gly-Gly was added to the mucosal bathing solution, the PD_t increased in a concentration-dependent manner as in the case of addition of glycine (Fig. 2). Both the Gly-Gly-induced and glycine-induced changes in PD_t were saturable when the concentration of added substances was increased. The double-reciplocal plot of the change in PD_t and concentration was linear for both Gly-Gly and glycine (data not shown), indicating that the changes in PD_t induced by both substances conform to Michaelis-Menten kinetics. The values of the maximum change in PD_t and the half

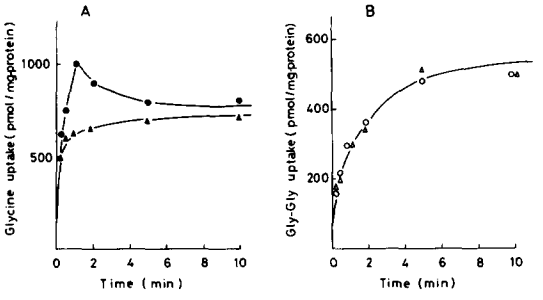

Fig. 1. The time-course of uptake of glycine (A) and Gly-Gly (B) into brush-border membrane vesicles in the presence and absence of an Na^+ gradient. The concentration of the test substances in the incubation medium was 0.1 mM. In (A), ● indicates the uptake in the presence of 100 mM NaSCN; ▲ in the presence of 100 mM KSCN. In (B), ○ indicates the uptake in the presence of 100 mM NaSCN; △ in the presence of 100 mM KSCN.

TABLE II
EFFECTS OF GLY-GLY AND GLYCINE ON Na^+ INFLUX ACROSS THE MUCOSAL BORDER OF GUINEA-PIG ILEUM

The everted preparations were preincubated in the standard medium (100 mM Na^+) for 20 min, and then the preparations were transferred to another medium containing $^{24}Na^+$ or $^{24}Na^+$ plus glycine or Gly-Gly and incubated for 1 min. The osmolality of the medium containing Gly-Gly or glycine was made identical with that of the standard medium by removing an equimolar amount of D-mannitol. The data are given as means ± S.E., n indicates the number of observations (different animals).

Addition	n	Na^+ influx ($nmol \cdot min^{-1} \cdot cm^{-2}$)	Increase in Na^+ influx from control value ($nmol \cdot min^{-1} \cdot cm^{-2}$)
None (control)	12	256.4 ± 10.6	
10 mM Gly-Gly	12	303.1 ± 10.8	46.7 ± 6.92
10 mM Glycine	12	322.1 ± 9.88	65.6 ± 10.9

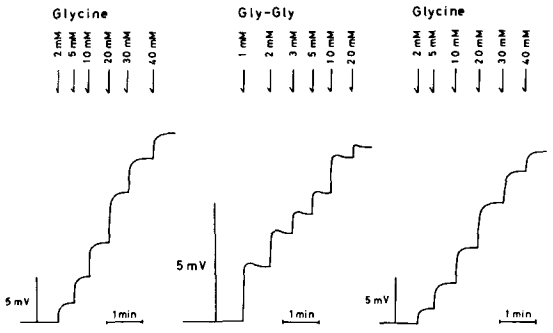

Fig. 2. The increases in the transmural potential (PD_t) of guinea-pig ileum evoked by addition of glycine and Gly-Gly to the mucosal bathing solution. Effects of cumulative increases in concentration of the added substrate are shown. All tracings were recorded from the same preparation successively. Note that the changes in PD_t induced by both glycine and Gly-Gly are saturable and the glycine-induced PD_t changes are reproducible. Amplification magnitude was increased 2.5-times when changes in PD_t induced by Gly-Gly were recorded. The level of the spontaneous PD_t (control level of PD_t) was about 3 mV, the mucosal side being negative.

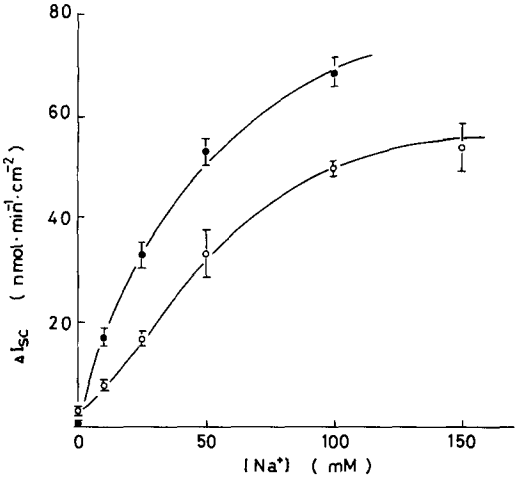

Fig. 3. Glycine- (●) and Gly-Gly- (○) dependent increases in short-circuit current (ΔI_{sc}) as a function of the concentration of Na^+ in the incubation medium. Reduction of Na^+ concentration was carried out by replacing with mannitol without changing the osmolality.

saturation concentration were significantly different between Gly-Gly and glycine as observed in kinetic studies of influxes of these two substances [16]. The values of the maximum change in PD_t and K_t for Gly-Gly were 6.1 ± 0.9 mV and 2.6 ± 0.4 mM ($n = 4$), and those for glycine were 28.8 ± 3.4 mV and 31.0 ± 2.3 mM, respectively. Such a significant difference between the values of the maximum change in PD_t indicates that the electrogenic processes responsible for the generation of PD_t changes by Gly-Gly and glycine are separate and independent. Furthermore, it should be pointed out that the electrically estimated value of K_t for Gly-Gly was about the same as that estimated from the data of flux measurements reported in our previous paper [14,16]. Such an identity of the K_t values was also confirmed for glycine.

Increase in short-circuit current induced by Gly-Gly

The short-circuit current I_{sc}, across the intestinal wall was measured at various Na^+ concentrations, and the effects of Gly-Gly and glycine on I_{sc} were studied kinetically. In the absence of Na^+, glycine induced no change in I_{sc}, while it increased I_{sc} markedly when Na^+ was present in the medium (Fig. 3). The increase in I_{sc} (ΔI_{sc}) induced by a certain fixed concentration of glycine obeyed a Michaelis-Menten-type relation to the Na^+ concentration over the range of 0 to 100 mM Na^+. In the presence of Gly-Gly, on the other hand, the increase in I_{sc} with increase in Na^+ concentration was found to be sigmoid. Also, in the absence of Na^+, Gly-Gly caused a small increase in I_{sc}. This small increase in I_{sc} was not abolished, even when one of the other ionic constituents, K^+, Ca^{2+}, Mg^{2+}, SO_4^{2-}, HCO_3^- or $H_2PO_4^-$, was omitted or replaced by other ion species. This increase showed a pH-dependence, the optimum pH being 7. At the present time, the genesis of this small Na^+-independent electrical change is unknown. The fraction of this Na^+-independent increase within the maximal increase in I_{sc} was very small. Therefore, we neglected this fraction in the following discussion.

The kinetic behavior of the Gly-Gly-induced ΔI_{sc} as a function of $[Na^+]$ was analyzed according to Garay and Garrahan [19] who presented a kinetic equation for Na^+ transport by $(Na^+ + K^+)$-ATPase of the red blood cell membrane. They assumed that multiple Na^+ sites on a single transporter have the same affinity constant and that there was no mutual interaction between the sites.

Based on these assumptions, they derived an equation for the probability of occupation of all Na^+ sites by Na^+ on a single transporter at one moment as a function of Na^+ concentration. They also assumed that translocation of Na^+ occurred only when all sites were occupied by Na^+. The final equation derived by them to describe the relation of Na^+ flux and Na^+ concentration is as follows:

$$\frac{[Na^+]}{J^{1/n}} = \frac{K_{Na}}{J_{max}^{1/n}} + \frac{[Na^+]}{J_{max}^{1/n}} \quad (1)$$

where $[Na^+]$ is the concentration of Na^+, J the flux of Na^+, K_{Na} the dissociation constant of the binding reaction of Na^+, J_{max} the maximum flux of Na^+, and n the number of Na^+ sites. Eq. 1 means that the plot of $[Na^+]/J^{1/n}$ against $[Na^+]$ yields a straight line with a slope of $1/J_{max}^{1/n}$ and the intercept at the abscissa corresponds to $-K_{Na}$.

Fig. 4 shows the plot of our data shown in Fig. 3 according to Eqn. 1, assuming $n = 1$. The line for $[Na^+]/\Delta I_{sc}$ vs. $[Na^+]$ was linear in the case of glycine ($\gamma = 0.959$) but non-linear (concave) in the case of Gly-Gly. When n was taken to be 2, the line relating $[Na^+]/\Delta I_{sc}^{1/2}$ to $[Na^+]$ for Gly-Gly became straight (data not shown). From the linear relationship obtained, the values of K_{Na} and J_{max} could be estimated to be 23 mM and 72.6 nmol · $min^{-1} \cdot cm^{-2}$, respectively. The results of this

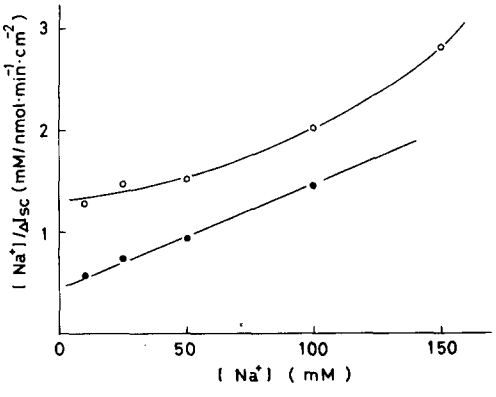

Fig. 4. Kinetic analysis of the effect of Na^+ on the glycine- (●) and Gly-Gly- (○) induced ΔI_{sc}. The data shown in Fig. 4. were re-plotted according to Eqn. 1 in the text. In this figure, calculations were made by assuming that n in Eqn. 1 was unity. The curve for the results of Gly-Gly-induced ΔI_{sc} was drawn by eye.

kinetic analysis susggest that the carrier for glycine has a single binding site ($n = 1$) for Na^+, whereas the carrier for Gly-Gly has two separate Na^+ binding sites ($n = 2$) and each carrier transfers two Na^+ at the same time when the carrier is loaded with the peptide. It should be pointed out that Gly-Gly influx from 10 mM Gly-Gly solution is 33.9 ± 4.5 nmol · $min^{-1} \cdot cm^{-2}$ ($n = 7$), thus the J_{max} for Na^+ in the presence of 10 mM Gly-Gly is about twice the Gly-Gly influx. However, this ratio does not mean the stoichiometry of transport, since Gly-Gly influx is entirely independent on Na^+ concentration. The values of ΔI_{sc} induced by 10 mM Gly-Gly and 10 mM glycine at 100 mM Na^+ were 49.5 ± 1.6 and 67.8 ± 2.9 nmol · $min^{-1} \cdot cm^{-2}$, these values being well in accord with the values of increases in Na^+ influx measured with $^{24}Na^+$ at 100 mM Na^+ (cf. Table II).

Another analysis of the data is based on the Hill equation. Implicit in the formulation of the Hill equation is the assumption of cooperativity between multiple substrate binding sites. According to the Hill equation, the relationship between ΔI_{sc} and $[Na^+]$ can be described as follows:

$$\log\left(\frac{\Delta I_{sc}}{\Delta I_{sc}^{max} - \Delta I_{sc}}\right) = h \cdot \log[Na^+] - \log K \quad (2)$$

where ΔI_{sc}^{max} is the maximum value of ΔI_{sc}, h is the Hill coefficient, and K is a constant comprising the interaction factors between binding sites and the intrinsic association constant [20]. In the Hill analysis, we needed an independent estimate of ΔI_{sc}^{max}. In the present analysis, we employed the value of ΔI_{sc}^{max} estimated from the Garay and Garrahan analysis as described above. The Hill plots of the data presented in Fig. 4 yielded the Hill coefficient of 1.0 for glycine-induced ΔI_{sc} and 1.5 for Gly-Gly-induced ΔI_{sc}, as shown in Fig. 5.

Thus, the results of kinetic analysis based on the principle presented by Garay and Garrahan [19] revealed that the number of Na^+ sites (n) on the single Gly-Gly carrier is 2. The Hill coefficient estimated from the same data ($h = 1.5$) also suggests the presence of at least two sites for Na^+. In the case of glycine, the similar kinetic analysis gave $n = 1$, and the Hill coefficient was unity. These results indicate that the number of Na^+ sites is different for glycine and Gly-Gly carriers.

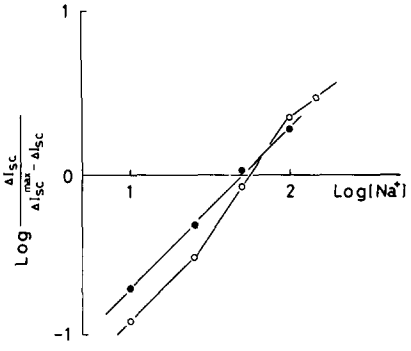

Fig. 5. The Hill plot of the glycine-induced (●) and Gly-Gly-induced (○) increases in I_{sc}. The data shown in Fig. 4 were re-plotted according to Eqn. 2 in the text.

Discussion

The results of the present study clearly indicate that the interaction of Gly-Gly and Na^+ at the mucosal border of the small intestine is non-mutual. Gly-Gly transport is entirely independent of the presence of Na^+ while Gly-Gly added to the mucosal solution obviously stimulates Na^+ influx across the same border. Such a non-mutual stimulation is a quite different feature from the mutual stimulation seen in usual Na^+-linked cotransport of organic solutes, e.g., D-glucose and L-amino-acids.

The Gly-Gly-induced changes in PD_t revealed a Michaelis-Menten type relation to Gly-Gly concentration when the external Na^+ concentration was constant. This type of behavior of the changes in PD_t is very similar to that of the PD_t changes induced by D-glucose and L-type amino acids as demonstrated by many previous authors [21–23]. Moreover, the value of the half-saturation concentration for Gly-Gly estimated from the electrical data was approximately the same as that estimated from the influx data. These findings suggest that the stimulation of Na^+ influx by Gly-Gly takes place on the carrier of Gly-Gly at the brush-border membrane. This is also supported by the fact that the maximum PD_t change induced by Gly-Gly coincides with that induced by Gly-Gly-Gly (data not shown) which is fully competitive with Gly-Gly [14]. Moreover, the stimulation of Na^+ transport or related phenomena, such as changes in PD_t and I_{sc}, were not observed in the guinea-pig colon or other Na^+-transporting epithelia, e.g., amphibian renal proximal tubule (Himukai and Hoshi, unpublished observation). These findings may exclude the possibility that the observed stimulation of Na^+ transport by Gly-Gly is a nonspecific effect of Gly-Gly. There may be an argument that the peptide transport might activate a parallel conductive pathway for Na^+ which is closely associated with the peptide carrier but separated in molecular structure. This possibility also seems unlikely, since it becomes difficult to explain the quantitative aspects of the stimulation of Na^+ transport by Gly-Gly when we assume the separate molecules. Moreover, there has been no report demonstrating the existance of a specific Na^+ channel, such as the amiloride-sensitive Na^+ channel. In fact, the Gly-Gly-induced electrical phenomena are entirely insensitive to amiloride (Himukai, unpublished observation).

Recent investigations of dipeptide transport have shown that many kinds of dipeptide are transported by Na^+-independent mechanisms across the mucosal border of the small intestine. This case has been proved for Gly-Leu [8,12,13], Leu-Gly [8], carnosine [7], Gly-Phe [11] and Gly-Pro [9,10]. On the other hand, the ability of di- and tripeptides to induce an immediate increment of the transmural potential has been demonstrated from an early investigation [24]. Moreover, the recent study of Boyd and Ward [25] demonstrated that all dipeptides tested by them, i.e., carnosine, Gly-Pro, Leu-Leu and Gly-Gly, caused an immediate and reversible depolarization of the luminal membrane of the intestinal cells and a concomitant increase in the luminal membrane electrical conductance. In view of these observations, together with the present findings, it is suggested that the non-mutual stimulation, as observed in the present study, may be a quite common feature among many kinds of dipeptide.

The cell membrane depolarization associated with an increase in the membrane conductance due to cotransport of Na^+ and sugars or amino acids have been observed in both the small intestine [26] and the renal proximal tubule [27]. These electrical phenomena are interpreted as the result of the increase in Na^+ conductance of the luminal membrane due to the formation of Na^+ pathways through the cotransport carriers [28].

The size and the polarity of the changes in the transmembrane potential induced by the sugars and amino acids are dependent on the initial level of the transmembrane potential, and the reversal of the induced potential change is observable when the initial level of transmembrane potentials was raised (depolarized) above a certain level [28]. Probably, the binding of a transported solute to its carrier site induces binding site(s) for Na^+ in an allosteric manner, thereby Na^+ becomes more permeable through the membrane. Alternatively, loading of the carrier with a transported solute may open the gate for Na^+ so that Na^+ becomes able to access its binding site(s) on or within the carrier. The enhancement of Na^+ influx and the associated electrical events induced by Gly-Gly suggests that the carrier for Gly-Gly has a similar gate-opening mechanism or mechanism of Na^+-site induction. In this regard, the Gly-Gly carrier appears to be somewhat similar to known cotransport carriers, e.g., the Na^+/glucose or Na^+/amino acid cotransporters. However, owing to the lack of coupling between Gly-Gly and Na^+ transport, Gly-Gly can not be driven by the induced Na^+ flow. Accordingly, the transport of Gly-Gly across the brush-border membrane exhibits characteristics of the uniport or facilitated diffusion.

It is obvious from the above discussion that the physiological significance of the enhancement of Na^+ transport by Gly-Gly is somewhat different from that of stimulation of Na^+ transport in the usual Na^+-linked cotransport. In the latter, Na^+ has at least three important effects. First, an Na^+ electrochemical potential gradient becomes utilizable as the driving force for uphill movement of the counter solute by virtue of binding of Na^+ to a common carrier [29,30]. Secondly, the binding of Na^+ to its carrier site may activate the binding site for the counter solute, as suggested by Alvarado and Mahmood [31] and Sepúlveda and Robinson [32]. The third effect is the stimulation of fluid absorption during the transport of the cotransported solute. In the case of interaction of Gly-Gly and Na^+, only the third effect seems to be of importance. We confirmed that Gly-Gly induced a stimulation of volume flow (J_v) more than that expected from isotonic transfer of water with twice the Gly-Gly influx (Gly-Gly is rapidly split into glycine within the cells [16]). The increases in weight of closed everted sacs incubated in the solution containing 100 mM NaCl were compared in the presence and absence of Gly-Gly or glycine. Gly-Gly added to the mucosal solution at 10 mM increased J_v by about 60%, i.e., from 3.92 ± 0.47 to 6.29 ± 0.96 ml $\cdot$ h^{-1} $\cdot$ g^{-1} dry wt. ($n = 5$), while 10 mM glycine enhanced J_v by 75%, i.e., from 4.14 ± 0.36 to 7.25 ± 0.51 ml $\cdot$ h^{-1} $\cdot$ g^{-1} dry wt. ($n = 5$). The ratio of the Gly-Gly-dependent increase in J_v to the glycine-induced increase in J_v ($2.37/3.11 = 0.76$) was near the ratio of the sum of twice the Gly-Gly influx (68 nmol $\cdot$ min^{-1} $\cdot$ cm^{-2}) and Gly-Gly-dependent increase in Na^+ influx (47 nmol $\cdot$ min^{-1} $\cdot$ cm^{-2}) to that of glycine influx (75 nmol $\cdot$ min^{-1} $\cdot$ cm^{-2}) and glycine-dependent increase in Na^+ influx (66 nmol $\cdot$ min^{-1} $\cdot$ cm^{-2}), i.e., $115/141 = 0.82$.

Hellier et al. [33] investigated the effects of Gly-Gly, Gly-Ala and glycine on absorption of Na^+ and water from the intestinal lumen, using jejunal perfusion in man. They found that both peptides and glycine significantly enhanced fluid absorption and showed that the ratio of transferred glycine and Na^+ was 1 : 1, whereas Gly-Gly : Na^+ was 1 : 2. Our results indicate that the ratio of transferred Gly-Gly and Na^+ varies depending on the Na^+ concentration, but, at a sufficiently high concentration of Na^+, the ratio of Gly-Gly influx to Gly-Gly-induced ΔI_{sc} approaches 1 : 2. Since Hellier et al. [33] carried out their studies at normal Na^+ concentration (150 mM), the ratio obtained by them is well in accord with that expected from our data obtained in the present study. However, it should be pointed out that the value of 1 : 2 ratio ($J_{Gly\text{-}Gly} : \Delta J_{Na^+}$) would be fortuitous, since there is no direct coupling between Gly-Gly and Na^+ influxes as demonstrated in the present study.

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