View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

ELSEVIER

Biochimica et Biophysica Acta 1235 (1995) 93-99

BBA Biochimica et Biophysica Acta

Transport of glycine and lysine on the chloride-dependent β-alanine $(B^{0,+})$ carrier in rabbit small intestine

Lars Kristian Munck *, Bjarne Gyldenløve Munck

Department of Medical Physiology, University of Copenhagen, The Panum Institute, 12.4, Blegdamsvej 3C, DK-2200 Copenhagen, Denmark

Received 8 September 1994; accepted 8 December 1994

Abstract

Transport of glycine, lysine and β-alanine in rabbit, guinea pig and rat small intestine has been examined by measurements of the unidirectional influx across the brush border membrane of the intact epithelium. In rabbit distal ileum the chloride-dependent fraction of glycine transport, and all sodium- and chloride-dependent lysine transport is carried on the β-alanine carrier. Lysine eliminates all saturable, sodium-independent transport of glycine. The saturable, sodium-dependent, and lysine resistant influx of glycine is characterized by a $K_{1/2}^{Gly}$ of 60 mM. Glycine transport in the mid intestine of the guinea pig is chloride-independent and in the rat only a minute fraction may be chloride-dependent. These species do not possess an equivalent of the rabbit β-alanine carrier. In conclusion, glycine transport in rabbit distal ileum is by the sodium-dependent carrier of neutral amino acids, by the sodium-independent lysine carrier, and by the sodium- and chloride-dependent β-alanine carrier which closely resembles the $B^{0,+}$ carrier described in mouse blastocysts. All sodium dependent lysine transport in rabbit distal ileum is by the chloride- and sodium-dependent β-alanine carrier. It is proposed that the β-alanine carrier in rabbit distal ileum be renamed the $B^{0,+}$ carrier.

Keywords: Biological transport; Amino acid; Chloride; β-Alanine; (Guinea-pig); (Rat); (Rabbit)

1. Introduction

The rabbit distal ileum is equipped with a high-affinity amino acid carrier with a broad specificity termed the β-alanine carrier which transports alanine [1,2]. Cross inhibition studies suggest transport of leucine and lysine as well [1,2]. Inhibition studies indicate that the β-alanine carrier has a considerably higher affinity for the non-methylated α-amino-monocarboxylic acids alanine, leucine, serine, aminoisobutyric acid and the cationic amino acid lysine, than does the carriers of dipolar and cationic amino acids [1]. The carrier is chloride- and sodium-dependent [3] and differs from the very-high-affinity, low-capacity carrier of β-amino acids present throughout the rabbit small intestine [3-6].

The β-alanine carrier exhibits a striking resemblance to the $B^{0,+}$ carrier described in mouse blastocysts [7]. The $B^{0,+}$ carrier has a broad specificity accepting bipolar and cationic amino acids and β-alanine [7,8] and is both sodium- and chloride-dependent [9]. Leucine influx in rabbit distal ileum measured at 0.1 mM leucine is partly chloride-dependent [10] providing further indication of an identity of these two carriers. Here we complete this comparison by examining the chloride-dependence of glycine and lysine and by examining the transport of glycine in detail in rabbit small intestine. In addition, it is examined whether the β-alanine carrier could be the only sodium-dependent carrier of lysine in which case the absence of the β-alanine carrier in the jejunum [6] could explain the discrepancy between the observation of sodium-dependent lysine transport by intact ileal mucosa [11,12] and complete sodium-independent transport in jejunal brush-border membrane vesicles [13].

β-Alanine transport in the rat jejunum is chloride-independent, and in the guinea pig jejunum β-alanine is transported by the taurine carrier [14]. Here we examine whether an equivalent of the β-alanine or $B^{0,+}$ carrier exists in the small intestine of these species.

2. Materials and methods

2.1. Animals and materials

Female albino rabbits with a body weight of 2500-3000 g, female guinea pigs of 400 g and male Wistar rats of 200

* Corresponding author. Fax: +45 3532 7420.

0005-2736/95/$09.50 © 1995 Elsevier Science B.V. All rights reserved
SSDI 0005-2736(94)00309-2

g were used. The animals were raised and maintained with free access to water and food. Rabbits were killed by intravenous phenobarbital while rats and guinea pigs were anesthetized by intraperitoneal phenobarbital and killed by opening of the thorax.

All solutions were made from a phosphate buffer with a composition in mM of Na^+, 140; K^+, 8; Ca^{2+}, 2.6; Mg^{2+}, 1; Cl^-, 140; phosphate, 8; SO_4^{2-}, 1. The concentrations of sodium and chloride were varied by substitution with *N*-methyl-D-glucamine HCl and isethionate, respectively. pH was kept at 7.4. In the inhibition experiments the osmotic concentrations were kept constant within each series of experiments by keeping the sum of inhibitor and D-mannitol constant. 5 mM D-glucose was present in all solutions and served as a substrate and to reduce the effect of the amino acids on the potential difference across the luminal membrane. ^{14}C-labeled amino acids and ^{3}H-labeled poly(ethylene glycol) (^{3}H-PEG; mol. wt. 4000) were purchased from DuPont NEN Research. The chloride concentrations in the retracted incubation fluids were determined by chloride titration (CMT 10, Radiometer, Copenhagen).

2.2. Unidirectional influx across the brush-border membrane

Influx across the brush-border membrane of intact distal ileum from all species (0–30 cm from the ileocecal junction) and mid intestine of the guinea pig and rat was measured as previously described [6,15]. The excised intestine was mounted between Lucite plates such that the mucosa became exposed in the bottom of wells in which the solution was oxygenated and stirred by high rates of 100% O_2-flow at 37° C. Following preincubation for 20 min the tissues were incubated for 0.50 min. The incubation was stopped by flushing with ice-cold 300 mM D-mannitol, the exposed tissue cut out and blotted before extraction for 12 h in 0.1 mM HNO_3. The extract and the retracted incubation fluid was analyzed in a liquid scintillation counter (TRI-CARB 2200CA, Packard). The content of ^{3}H-PEG-4000 in the tissue extract was used to correct for extracellular contamination, and thus corrected, the content of ^{14}C-activity in the tissue extract was used to calculate the rate of amino acid influx across the brush-border membrane.

The unidirectional influx across the brush-border membrane (J_{mc}) was measured at different substrate concentrations, $[A]_m$, and at different inhibitor concentrations, $[I]_m$, in the mucosal bathing solutions. The results are pooled data from two to six animals and are given as mean ± S.E. with the number of observations in parentheses. Fluxes were measured in a paired design and compared using the paired Student's *t*-test. One way ANOVA was used for comparison of more than two groups and the effect of two factors on fluxes were compared using two-way ANOVA. The Student-Neuman-Keuls test or the Kruskal-Wallis test was used for multiple comparisons (Sigma-Stat; Jandel Scientific). A P value of 0.05 was used as the level of statistically significance throughout.

Estimates of the transport kinetics (± S.D.) for glycine were made by nonlinear least-square fitting (SigmaPlot 4.0; Jandel Scientific) of the experimentally determined relationships between J_{mc}^{A} and $[A]_m$ weighted by the inverse of the variance (1/S.D.) to models of one or two saturable processes with and without a nonsaturable component:

$$J_{mc} = \frac{J_{max}[A]_m}{K_{1/2} + [A]_m + \frac{[I]K_{1/2}}{K_i}} + P[A]_m \quad (1)$$

where P is the (non-saturable) diffusive permeability of A in cm/h. J_{mc}^{A} is given in μmol/cm^2 (serosal area) per h. The apparent affinity constant at which transport of A by the carrier is half maximal, $K_{1/2}$, and the inhibitory constant, K_i, are in mM. The estimates were evaluated by the even distribution of residuals and by the Chi square test with df equal to the number of experimental points less the number of parameters estimated.

3. Results

3.1. Chloride-dependence of glycine influx in rabbit distal ileum

Preliminary experiments revealed that the influx of glycine measured at 1 mM glycine in the presence 140 mM Cl^- and 0 mM Cl^- was 0.73 ± 0.06 and 0.56 ± 0.05 μmol/cm^2 per h, respectively ($n = 15$; $P = 0.002$) indicating that glycine transport in rabbit distal ileum is in part by a chloride-dependent carrier. This might reflect transport by the imino acid carrier or by the β-alanine carrier for which K_i^{Gly} is 0.20 mM [1]. Whether transport by the chloride-dependent imino acid carrier [10] could contribute to a chloride-dependent transport of glycine was examined by determining glycine inhibition of MeAIB influx and vice versa. The results given in Fig. 1 correspond to a K_i^{Gly} of 179 ± 15 mM. With 5 μmol/cm^2 per h as the maximal transport capacity for the imino acid carrier [1] a K_i^{Gly} of 179 predicts a contribution by this carrier to glycine influx of 0.03 μmol/cm^2 per h at 1 mM glycine. Consistent with this, MeAIB at ten times its $K_{1/2}$ did not significantly affect the transport of glycine (Fig. 1).

The role of the β-alanine carrier as a chloride-dependent transporter of glycine was examined by paired measurements of J_{mc}^{Gly} at 1 mM glycine and 140 mM NaCl with 0, 80, or 160 mM β-alanine + 160, 80, or 0 mM D-mannitol present: a J_{mc}^{Gly} of 1.05 ± 0.09 μmol/cm^2 per h was reduced to 0.52 ± 0.08 and 0.34 ± 0.04 μmol/cm^2 per h, respectively ($n = 6$; $P < 0.05$ by one-way ANOVA). The apparent discrepancy between the 24% reduction of glycine influx by removing chloride and this 68% inhibi-

tion by β-alanine is potentially confusing. It probably reflects the unusual inter-individual variation in β-alanine transport observed repeatedly [1,3] and the low $K_{1/2}^{Cl}$ for activation of β-alanine carrier [3]. This interpretation was examined in two series of paired experiments. First, the chloride-dependence of glycine influx (1 mM) was reexamined in the presence of 160 mM β-alanine. Given a $K_{1/2}^{\beta\text{-ala}}$ of 2.0 mM and a K_i^{Gly} of 0.20 mM [1] this β-alanine concentration should inhibit glycine influx on the β-alanine carrier by approx. 90%. Indeed, in the presence of 160 mM β-alanine J_{mc}^{Gly} measured at 140 and 0 mM Cl^- was 0.274 ± 0.013 and 0.240 ± 0.010 $\mu mol/cm^2$ per h, respectively ($n = 16$; $P = 0.04$). Second, glycine influx was measured at 140 and 0 mM Cl^- with 160 mM β-alanine or D-mannitol present (Fig. 2). The data demonstrate that β-alanine and removal of chloride reduce glycine influx by 65% and 50%, respectively. The chloride concentrations in the withdrawn incubation solutions were between 0 and 2 mM. Since the $K_{1/2}^{Cl}$ for activation of β-alanine carrier is 7.5 mM [3] the β-alanine carrier would be partly activated in the nominally zero mM Cl^- studies, which explains the difference in degree of inhibition. Thus, the chloride-dependent influx of glycine is carried on the β-alanine carrier.

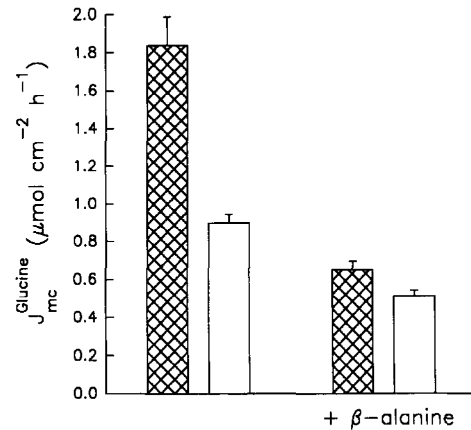

Fig. 2. Chloride-dependence of glycine influx in rabbit distal ileum. The influx of glycine was measured at 1 mM glycine in the presence of 0 mM Cl^- (open bars) or 140 mM Cl^- (cross hatched bars). Results obtained with 160 mM D-mannitol present are shown to the left and those obtained with 160 mM β-alanine to the right. Results are means ± S.E. of eight observations. By two-way ANOVA an effect of chloride removal and of β-alanine and of an interaction between these two is detected ($P < 0.001$). By all pairwise comparison both the removal of chloride, the addition of β-alanine and removal of chloride in the presence of β-alanine reduce lysine influx ($P < 0.05$).

3.2. Additional pathways for glycine influx in rabbit distal ileum

Sodium-dependent transport of glycine in rabbit distal ileum has been described as dominated by a process with a $K_{1/2}$ of 23 mM and J_{max} of 4.5 $\mu mol/cm^2$ per h in which was hidden a transport with a $K_{1/2}$ of 0.8 mM and a J_{max} of 0.1 $\mu mol/cm^2$ per h [16]. We have in the preceding identified this high-affinity carrier as the β-alanine carrier. Next, we examine whether transport of glycine conforms to the general scheme for transport of neutral amino acids [1,6,11–13,17].

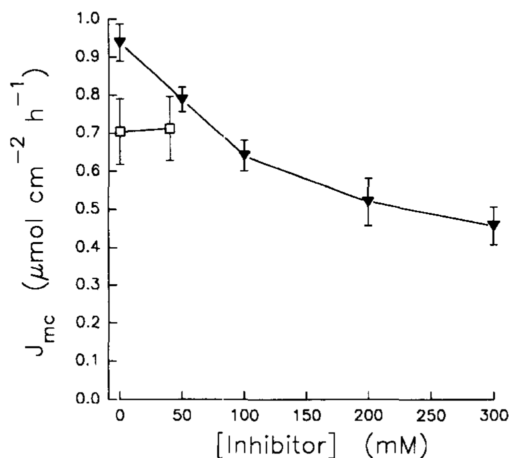

Fig. 1. Interaction of glycine with the imino acid carrier in rabbit distal ileum. The influx of MeAIB (▼) was measured at 1 mM MeAIB in the presence of 0–300 mM glycine – 300–0 mM D-mannitol (means ± S.E. of eight or nine observations; $K_i^{Gly} = 179 \pm 15$ mM, $n = 4$). The influx of glycine (□) was measured at 1 mM glycine in the presence of 0 or 40 mM MeAIB (means ± S.E. of nine observations; N.S.).

Glycine influx (1 mM) measured in paired experiments at 140 mM NaCl was 0.83 ± 0.5 $\mu mol/cm^2$ per h without inhibitor, 0.33 ± 0.03 $\mu mol/cm^2$ per h with 160 mM β-alanine present, and 0.11 ± 0.01 with 160 mM β-alanine and 100 mM lysine present ($n = 4$; $P < 0.05$ for both inhibitors by one-way ANOVA). The osmotic concentration was kept constant by addition of D-mannitol. The results demonstrate that not all of the lysine inhibitable fraction of J_{mc}^{Gly} is carried by the β-alanine carrier. Next, J_{mc}^{Gly} was measured at 1 mM glycine and varying lysine concentrations (Fig. 3). Maximum inhibition was obtained at 100 mM lysine as previously demonstrated for alanine, leucine and phenylalanine [11,13,18]. In paired experiments performed at 0 mM Na^+ and 1 mM glycine a J_{mc}^{Gly} of 0.063 ± 0.007 $\mu mol/cm^2$ per h was reduced to 0.023 $\pm$ 0.008 $\mu mol/cm^2$ per h by addition of 100 mM lysine ($P < 0.05$ by one-way ANOVA) while J_{mc}^{Gly} was 0.013 ± 0.0003, 0.027 ± 0.008, and 0.021 ± 0.004 $\mu mol/cm^2$ per h with the further addition of 50, 100, and 200 mM glycine (NS). The osmotic concentration was kept constant by addition of D-mannitol. These results show that at 0 mM Na^+ all saturable transport of glycine is eliminated by 100 mM lysine.

The kinetics of lysine-resistant glycine influx were determined by measuring J_{mc}^{Gly} at 140 mM Na^+ in the presence of 100 mM lysine (Fig. 4). In addition, Fig. 4 shows the above described sodium-independent, lysine resistant glycine fluxes as a function of glycine concentration with the best fit of these data to a non-saturable process (the diffusive permeability $P = 0.022$ cm/h). Us-

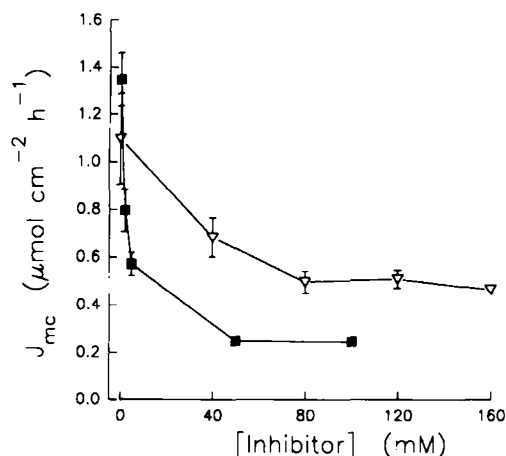

Fig. 3. Interaction between transport of glycine and lysine in rabbit distal ileum. The influx of glycine (1 mM; ■) was measured at 140 mM NaCl in the presence of 0–100 mM lysine and 100–0 mM D-mannitol (means ± S.E.; $n = 6$ or 7). Lysine influx (1 mM; ▿) was measured at 140 mM NaCl in the presence of 0–160 mM glycine and 160–0 mM D-mannitol ($n = 4$).

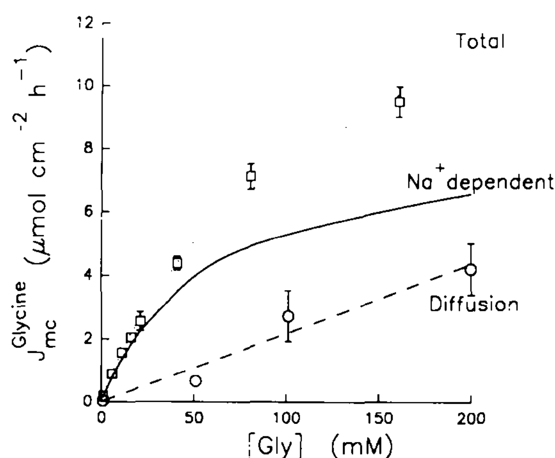

Fig. 4. Lysine-resistant glycine influx in rabbit distal ileum by a low affinity carrier. Glycine influx measured at 140 mM NaCl and 8 concentrations of glycine between 1–161 mM + 161–1 mM D-mannitol in the presence of 100 mM lysine (□ and dotted line; means ± S.E. of seven or eight observations). The sodium-independent, lysine resistant glycine fluxes measured at 0 mM Na^+, 1–200 mM glycine, 200–1 mM D-mannitol and 100 mM lysine were fitted to a nonsaturable process (○ and broken line; means ± S.E. of six or seven observations). The saturable, sodium-dependent and lysine resistant fluxes obtained by curve subtraction were fitted by nonlinear regression to one saturable process (solid line) given by Eq. (2).

ing this as an estimate of diffusive contribution to J_{mc}^{Gly} the saturable, sodium-dependent and lysine resistant J_{mc}^{Gly} was best (Chi-square value of 0.838, $df = 6$, $P = 0.975$) described by one saturable process:

$$J_{mc}^{Gly} = \frac{(8.5 \pm 0.5)[Gly]}{(58.2 \pm 7.7) + [Gly]} \tag{2}$$

3.3. Sodium- and chloride-dependence of lysine influx in rabbit distal ileum

Neutral and cationic amino acids share means of sodium-dependent as well as sodium-independent transport in rabbit distal ileum [11,12,17] but probably only sodium-independent means in rabbit jejunum [13] which also does not possess an equivalent of the ileal β-alanine carrier [1,3,13]. Mutual inhibition between lysine and β-alanine has been observed in the millimolar range [1]. In addition, mutual inhibition between lysine and glycine is demonstrated in Fig. 3. With this background the demonstration above of chloride-dependent transport of an α-amino-monocarboxylic acid by the β-alanine carrier posed the question whether also cationic amino acids were transported by this carrier in a chloride-dependent manner, and kindled the question whether the β-alanine carrier were their only means of sodium-dependent transport.

The chloride-dependence was examined by paired measurements of J_{mc}^{Lys} at 0.3 mM lysine (Fig. 5) and the sodium-dependence was examined in paired experiments at 1 mM lysine (Fig. 6). The results show that both the chloride-dependent and the sodium-dependent contribution to transport of lysine are of the same magnitude as the transport which can be ascribed to the β-alanine carrier.

Because very high inhibitor concentrations were used control experiments was performed in which lysine influx was measured without the potential disturbing influence of a hyperosmotic mucosal solution. J_{mc}^{Lys} measured at 1 mM lysine without β-alanine present was 1.60 ± 0.09 $\mu mol/cm^2$ per h at 140 mM Na^+ and 1.01 ± 0.09 at 0 mM Na^+ after preincubation at 140 mM Na^+ followed by a wash with 0 mM Na^+ ($n = 8$; $P = 0.003$). These results

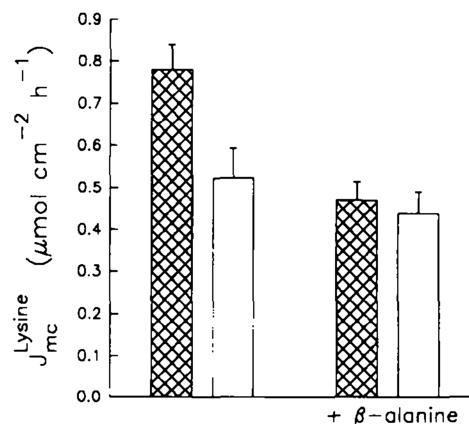

Fig. 5. Chloride-dependence of lysine influx in rabbit distal ileum. The influx of lysine was measured at 0.3 mM lysine in the presence of 0 mM Cl^- (open bars) or 140 mM Cl^- (cross hatched bars). Results obtained with 160 mM D-mannitol present are shown to the left and those obtained with 160 mM β-alanine to the right. Results are means ± S.E. of eight observations. By two-way ANOVA both the removal of chloride and the addition of β-alanine reduce lysine influx ($P < 0.05$). Removal of chloride in the presence of β-alanine does not reduce lysine influx further (N.S.).

Table 1
Influx of β-alanine in the distal ileum of the guinea pig and the rat

[Inhibitor]	[Cl⁻] (mM)	Guinea pig (nmol/cm² per h)	Rat (nmol/cm² per h)
None	140	49 ± 13	67 ± 12
	0	40 ± 10	44 ± 7
40 mM leucine	140	45 ± 21	16 ± 5
	0	21 ± 11	25 ± 7
100 mM lysine	140	41 ± 6	–
	0	39 ± 8	–

β-Alanine influx was measured at 1 mM β-alanine, 140 mM Na^+ and the Cl^- concentrations stated without inhibitor and in the presence of 40 mM leucine or 100 mM lysine as inhibitors. D-Mannitol was added to maintain isosmolarity. Data are means ± S.E. of 11 and 5 paired measurements in the rat and guinea pig, respectively. Analyzed by two-way ANOVA there was no effect of chloride removal or inhibitors in the guinea pig. In the rat there was an effect of leucine ($P < 0.001$) which did not reach statistical significance in the pairwise comparison.

are within the range of the results given above. In addition, a direct test of the effect of a hyperosmotic solution was performed in which the differences in osmotic concentration was not corrected; the influx of lysine measured at 1 mM lysine and 140 mM NaCl was 1.61 ± 0.07 and 1.75 ± 0.10 in the presence of 0 and 300 mM D-mannitol, respectively ($n = 12$; N.S.).

3.4. Chloride-dependence of glycine influx in guinea pig and rat

Glycine influx (1 mM) in the guinea pig mid intestine was 0.215 ± 0.009 and 0.207 ± 0.006 μmol/cm² per h measured at 140 mM and 0 mM Cl^-, respectively ($n = 8$; NS). In the rat mid intestine a very small, but statistically significant fraction of glycine influx was chloride-dependent; J_{mc}^{Gly} was 0.218 ± 0.015 and 0.179 ± 0.011 μmol/cm² per h measured at 140 mM and 0 mM Cl^-, respectively ($n = 12$; $P = 0.004$).

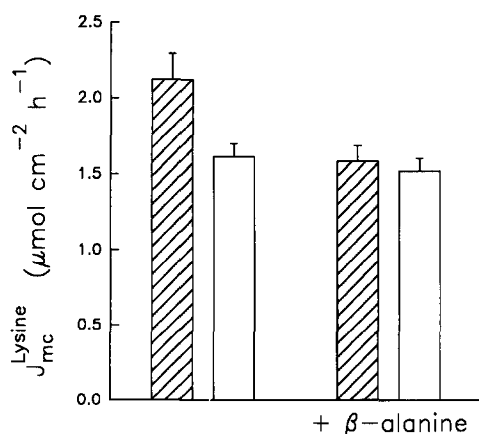

Fig. 6. Sodium-dependence of lysine influx in rabbit distal ileum. The influx of lysine (1 mM) was measured in paired experiments at 140 mM Na^+ (single hatched bars) and at 0 mM Na^+ (open bars) after preincubation at 140 mM Na^+ followed by a wash with 0 mM Na^+ (means ± S.E.; $n = 9$). The measurements were performed in the presence of 300 mM D-mannitol (left) and 300 mM β-alanine (right). Lysine influx is inhibited both by β-alanine and by the absence of sodium ($P < 0.05$ by two-way ANOVA) while the sodium-independent lysine influx is unaffected by β-alanine (N.S.).

The rat imino acid carrier is chloride-independent and accepts glycine and taurine. Only the separate high-affinity carrier of taurine and β-alanine is chloride-dependent [14,19]. Taurine influx measured at 5 μM taurine was 1.30 ± 0.09 and 1.37 ± 0.10 nmol/cm² per h in the absence and presence of 1 mM glycine, respectively ($n = 8$; N.S.), excluding the possibility of an interaction between glycine and taurine on the chloride-dependent high-affinity carrier.

Although the rat and guinea pig small intestine do not show the dramatic increase in transport capacity in aboral direction [14] seen in the rabbit [6] it could not be excluded that the distal ileum of these species possessed an equivalent to the β-alanine carrier. This possibility was addressed in both animals by paired measurements of β-alanine influx in the distal ileum (Table 1). In both species the β-alanine influx was minute. In the guinea pig there was no effect of chloride substitution or inhibitors. In the rat the leucine inhibitable β-alanine transport is chloride-independent. Thus, neither the rat nor the guinea pig posses an equivalent to the β-alanine carrier.

4. Discussion

4.1. Glycine transport

Glycine transport by rabbit distal ileum has been characterized as a low-affinity, high-capacity sodium-dependent process in which was hidden a low-capacity, high-affinity contribution [16]. Inhibition studies have shown that lysine and glycine have approximately the same affinity for the β-alanine carrier [1]. With the further results presented here it is demonstrated that transport of glycine conforms to the transport scheme of other bipolar α-amino-monocarboxylic acids: transport of alanine, leucine and phenylalanine has been resolved in (1) a sodium-dependent, lysine inhibitable fraction probably mediated by

the β-alanine carrier, (2) a sodium-independent, saturable fraction completely inhibitable by lysine, (3) a sodium-dependent, lysine resistant contribution attributed to the sodium-dependent carrier of neutral amino acids, and (4) a diffusive contribution defined as the sodium-independent and lysine resistant contribution [1,11–13,17,18].

The present study demonstrates mutual inhibition between the influx of glycine and lysine (Fig. 1) and shows that approx. 60% of the sodium-dependent glycine transport is inhibited by β-alanine and an even greater fraction is inhibitable by lysine. The chloride-dependent glycine transporter is shown to be identical to the β-alanine carrier (Fig. 2 and text). The other chloride-dependent carrier, the imino acid carrier, does not contribute to the transport of glycine (Fig. 1), and the capacity of the β-amino acid carrier is too small to account for the transport [3–5]. The small sodium-independent fraction of glycine influx was completely inhibited by lysine which indicates that the rabbit small intestine possess an equivalent of the $b^{0,+}$ carrier described for mouse blastocysts [8]. In addition, glycine was transported by a lysine resistant and sodium-dependent, low-affinity carrier with a $K_{1/2}^{Gly}$ of approx. 60 mM. The difference between this estimate and earlier estimate of 23 mM [16] is not surprising since the latter was obtained without correction for the contribution by the β-alanine carrier.

Clearly the distribution of glycine transport on these three secondary active transporters and diffusive transport depends on the chosen concentrations. But it is evident that at the physiological relevant concentration of 1 mM the basal contribution is that of the β-alanine carrier followed by the sodium-independent contribution and the lysine resistant, sodium-dependent contribution with only a very small diffusive fraction. An apparent discrepancy is revealed by the glycine influx being higher in the absence of chloride than at 140 mM chloride plus 160 mM β-alanine and at 0 mM chloride plus 160 mM β-alanine. However, during incubation at 0 mM chloride the tissue itself remains a source for diffusion of chloride into the incubation fluid as indicated by the detection of chloride in the withdrawn test solutions which also underestimates the concentration at the transport site. 1 mM glycine is 5-times its K_i against β-alanine influx observed at 140 mM NaCl and can therefore be expected to give rise to a significant transport which will not be completely inhibitable by 160 mM β-alanine, 80-times the $K_{1/2}^{\beta\text{-ala}}$. It is therefore reasonable to take the combined effect of chloride substitution and 160 mM β-alanine as a measure of the chloride-dependent fraction of glycine transport on the β-alanine carrier.

4.2. Lysine transport

Mutual inhibition between lysine and β-alanine has been demonstrated in rabbit ileum [1]. Except in its distal section rabbit jejunal transport of β-alanine can be accounted for by the taurine carrier [4], since the β-alanine carrier is absent [3,6]. Similarly, sodium-dependent transport of lysine is probably absent in the rabbit jejunum [13] although clearly present in distal ileum [11] and distal jejunum [6]. It is, therefore, possible that the β-alanine carrier could be not only a chloride-dependent carrier of lysine but also exclusively responsible for its sodium-dependent transport. This was confirmed by the data of Figs. 5 and 6. Fig. 5 demonstrates a chloride-dependence which quantitatively corresponds to the previously described characteristics of the β-alanine carrier, a maximum rate of transport of 0.8 $\mu mol/cm^2$ per h and a K_i^{Lys} of 0.4 mM. In contrast to the glycine series the effect of β-alanine and removal of chloride could not be secluded. This difference is likely to be caused by the ratio between substrate concentration and the affinity constant. Likewise, the effect of sodium removal and of β-alanine addition were identical and inseparable (Fig. 6).

The demonstration of chloride-dependent transport of glycine and lysine by the β-alanine carrier, together with the previous observation of a small but statistically significant chloride-dependence of leucine influx measured at 0.1 mM leucine [10], indicates that the previously demonstrated chloride-dependence of the β-alanine carrier in rabbit distal ileum [3] is a property of the carrier itself and not of any particular substrate.

Because of a $K_{1/2}^{\beta\text{-ala}}$ of 2 mM and K_i values for glycine and lysine actions on the β-alanine carrier of 0.2–0.4 mM [1] very high concentrations of β-alanine were needed to fully suppress transport of 1 mM glycine and lysine by this carrier. The emerging high osmolarities might affect the amino acid fluxes through activation of volume regulatory responses [20] or through solvent drag [21]. However, the data reported here demonstrate similar values of lysine influx in the presence of 0 and 300 mM D-mannitol, indicating that such an osmotic effect is not present when intact epithelia are exposed to hyperosmotic solutions for only 30 seconds. A conclusive demonstration of this fact was the demonstration of identical lysine fluxes measured at 1 mM lysine in paired experiments with 0 or 300 mM D-mannitol present.

4.3. Identity of the β-alanine carrier and the $B^{0,+}$ carrier

Sodium-dependence of β-alanine influx on the β-alanine carrier has been demonstrated using choline as sodium substitute [1]. Furthermore, transport of β-alanine by the β-alanine carrier is chloride-dependent and bipolar α-amino acids as well as cationic amino acids have a high-affinity for this carrier [1,3]. These results are confirmed here and it is demonstrated that their transport is chloride-dependent. These are the characteristics of the $B^{0,+}$ carrier described in mouse blastocysts [7–9] suggesting a very close resemblance between these carriers. Van Winkle's group used both lithium and choline as sodium substitutes [7–9]. We, therefore, suggest that the '$B^{0,+}$

carrier' be substituted for the 'β-alanine carrier' in epithelial tissue since this term is too easily confused with the term β-amino acid carrier used for the chloride- and sodium-dependent carrier of taurine and β-alanine.

4.4. Lack of a $B^{0,+}$ carrier in rat and guinea pig small intestine

Mutual inhibition between lysine and leucine, alanine and methionine has been observed in the rat small intestine in the presence of sodium [22]. A minor part of glycine influx in rat mid intestine is chloride-dependent. However, this part of the rat small intestine does not possess a chloride-dependent β-alanine carrier and glycine does not interfere with the taurine carrier, the only chloride-dependent amino acid carrier so far described in the rat [14,19]. The possibility that rat ileum like rabbit ileum were equipped with an equivalent of the $B^{0,+}$ carrier can be excluded on the basis of the data on β-alanine transport in rat distal ileum. As shown in Table 1 a leucine inhibition but no chloride-dependence was demonstrated. The chloride-dependent fraction of glycine influx is so small that it appears biologically insignificant and we are unable to ascribe it to a specific carrier in the rat small intestine. In the mid small intestine of guinea pig glycine transport is chloride-independent. In the distal ileum transport of β-alanine measured at 1 mM is chloride-independent and not inhibitable by lysine or leucine (Table 1). Thus, neither the rat nor the guinea pig small intestine possess an equivalent to the $B^{0,+}$ carrier.

Acknowledgements

This study was supported by the NOVO foundation, the Danish Medical Research Council, and the Danish Medical Association.

References

[1] Munck, B.G. (1985) J. Membr. Biol. 83, 15–24.
[2] Andersen, V. and Munck, B.G. (1987) Biochim. Biophys. Acta 902, 145–148.
[3] Munck, L.K. and Munck, B.G. (1992) Am. J. Physiol. 262, G609–G615.
[4] Miyamoto, Y., Nakamura, H., Hoshi, T., Ganapathy, V. and Leibach, F.H. (1990) Am. J. Physiol. 259, G372–G379.
[5] Miyamoto, Y., Tiruppathi, C., Ganapathy, V. and Leibach, F.H. (1989) Am. J. Physiol. 257, G65–G72.
[6] Munck, L.K. and Munck, B.G. (1992) Biochim. Biophys. Acta 1116, 83–90.
[7] Van Winkle, L.J., Christensen, H.N. and Campione, A.L. (1985) J. Biol. Chem. 260, 12118–12123.
[8] Van Winkle, L.J. (1988) Biochim. Biophys. Acta 947, 173–208.
[9] Van Winkle, L.J., Haghighat, N., Campione, A.L. and Gorman, J.M. (1988) Biochim. Biophys. Acta 941, 241–256.
[10] Munck, L.K. and Munck, B.G. (1990) Biochim. Biophys. Acta 1027, 17–20.
[11] Munck, B.G. (1985) J. Membr. Biol. 83, 1–13.
[12] Paterson, J.Y., Sepulveda, F.V. and Smith, M.W. (1981) J. Physiol. 319, 345–354.
[13] Stevens, B.R., Ross, H.J. and Wright, E.M. (1982) J. Membr. Biol. 66, 213–225.
[14] Munck, L.K. and Munck, B.G. (1994) Am. J. Physiol. 266, R997–R1007.
[15] Schultz, S.G., Curran, P.F., Chez, R.A. and Fuisz, R.E. (1967) J. Gen. Physiol. 50, 1241–1260.
[16] Peterson, S.C., Goldner, A.M. and Curran, P.F. (1970) Am. J. Physiol. 219, 1027–1032.
[17] Paterson, J.Y., Sepulveda, F.V. and Smith, M.W. (1979) J. Physiol. 292, 339–350.
[18] Munck, B.G. and Munck, L.K. (1994) J. Physiol. 480, 99–107.
[19] Barnard, J.A., Thaxter, S., Kikuchi, K. and Ghishan, F.K. (1988) Am. J. Physiol. 254, G334–G338.
[20] Handler, J.S. and Kwon, H.M. (1993) Am. J. Physiol. 265, C1449–C1455.
[21] Pappenheimer, J.R. (1990) Am. J. Physiol. 259, G290–G299.
[22] Munck, B.G. and Rasmussen, S.N. (1975) Biochim. Biophys. Acta 389, 261–280.