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Transport of glycyl-L-proline in intestinal brush-border membrane vesicles of the suckling rat: characteristics and maturation

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Transport of the dipeptide glycine-L-proline (Gly-L-Pro) in the developing intestine of suckling rats and its subsequent maturation in adult rats was examined using the brush-border membrane vesicles (BBMV) technique. Uptake of Gly-L-Pro by BBMV was mainly the result of transport into the intravesicular space with little binding to membrane surfaces. Transport of Gly-L-Pro in BBMV of suckling rats was: (1) Na^+ independent; (2) pH dependent with maximum uptake at an incubation buffer pH of 5.0; (3) saturable as a function of concentration (apparent $K_m = 21.5 \pm 7.9$ mM, $V_{max} = 8.6 \pm 1.5$ nmol/mg protein per 10 s); (4) inhibited by other di- and tripeptides; and (5) stimulated and inhibited by inducing a negative and positive intravesicular membrane electrical potential, respectively. Similarly, transport of Gly-L-Pro in intestinal BBMV of adult rats was saturable as a function of concentration (apparent $K_m = 17.4 \pm 8.6$ mM, $V_{max} = 9.1 \pm 2.1$ nmol/mg protein per 10 s) and was stimulated and inhibited by inducing a relatively negative and positive intravesicular membrane potential, respectively. No difference in the transport kinetic parameters of Gly-L-Pro was observed in suckling and adult rats, indicating a similar activity (and/or number) and affinity of the transport carrier in the two age groups. These results demonstrate that the transport of Gly-L-Pro is by a carrier-mediated process which is fully developed at the suckling period. Furthermore, the process is H^+-dependent but not Na^+-dependent, electrogenic and most probably occurs by a Gly-L-Pro/H^+ cotransport mechanism.

Introduction

The infancy period is characterized by rapid growth and development. Both of these processes depend largely on an adequate supply of nutrients and efficient absorption in the intestine. For this reason and because of the many adverse long-term effects of malnutrition in early life, studies on neonatal intestinal functions and their maturation have been of great interest. The structure and function of the gastrointestinal tract undergo ontogeny during the early stages of life [1,2]. This includes changes in the mechanism and capacity of transport of nutrients and substrates. These changes, however, do not follow a unified pattern. For example, transport of bile salts and calcium in suckling rats occur by a diffusion process that evolves with age to become an active process in adulthood [3–5]. Transport of glucose and folate,

Abbreviations: BBMV, brush-border membrane vesicles; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mes, 4-morpholineethanesulfonic acid.

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on the other hand, is by an active, carrier-mediated system both in suckling and adult rats [6,7]. Moreover, intestinal transport of folate, riboflavin, glucose and calcium is higher in the suckling period and decreases with maturation [4,6–8]. In contrast, transport of bile salts is lower in the suckling period and increases with maturation [3,5].

In recent years it has been well established that small peptides (di- and tripeptides) are transported from the intestinal lumen into the enterocyte by an efficient, specialized carrier-mediated process which is different from that of free amino acids [9–16]. Most of the previously reported studies, however, were performed in adult animals. On the other hand, only limited studies are available, describing the mechanism and characteristics of dipeptide transport in the developing intestine of suckling animals and its subsequent maturation in adulthood [17,18]. Furthermore, although the general characteristics (driving force, electrical nature, effect of pH, etc.) of the transport process of dipeptides across the intestinal brush-border membrane have been examined using brush-border membrane vesicles (BBMV), the available data are conflicting [11–14,16]. In this study, we examined the characteristics of the transport process of glycine-L-proline (Gly-L-Pro) in intestinal BBMV of suckling rats and determined its subsequent maturation in adult rats. We used Gly-L-Pro as a representative dipeptide because of its relative resistance to enzymatic hydrolysis [15].

Materials and Methods

[1-^{14}C]Glycyl-L-proline (spec. act. 6 Ci/mmol) was purchased (custom made) from Amersham (Arlington Heights, IL). [2-^{3}H]Glycine (spec. act. 43 Ci/mmol) was purchased from New England Nuclear Boston, MA. Valinomycin, glycine, di- and tripeptides and all other chemicals were purchased from Sigma Chemical Company (St. Louis, MO) and were of analytical quality. Cellulose nitrate filters (pore size 0.45 μM) were purchased from Sartorius Filters (Haywood, CA).

Preparation of BBMV and transport studies. Sprague-Dawley rats were used in this study. Suckling (15 days old) and adult (90 days old) rats were purchased from Sasco (Omaha, NE) and were fed Purina rat chow and tap water ad libitum.

Rats were killed by an overdose of ether. The jejunum was removed and the mucosa was scraped (in suckling rats the jejunum was the 20 cm distal to the initial 8 cm in the small intestine; in adult rats, the jejunum was the 30 cm distal to the initial 12 cm of the small intestine). BBMV were prepared from the mucosal scraping by a modification of Kessler's divalent cation (Mg^{2+}) precipitation technique [19] as described in detail by us previously [20,21]. The BBMV technique has been shown to be a suitable for the study of transport process in the developing intestine of suckling rats and their subsequent maturation [3,20]. Isolated BBMV were suspended in the desired volume of the transport (intravesicular) buffer (280 mM mannitol and 20 mM Hepes/Tris, pH 7.4); changes in this buffer are mentioned in the figure legends). The suspension was incubated for 1 h at room temperature to load the inside of the vesicles. Transport studies were performed at 37 °C by a rapid-filtration technique [22]. Reaction was initiated by adding a 20 μl aliquot of membrane vesicle suspension to 80 μl of incubation buffer (final concentrations: 100 mM NaCl or KCl, 80 mM mannitol and 20 mM Hepes/Tris or Mes; changes in this buffer are mentioned in the figure legends) containing various amounts of radiolabelled and unlabelled substrate plus other constituents. The preincubation and incubation media, unless otherwise stated, were always isoosmotic. The reaction was terminated by the addition of 1 ml of ice-cold stop solution (100 mM NaCl, 100 mM mannitol, 10 mM K_2HPO_4, pH 7.4). The cold, diluted reaction mixture was immediately pipetted onto a prewetted filter and kept under suction. The filter was rinsed with 5 ml of ice-cold stop solution and then dissolved in 5 ml ACS scintillation cocktail (Amersham). Radioactivity was counted in a liquid scintillation counter (Beckman Instruments, Model LS 3801, Irvine, CA). Nonspecific binding of the substrate to the filter (background) was determined by filtering a reaction mixture that contained an identical solution but no vesicles and was subtracted from the transport data. Transport results are expressed as mean ± S.E. (nmol/mg protein). Transport studies were performed at least in triplicate usually on two

separate BBMV preparations isolated on different days. Protein concentration was measured by the method by Lowry et al. [23] using bovine serum albumin as a standard.

The purity and suitability for transport studies of intestinal BBMV prepared from the intestine of rats of different ages has been shown by us previously [20].

Results

Binding vs. transport

To differentiate between binding of Gly-L-Pro to membrane surfaces and transport into the intravesicular compartment, we examined the uptake of the substrate as a function of changing incubation medium osmolarity. Fig. 1 shows the relationship between uptake at 10 s of incubation of 1 mM Gly-L-Pro by intestinal BBMV of suckling rats and 1/osmolarity of the incubation medium (the osmolarity was varied by changing the mannitol concentration). The relationship was found to be linear with a correlation coefficient (r) of 0.93. Extrapolating the line to infinite osmolarity showed minimal uptake of Gly-L-Pro (intercept = 0.025). These results indicate that at 10 s and under isotonic conditions 92% of Gly-L-Pro taken up by BBMV is the result of transport into the intravesicular space and the remaining 8% represents binding to membrane surfaces. (We also examined the effect of incubation osmolarity on uptake following incubation of intestinal BBMV for 90 min with 1 mM Gly-L-Pro. The results showed that under isotonic conditions 93.4% of the radiolabelled compound(s) taken up by the vesicles was the result of transport into an osmotically active intravesicular compartment).

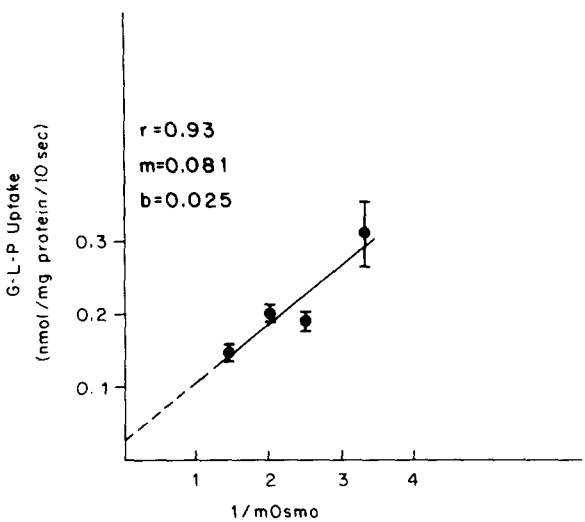

Fig. 1. Effect of incubation medium osmolarity on Gly-L-Pro transport. BBMV prepared from the intestine of suckling rats were preloaded with a buffer of 280 mM mannitol and 20 mM Hepes/Tris (pH 7.4). Incubation was performed for 10 s at 37°C in an incubation buffer of 100 mM NaCl, 20 mM Hepes/Mes (pH 5.0) and a sufficient amount of mannitol to give the indicated osmolarity. Gly-L-Pro (1 mM) was added to the incubation medium at the onset of incubation. Each data point represents the mean ± S.E. of at least three experiments. $y = mx + b$, where m = slope, $b = y$ intercept. r represents correlation coefficient.

In another study, we examined the uptake of 1 mM Gly-L-Pro at 37°C and compared the results to that at 4°C. Uptake of 0.50 ± 0.09 and 0.084 ± 0.02 nmol/mg protein per 10 s were recorded for Gly-L-Pro at 37°C and 4°C, respectively ($P < 0.01$). Since uptake at 4°C most probably represents binding to membrane surfaces, these results further suggest that uptake of Gly-L-Pro by BBMV at 37°C under isotonic conditions is mainly the result of transport of the substrate into the intravesicular space with little binding to membrane surfaces.

Effect of Na^+

The transport of 1 mM Gly-L-Pro in BBMV of suckling rats was determined in the presence of a Na^+ and a K^+ gradient (outside = 100 mM, inside = 0 mM) (the incubation buffer was 100 mM NaCl or KCl, 80 mM mannitol and 20 mM Hepes/Mes, pH 5; the transport buffer was 280 mM mannitol and 20 mM Hepes/Tris, pH 7.4). The results (Fig. 2) show that in both cases the transport of Gly-L-Pro is rapid and linear for approx. 60 s of incubation. A transient accumulation ('overshoot') of Gly-L-Pro in BBMV was observed in both cases with a peak value at around 1 min of incubation. Following that, accumulation decreased, indicating an efflux of the substrate from vesicles. No significant difference in Gly-L-Pro uptake was observed in the presence of a Na^+ or a K^+ gradient at any time point examined. (Transport of Gly-L-Pro was also not significantly different in the presence of a Na^+ and a K^+ gradient at $pH_i = pH_o = 7.4$ and no 'overshoot'

was observed, data not shown.) We elected to use a 10 s incubation time in all our subsequent studies, because it is within the initial linear rate of uptake and to minimize any possible metabolism of Gly-L-Pro (previous studies have established that negligible metabolism of Gly-L-Pro takes place in intestinal BBMV during this period of incubation, see Refs. 12 and 14).

In contrast to the Na^+-independent transport of Gly-L-Pro in BBMV, transport of glycine under identical conditions to that described above with Gly-L-Pro was found to be Na^+ dependent (Fig. 3). In the presence of a Na^+ gradient, transport of glycine was rapid and a transient accumulation ('overshoot') was observed with a peak value at around 60 s of incubation. Following that, uptake of glycine decreased, indicating efflux from vesicles and equilibrium was reached after 30 min of incubation. In the presence of a K^+ gradient (outside = 100 mM, inside = 0 mM) transport of glycine was slower and without an overshoot. Equilibrium values were reached after approx. 40 s of incubation.

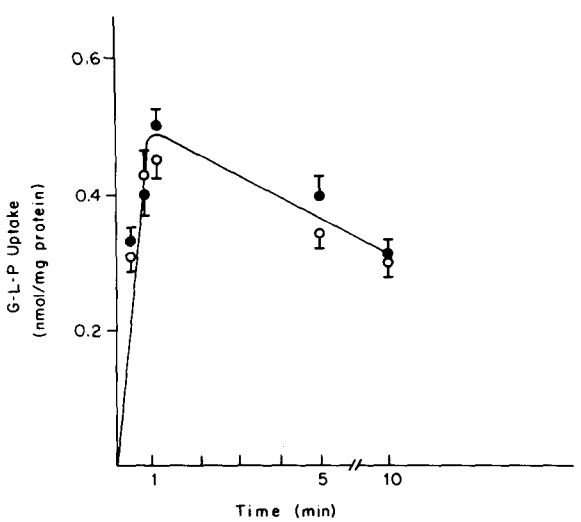

Fig. 2. Transport of Gly-L-Pro in the presence of a Na^+ and a K^+ gradient as a function of time. BBMV prepared from the intestine of suckling rats were preloaded with a buffer of 280 mM mannitol and 20 mM Hepes/Tris (pH 7.4). Incubation was performed in an incubation buffer of 100 mM NaCl (●) or KCl (○), 80 mM mannitol and 20 mM Hepes/Mes (pH 5.0). Gly-L-Pro (1 mM) was added to the incubation buffer at the onset of incubation. Each data point represents the mean ± S.E. of at least three experiments.

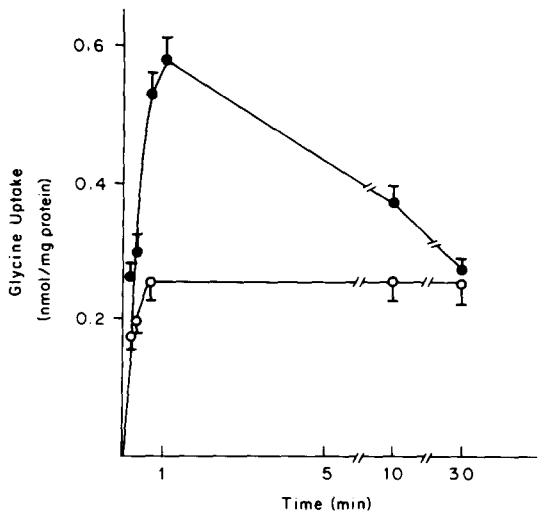

Fig. 3. Transport of glycine in the presence of a Na^+ and a K^+ gradient as a function of time. BBMV prepared from the intestine of suckling rats were preloaded with a buffer of 280 mM mannitol and 20 mM Hepes/Tris (pH 7.4). Incubation was performed in an incubation buffer of 100 mM NaCl (●) or KCl (○), 80 mM mannitol and 20 mM Hepes/Mes (pH 5.0). Glycine (1 mM) was added to the incubation buffer at the onset of incubation. Each data point represents the mean ± S.E. of at least three experiments.

Effect of pH

The effect of pH on the transport of Gly-L-Pro in intestinal BBMV is controversial [11,12,14,16]. Both stimulation and inhibition of dipeptide transport has been reported upon increasing H^+ concentration in the incubation medium [11,12,14,16]. In this study, we examined the effect of changing the pH value of the incubation buffer on the transport of 1 mM Gly-L-Pro into intestinal BBMV of suckling rats. The incubation buffer pH was varied by adjusting the composition of the buffering system (Hepes/Tris or Mes). Transport (intravesicular) buffer pH was 7.4 in all cases. The results (Fig. 4) show that decreasing the incubation buffer pH from 8 was associated with an increase in Gly-L-Pro transport which reached its highest level at pH 5.0. A further decrease in the incubation buffer pH lead to a decrease in Gly-L-Pro transport.

Effect of concentration

The transport of Gly-L-Pro in intestinal BBMV of suckling and adult rats was examined as a function of increasing the substrate concentration

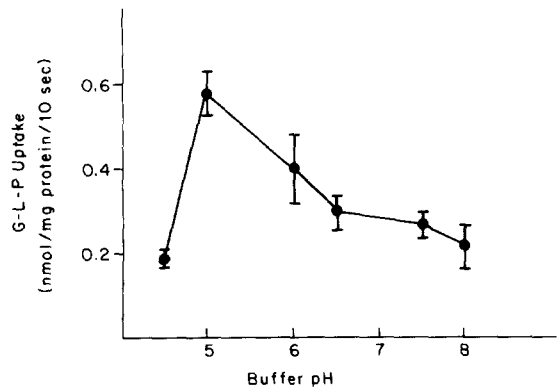

Fig. 4. Effect of incubation buffer pH on transport of Gly-L-Pro in intestinal BBMV of suckling rats. BBMV were preloaded with a buffer of 280 mM mannitol and 20 mM Hepes/Tris (pH 7.4). Incubation was performed for 10 s in an incubation buffer of 100 mM NaCl, 80 mM mannitol and 20 mM Hepes/Tris or Mes. Gly-L-Pro (1 mM) was added to the incubation medium at the onset of incubation. Each data point represents the mean ± S.E. of at least three experiments.

(1–35 mM) in the incubation medium. In both cases, saturation in the transport of Gly-L-Pro was observed (Figs. 5a and b). Transport kinetic parameters (apparent K_m and V_{max}) were calculated by a computerized model of the Michaelis-Wilkinson [24]. Apparent K_m values of 21.5 ± 7.9 and 17.4 ± 8.6 mM and V_{max} values of 8.6 ± 1.5 and 9.1 ± 2.1 nmol/mg protein per 10 s were calculated for suckling and adult rats, respectively.

TABLE I

EFFECT OF DI- AND TRIPEPTIDES AND FREE AMINO ACID ON TRANSPORT OF GLY-L-PRO

BBMV prepared from the intestine of suckling rats were preloaded with a buffer of 280 mM mannitol and 20 mM Hepes/Tris (pH 7.4). Incubation was performed for 10 s in an incubation buffer of 100 mM KCl, 20 mM mannitol and 20 mM Hepes/Mes, pH 5.0. Gly-L-Pro (1 mM) and the compound under study were added to the incubation medium at the onset of incubation. Each data point is the mean ± S.E. of at least three experiments. *P* values were determined using the Student's *t*-test. Comparison was made relative to control.

Compound	(mM)	Gly-L-Pro transport (nmol/mg protein per 10 s)	*P*
Control		0.280 ± 0.02	
Gly-L-Pro	(35)	0.162 ± 0.03	< 0.01
Gly-L-Leu	(60)	0.168 ± 0.05	< 0.01
Gly-L-His	(60)	0.083 ± 0.01	< 0.01
Gly-D-Phe	(60)	0.168 ± 0.04	< 0.01
Gly-L-Phe-L-Ala	(60)	0.174 ± 0.02	< 0.01
Gly-L-Leu-L-Tyr	(60)	118 ± 0.02	< 0.01
Gly	(60)	0.24 ± 0.07	n.s.

Effect of other peptides

In this study, we examined the effect of unlabelled Gly-L-Pro, certain di- and tripeptides and that of glycine on the transport of 1 mM [^{14}C]Gly-L-Pro. The results (Table I) show that all compounds examined, except glycine, significantly inhibit the transport of Gly-L-Pro.

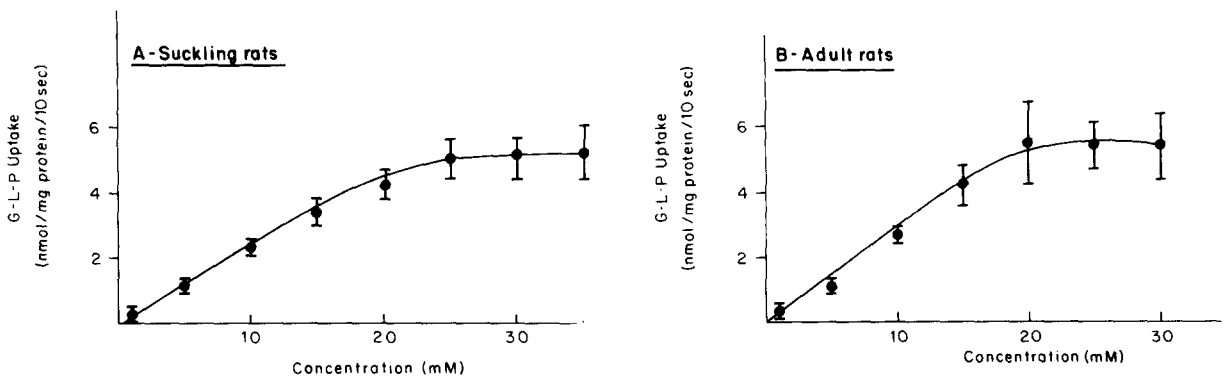

Fig. 5. Transport of G-L-P as a function of concentration in intestinal BBMV of (A) suckling and (B) adult rats. BBMV were preloaded with a buffer of 280 mM mannitol and 20 mM Hepes/Tris (pH 7.4). Incubation was performed for 10 s in an incubation buffer of 100 mM NaCl, 80 mM mannitol and 20 mM Hepes/Mes (pH 5.0) in the presence of different concentrations of Gly-L-Pro. Each data point represents the mean ± S.E. of at least three experiments.

Effect of transmembrane electrical potential

Conflicting data exist regarding the electrical nature of dipeptide transport in intestinal BBMV [11,12,14,16]. In this study, we examined the effect of imposing a transmembrane electrical potential on the transport of the dipeptide Gly-L-Pro in BBMV of suckling and adult rats. An established methodology was used which has been shown by us and others to induce alterations in transmembrane electrical potential in membrane vesicles [1,16,20,21,25,26]. A positive and a negative transmembrane electrical potential was generated with the use of the K^+ ionophore valinomycin (10 μg/mg protein) and an inwardly and an outwardly directed K^+ gradient, respectively.

In a first set of experiments, we examined the effect of imposing a relatively positive intravesicular compartment on Gly-L-Pro transport. This was done by examining the transport of 1 mM Gly-L-Pro in intestinal BBMV of suckling rats in the presence of valinomycin in the incubation medium and the presence of an inwardly directed K^+ gradient ($K_o = 100$ mM, $K_i = 0$ mM) (the incubation buffer was 100 mM KCl, 80 mM mannitol and 20 mM Hepes/Mes, pH 5; the transport buffer was 280 mM mannitol and 20 mM Hepes/Tris, pH 7.4). The results were compared to experiments performed simultaneously and in the presence of valinomycin, but in the absence of a K^+ gradient ($K_o = K_i = 100$ mM) (the incubation buffer was 100 mM KCl, 80 mM mannitol, 20 mM Hepes/Mes, pH 5; the transport buffer was 100 mM KCl, 80 mM mannitol and 20 mM Hepes/Tris, pH 7.4), i.e., a 'voltage clamp' condition. The rapid diffusion of K^+ in the first experiment will generate a transmembrane electrical potential with a relatively positive intravesicular compartment (as compared to the second experiment) thereby affecting any electrogenic component of Gly-L-Pro transport. The results show the transport of 1 mM Gly-L-Pro in BBMV of suckling rats to be significantly ($P < 0.01$) lower in the first experiment as compared to the second (transport of 0.149 ± 0.027 and 0.241 ± 0.011 nmol/mg protein per 10 s, respectively). Similarly, transport of 1 mM Gly-L-Pro in BBMV of adult rats was significantly ($P < 0.01$) lower in the first experiment as compared to the second (transport of 0.15 ± 0.005 and 0.21 ± 0.01 nmol/mg protein per 10 s, respectively).

In a second set of experiments we examined the effect of imposing a relatively negative intravesicular compartment on the transport of Gly-L-Pro into BBMV. This was done be examining the transport of 1 mM Gly-L-Pro in intestinal BBMV of suckling rats in the presence of valinomycin in the incubation medium and the presence of an outwardly directed K^+ gradient ($K_o^+ = 0$ mM, $K_i^+ = 100$ mM) (the incubation buffer was 280 mM mannitol and 20 mM Hepes/Mes, pH 5.0; the transport buffer was 100 mM KCl, 80 mM mannitol and 20 mM Hepes/Tris, pH 7.4). The results were compared to experiments performed simultaneously and in the presence of valinomycin, but in the absence of a K^+ gradient ($K_o^+ = K_i^+ = 100$ mM) (the incubation buffer was 100 mM KCl, 80 mM mannitol and 20 mM Hepes/Mes pH 5.0; the transport buffer was 100 mM KCl, 80 mM mannitol and 20 mM Hepes/Tris pH 7.4). The rapid diffusion of K^+ out of the vesicles in the first experiment will generate a relatively negative intravesicular compartment (as compared to the second experiment) thereby affecting any electrogenic component of Gly-L-Pro transport. The results showed that the transport of 1 mM Gly-L-Pro in BBMV of suckling rats was significantly ($P < 0.05$) higher in the first experiment as compared to the second (transport of 0.441 ± 0.065 and 0.252 ± 0.049 nmol/mg protein per 10 s, respectively). Similarly, transport of 1 mM Gly-L-Pro in BBMV of adult rats was significantly ($P < 0.01$) higher in the first experiment as compared to the second (transport of 0.314 ± 0.035 and 0.217 ± 0.031 nmol/mg protein, respectively).

Discussion

The present study examined the transport process of dipeptides in the developing intestine of suckling rats and determined its subsequent maturation in adult rats using Gly-L-Pro as a model substrate. The study of dipeptide transport during the suckling period has significant nutritional implications during an active period of growth and development. Three lines of evidence suggest the importance of dipeptide transport.

First, following a meal, more di- and tripeptides are liberated in the intestinal lumen compared to free amino acids [9]. Second, patients with specific genetic amino acid transport defects such as Hartnup disease and cystinuria maintain a normal protein balance, presumably because of an efficient small dipeptide absorption [9,10,15]. Third, peptidase activities in the jejunum are less developed during the suckling period [1]. Therefore, we postulated that a well-developed dipeptide transport system may exist during early life. Our study was performed using an isolated, purified BBMV technique. This technique allows study of the transport events of a substrate at the membrane level, apart from possible effects of tissue metabolism and with great ability to manipulate the conditions on one or both sides of the membrane. First, we determined whether the uptake of Gly-L-Pro by BBMV is the result of transport of the substrate into the intravesicular compartment or is due to binding to membrane surfaces. We did so by examining Gly-L-Pro uptake as a function of the osmolarity of the incubation medium and at 4°C compared to 37°C. The results from both experiments indicated that the majority of Gly-L-Pro taken up by these vesicles is the result of transport into the intravesicular space with little binding to membrane surfaces. In another experiment, we found the transport of Gly-L-Pro to be linear for approx. 60 s of incubation with a distinct 'overshoot' phenomena with a peak value at around 1 min of incubation.

It has been shown that transport of dipeptides in intact intestinal tissue preparations (e.g., everted gut sacs) is Na^+ dependent, i.e., transport is inhibited by Na^+ removal from the incubation medium [9,15]. On the other hand, transport of dipeptides in intestinal BBMV of adult animals has been shown to be Na^+ independent [11,12,14]. Our present data (Fig. 2) show that transport of Gly-L-Pro in BBMV of suckling rats is also Na^+ independent. Transport of the free amino acid glycine (a constituent of the dipeptide Gly-L-Pro) in intestinal BBMV of suckling rats under identical conditions, however, was Na^+ dependent (Fig. 3). The contrasting effect of Na^+ on dipeptide transport in intact intestinal tissue preparations as compared to isolated BBMV indicates that the inhibitory effect of Na^+ removal from the incubation medium in the intact tissue preparation is a secondary phenomenon (this issue is addressed further below). Similar findings were reported by us and others on the effect of Na^+ on the transport of folate in gut everted sacs and intestinal BBMV [7,21,27].

Transport of Gly-L-Pro in BBMV was found to be pH dependent. The transport of Gly-L-Pro was increased with decreasing incubation buffer pH, with maximum uptake at pH 5.0 (Fig. 4). These findings confirm the previously reported findings of Ganapathy et al. [11] and Takuma et al. [16] but are contrary to the recently published findings of Rajendran et al. [14], who showed actual inhibition of Gly-L-Pro transport at incubation buffer pH 5.5 as compared to that at pH 7.5. Transport of Gly-L-Pro in BBMV of suckling rats was found to be saturable as a function of concentration, indicating the involvement of a carrier-mediated system in the transport process. Furthermore, the apparent K_m and V_{max} values of the transport process of Gly-L-Pro in BBMV of suckling rats were found to be similar to those of adult rats (apparent $K_m = 21.5 \pm 7.9$ and 17.4 ± 8.6 mM; $V_{max} = 8.6 \pm 1.5$ and 9.1 ± 2.1 nmol/mg protein per 10 s, respectively). These findings indicate that the number and/or activity and the affinity of the transport carrier of Gly-L-Pro is similar in the two age groups. The findings also indicate that the dipeptide transport system is fully developed in the suckling period. The findings that the apparent K_m and V_{max} values of Gly-L-Pro transport are similar in suckling and adult rats differ from the previously reported finding of Himukai et al. [18] in the guinea-pig intestine. These workers have shown, using an intact tissue preparation (everted segments), that the V_{max} of the transport process of the dipeptide glycylglycine is higher in the suckling animals as compared to that in adults. The cause of this difference is not known but could be due to species variations and/or to the fact that these workers used an intact intestinal tissue preparation where tissue hydrolysis of dipeptides is extensive. The ability of other di- and tripeptides to inhibit Gly-L-Pro transport in BBMV of suckling rats further confirms the existence of a specialized carrier-mediated system for Gly-L-Pro transport.

Transport of Gly-L-Pro in BBMV of both suck-

ling and adult rats was found to be electrogenic in nature. This conclusion is based on the observation that altering the transmembrane electrical potential with the use of valinomycin and a K^+ gradient (inwardly or outwardly directed) leads to alterations in Gly-L-Pro transport. These findings confirm the previously reported findings of Ganapathy et al. [11] and Takuma et al. [16], but is against the recently reported finding of Rajendran et al. [14] who showed a lack of effect of transmembrane electrical potential on Gly-L-Pro transport. Our findings that imposing a relatively positive intravesicular compartment inhibits Gly-L-Pro transport, while imposing a relatively negative intravesicular compartment increases Gly-L-Pro transport clearly indicate that the neutral Gly-L-Pro is transported into the BBMV with a cation. This cation is not Na^+ (see Fig. 2). These findings, together with the observations that decreasing the incubation buffer pH causes an increase in Gly-L-Pro transport (Fig. 4) and the transient 'overshoot' phenomenon occurs in Gly-L-Pro transport in the presence of an inwardly directed pH gradient ($pH_i = 7.4$, $pH_o = 5$) (see results), strongly suggest that Gly-L-Pro is most probably transported with H^+, as has been proposed previously [11,16]. The existence of a layer of H^+ at the luminal surface of the small intestine (the intestinal surface acid microclimate) has been well documented by us and others both in adult and suckling rats [28–30]. The intestinal surface acid microclimate is a normal physiological phenomenon of intact and viable intestine, which depends for its normal existence and maintenance on normal intracellular metabolism and extracellular Na^+ [28]. The intestinal surface acid microclimate may provide the acidic environment that is required for Gly-L-Pro transport in vivo. This suggestion may explain the inhibition in dipeptide transport in intact intestinal tissue preparations observed upon Na^+ removal from the incubation medium [9,15]. This could be mediated through inhibition of the intestinal surface acid microclimate with subsequent inhibition in dipeptide transport.

In summary, the present study shows that the transport process of Gly-L-Pro in rat intestinal BBMV is fully developed at the suckling age and occurs by a carrier-mediated system. This system displays similar kinetics to that of adult rat intestine. In addition, transport of Gly-L-Pro in intestinal BBMV is H^+ but not Na^+ dependent, electrogenic in nature and most probably occurs through a Gly-L-Pro/H^+ cotransport mechanism.

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