# Beta-amino acid transport in pig small intestine in vitro by a high-affinity, chloride-dependent carrier

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## Abstract

This study describes unidirectional influx of amino acids and D-glucose across the small intestinal brush-border membrane of fully weaned eight week old pigs. Influx is minimal in the duodenum and maximal in the distal and/or mid small intestine. Influx of $\beta$-alanine, taurine and $N$-methyl-aminoisobutyric acid is chloride-dependent. The activation stoichiometry for taurine influx is $1.0 \pm 0.2$ chloride/$2.4 \pm 0.3$ sodium/1 taurine. Influx of D-glucose, lysine, glycine and glutamate is chloride-independent. An ABC test demonstrates a common $\beta$-amino acid carrier: (a) the apparent affinity constant $K_{1/2}^{Taurine}$ is $44 \pm 13$ $\mu$M (means $\pm$ S.D.) and the inhibitory constant ($K_i^{Taurine}$) against $\beta$-alanine influx is $41 \pm 5$ $\mu$M (means $\pm$ S.E.). (b) $K_{1/2}^{\beta\text{-alanine}}$ is $97 \pm 23$ $\mu$M and $K_i^{\beta\text{-alanine}}$ against taurine influx is $160 \pm 22$ $\mu$M. (c) $K_i^{Hypotaurine}$ against taurine and $\beta$-alanine influx is $43 \pm 4$ ($n = 7$) and $22 \pm 5$ $\mu$M ($n = 7$), respectively. In conclusion, a high affinity, low capacity, sodium- and chloride-dependent carrier of $\beta$-amino acids is present in pig small intestine.

Keywords: Swine; Biological transport; Physiology; Amino acid; Glucose; Small intestine; Chloride

## 1. Introduction

Reference to the pig (Sus scrofa) in the literature on amino acid transport in the intestine is sparse. Yet the intestinal structure and function and the patterns of development are very similar in pigs and humans [1,2] suggesting that the pig could be a more relevant model of amino acid absorption in humans than rodents and the rabbit. Studies using everted sacs of pig jejunum demonstrated accumulation of neutral amino acids [3]. Neonatal pig colon is capable of transporting amino acids [4] and Michael Smith's group has provided extensive studies on colonic uptake of neutral amino acids using autoradiographic techniques and measurements of unidirectional, transmural fluxes in Ussing chambers [5–10]. More recently, uptake of methionine [11,12], threonine [13] and alanine [12,14] into jejunal brush-border membrane vesicles has been re-ported. An attempt to further characterize the transport system responsible for threonine uptake was hampered by the use of brush-border membrane vesicles which were not voltage clamped [13]. Additional attempts towards a systematic description of the amino acid carriers present in pig small intestine are lacking. Furthermore, only one study has addressed the question of regional distribution of amino acid transport along the pig small intestine using leucine and proline as probes [15].

A high affinity carrier of $\beta$-amino acids has been described in the brush-border membrane of all epithelia studied hitherto [16–25] except the adult cat [26]. In rabbit distal ileum an additional sodium-dependent carrier of $\beta$-alanine, bipolar and cationic amino acids with the characteristics of the $B^{0,+}$ carrier has been described [27].

The purpose of this study is to describe the regional distribution of amino acid transport using substrates of the different transport systems described in the brush-border membrane of enterocytes. As an initial step towards a systematic description of the amino acid carriers present in pig small intestine the chloride-dependence of these sub-

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strates is also reported. In addition, the β-amino acid transport system is described in detail.

## 2. Materials and methods

### 2.1. Animals and surgical procedures

Danish Landrace/Yorkshire crossbred 8-week-old, fully weaned female pigs (12–15 kg) were kept on a normal diet and switched to tap water containing 5 mM D-glucose 12 h prior to surgery. The pigs were sedated with an intramuscular injection of 5 mg $kg^{-1}$ azaperone (Sedaperone, Janssen, Denmark) and anaesthetized with pentobarbitone (10 mg $kg^{-1}$, i.v.) followed by halothane inhalation (2% in 2 l $min^{-1}$ $O_2$). Body temperature was maintained at 38°C.

A midline incision was made and segments of small intestine were removed at 0 and 75 min at which time the animal was killed with pentobarbitone (50 mg $kg^{-1}$, i.v.). For the topographic study the duodenum between the papilla Vateri and the ligament of Treitz was removed together with 10 cm segments of the small intestine 1 m distal to the ligament of Treitz (proximal jejunum), 5 m from Treitz (mid intestine) and 1 m from the ileocecal valve (distal ileum). The kinetics of D-glucose and leucine were determined in distal ileum while the mid intestine was used in all other experiments. Only normal epithelium without Peyer's patches, which occupied between one half and two thirds of the circumference in the distal ileum, was used. Control experiments revealed no difference in transport whether the tissue was removed at 0 or 75 min. The tissues were removed immediately after cutting the blood supply, stripped of the muscle layers, opened along the mesenteric border and placed in ice-cold glucose-free Ringer.

### 2.2. Materials

All solutions were made from a phosphate buffer with a pH of 7.4, and 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. 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 except when studying D-glucose fluxes. $^{14}$C-labeled amino acids and $^3$H-labeled polyethyleneglycol ($^3$H-PEG; mol.wt. 4000) were purchased from DuPont NEN Research.

### 2.3. Unidirectional influx across the brush-border membrane

Influx across the brush-border membrane of intact epithelium was measured as previously described [28,29]. Within 15 min after cutting the blood supply 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 30 s. 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 were analyzed in a liquid scintillation counter (TRI-CARB 2200CA, Packard). The content of $^3$H-PEG 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 ($J_{mc}^A$) was measured at different substrate concentrations, $[A]_m$, and at different inhibitor concentrations, $[I]_m$, in the mucosal bathing solutions. The results constitute pooled data from two animals or more and are given as means ± 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 transport along the intestine and for testing several inhibitors; the Student-Neuman-Keuls test was used for multiple comparisons (Sigma-Stat; Jandel Scientific). A P-value of 0.05 was used as the level of statistical significance throughout.

Estimates of the transport kinetics (± S.D.) 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, and for sodium-independent fluxes to a nonsaturable function alone:

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

where $P$ is the (non-saturable) diffusive permeability of A in cm $h^{-1}$. $J_{mc}^A$ is given in μmol or nmol $cm^{-2}$ (serosal area) $h^{-1}$. 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 or μM. The estimates were evaluated by the even distribution of residuals and by the Chi square test with d$f$ equal to the number of experimental points less the number of parameters estimated.

The kinetics of $Na^+$-and $Cl^-$-activation of $J_{mc}^{Tau}$ were estimated (± S.D.) by weighted non-linear least squares fitting to

$$J_{mc}^{Tau} = \frac{J_{max}^{Tau}[Na^+ \text{ or } Cl^-]^n}{K_{1/2}^n + [Na^+ \text{ or } Cl^-]^n}$$

$$(+ J_{mc}^{Tau} \text{ at } 0 \text{ mM } Na^+ \text{ or } Cl^-) \qquad (2)$$

where $J_{max}^{Tau}$ is $J_{mc}^{Tau}$ under maximal activation by chloride or sodium, $[Na^+$ or $Cl^-]$ is the concentration of the variable activator ion, and $n$ is the Hill coefficient.

## 3. Results

### 3.1. Regional distribution of amino acid transport

The unidirectional influx of D-glucose and L-amino acids in four regions of the small intestine was measured in paired experiments using concentrations in the range of the expected affinity constant for each substrate thereby minimizing the rate of nonsaturable transport (Table 1). Transport was minimal in the duodenum. Amino acid influx was fairly constant from proximal through distal small intestine with maximal rates in distal and/or mid small intestine. Influx of D-glucose, lysine and imino acids increased significantly in the distal small intestine (Table 1). Influx across epithelium covering Peyer's patches was not assessed.

### 3.2. Kinetics of D-glucose and leucine influx

In order to ascertain the viability of the tissue and to allow for comparison with results obtained with different techniques paired measurements of D-glucose influx were performed at 140 mM NaCl in the concentration range of 0.25–40 mM (Fig. 1). The best fit of these results to Eq. (1) for D-glucose (Chi square = 0.933, $df = 5$; $P = 0.97$) was

$$J_{mc}^{Glu} = \frac{(9.9 \pm 1.1)[\text{D-Glu}]}{(2.4 \pm 0.4) + [\text{D-Glu}]}$$
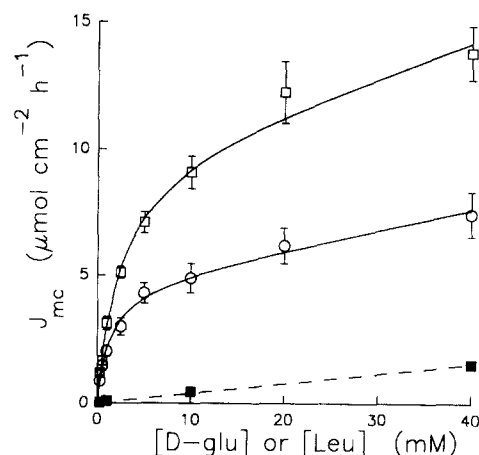$$+ 0.123 \pm 0.034[\text{D-Glu}]\,\mu\text{mol cm}^{-2}\,\text{h}^{-1} \qquad (3)$$



Fig. 1. Kinetics of D-glucose and leucine influx across the brush-border membrane of pig small intestine. Influx was determined in a paired design at 0.25–40 mM D-glucose (□) and leucine (○) in the presence of 140 mM NaCl, and for D-glucose also in the absence of mucosal sodium (■). Results are means ± S.E. of 5–8 observations. The full lines describe the best fit of measurements of total uptake as given in Eqs. (3) and (5). The broken line is the best fit of sodium-independent influx of D-glucose as given by Eq. (4).

The sodium-independent influx of D-glucose was measured at 0 mM sodium and at 0.25, 1, 10, and 40 mM D-glucose (Fig. 1). Transport could only be fitted to a linear (nonsaturable) process:

$$J_{mc}^{Glu} = 0.038 \pm 0.011[\text{D-Glu}]\,\mu\text{mol cm}^{-2}\,\text{h}^{-1} \qquad (4)$$

In a separate experiment $J_{mc}^{Glu}$ measured at 1 mM D-glucose was $2.751 \pm 0.202$ $\mu$mol cm$^{-2}$ h$^{-1}$ in the absence and $0.092 \pm 0.011$ $\mu$mol cm$^{-2}$ h$^{-1}$ in the presence of 0.1 mM phloridzin ($n = 4$; $P < 0.001$). Together these experiments demonstrate saturable, sodium-dependent transport of D-glucose in pig small intestine in vitro.

Table 1

Regional distribution of amino acid and D-glucose influx across the brush-border membrane of pig small intestine in vitro

| [Substrate] | Influx rate | | | |
| --- | --- | --- | --- | --- |
| | duodenum | proximal jejunum | mid intestine | distal ileum |
| MeAIB (1 mM) | 0.09 ± 0.02 | 0.11 ± 0.01 | 0.11 ± 0.02 | 0.18 ± 0.02 * |
| Proline (1 mM) | 0.18 ± 0.02 | 0.39 ± 0.01 | 0.44 ± 0.03 | 0.52 ± 0.04 * |
| Leucine (1 mM) | 0.66 ± 0.18 | 1.51 ± 0.07 * | 1.58 ± 0.25 * | 2.09 ± 0.42 * |
| Alanine (1 mM) | 0.43 ± 0.12 | 0.84 ± 0.04 | 1.26 ± 0.04 * | 1.23 ± 0.08 * |
| Lysine (1 mM) | 0.11 ± 0.01 | 0.20 ± 0.01 | 0.17 ± 0.02 | 0.25 ± 0.01 * |
| Taurine (5 $\mu$M) | 0.23 ± 0.07 | 0.70 ± 0.02 | 1.60 ± 0.10 * | 1.43 ± 0.16 * |
| $\beta$-Alanine (5 $\mu$M) | 0.43 ± 0.10 | 0.76 ± 0.12 | 0.73 ± 0.15 | 0.83 ± 0.07 |
| D-Glucose (1 mM) | 0.56 ± 0.04 | 0.99 ± 0.06 | 1.66 ± 0.12 | 3.51 ± 0.44 * |
| Glutamate (20 $\mu$M) | 15.67 ± 0.36 | 11.57 ± 0.80 | 42.39 ± 6.02 * | 43.41 ± 5.05 * |

The influx rates for $\beta$-amino acids and glutamate are given in nmol cm$^{-2}$ h$^{-1}$ and for the other substrates in $\mu$mol cm$^{-2}$ h$^{-1}$. The results are given as means ± S.E. of 4 (7 for alanine) paired observations in two animals. With the exception of $\beta$-alanine influx rates are minimal in the duodenum and maximal in the distal ileum and/or in the mid intestine (regions emphasized (*); $P < 0.05$ by one-way ANOVA).

Table 2

Chloride-dependence of amino acid and D-glucose influx across the brush-border membrane of pig small intestine

| [Substrate] | Influx rate | |
|---|---|---|
| | 140 mM NaCl | 0 mM Cl⁻ 140 mM Na⁺ |
| β-Alanine (5 μM) | 0.98 ± 0.06 | 0.47 ± 0.03 [a] |
| Taurine (5 μM) | 1.68 ± 0.11 | 0.35 ± 0.03 [a] |
| MeAIB (1 mM) | 0.122 ± 0.004 | 0.049 ± 0.006 [a] |
| Glycine (0.1 mM) | 38.1 ± 3.0 | 37.5 ± 0.9 |
| Lysine (0.1 mM) | 30.3 ± 1.8 | 31.8 ± 1.7 |
| Glutamate (20 μM) | 82.3 ± 6.4 | 81.7 ± 3.6 |
| D-Glucose (1 mM) | 2.28 ± 0.11 | 2.24 ± 0.06 |

The influx rates of MeAIB and D-glucose are given in $\mu$mol cm$^{-2}$ h$^{-1}$ and for the other substrates in nmol cm$^{-2}$ h$^{-1}$. The results are given as means ± S.E. of 8 paired observations in two animals.

[a] Denotes $P < 0.001$ by paired $t$-test.

For leucine the best fit to measurements at eight different leucine concentrations between 0.25 and 40 mM in the presence of 140 mM sodium (Chi square = 0.698, d$f$ = 5; $P$ = 0.98) was

$$J_{mc}^{Leu} = \frac{(4.7 \pm 0.4)[Leu]}{(1.3 \pm 0.2) + [Leu]}$$

$$+ 0.077 \pm 0.015[Leu] \, \mu mol \, cm^{-2} \, h^{-1} \qquad (5)$$

In a separate and paired experiment leucine influx measured at 1 mM was $1.30 \pm 0.07$ $\mu$mol cm$^{-2}$ h$^{-1}$ in the presence of 140 mM NaCl and $0.44 \pm 0.01$ $\mu$mol cm$^{-2}$ h$^{-1}$ in the presence of 0 mM sodium ($P < 0.001$; $n$ = 10). The addition of 100 mM lysine in the absence of sodium reduced leucine influx further to $0.21 \pm 0.01$ $\mu$mol cm$^{-2}$ h$^{-1}$ ($P < 0.001$; $n$ = 10). This demonstrates the existence of both sodium-dependent and sodium-independent leucine transport in pig small intestine and suggests the existence of a b$^{0,+}$ carrier [30].

### 3.3. Chloride-dependence of amino acid influx

The effect of chloride substitution on amino acid transport across the brush-border membrane of mid intestine was surveyed in paired experiments at 0 and 140 mM chloride (Table 2). Influx of 2 methyl-aminoisobutyric acid (MeAIB), taurine and β-alanine was chloride-dependent. Since 97% of D-glucose influx at 1 mM is sodium-dependent, its chloride-independence demonstrates that substituting isethionate for chloride does not affect sodium-dependent transport of sugars or amino acids non-specifically [25,31]. Influx of the bipolar amino acid glycine and the cationic amino acid lysine was chloride-independent demonstrating the absence of a B$^{0,+}$ carrier in pig mid small intestine [27].

Influx of taurine (5 μM) was measured in paired experiments as a function of sodium and chloride concentrations

(Fig. 2). For sodium-activation the best fit of these data to Eq. (2) was (Chi square = 0.949; d$f$ = 5; $P$ = 0.97)

$$J_{mc}^{Tau} = \frac{(1.51 \pm 0.06)[Na^+]^{2.4 \pm 0.3}}{(46.2 \pm 2.4)^{2.4 \pm 0.3} + [Na^+]^{2.4 \pm 0.3}}$$

$$+ 0.194 \, nmol \, cm^{-2} \, h^{-1} \qquad (6)$$

and for chloride activation (Chi square = 0.809; d$f$ = 5; $P$ = 0.98)

$$J_{mc}^{Tau} = \frac{(1.21 \pm 0.14)[Cl^-]^{1.0 \pm 0.2}}{(13.2 \pm 4.6)^{1.0 \pm 0.2} + [Cl^-]^{1.0 \pm 0.2}}$$

$$+ 0.357 \, nmol \, cm^{-2} \, h^{-1} \qquad (7)$$

The activation stoichiometry is $2.4 \pm 0.3$ sodium/$1.0 \pm 0.2$ chloride/1 taurine.

### 3.4. The β-amino acid carrier of pig small intestine

Influx of taurine was measured at ten different concentrations of taurine between 5 and 1280 μM in the presence of 140 mM NaCl (Fig. 3). The best fit (Chi square = 1.160; d$f$ = 7; $P$ > 0.99) of these data to Eq. (1) was

$$J_{mc}^{Tau} = \frac{(13.6 \pm 2.5)[Tau]}{(44.3 \pm 13.2) + [Tau]}$$

$$+ 0.037 \pm 0.004[Tau] \, nmol \, cm^{-2} \, h^{-1} \qquad (8)$$

Taurine influx was also measured at 40 to 640 μM taurine in the absence of sodium (Fig. 3). These sodium-independent fluxes could only be fitted to the linear term of Eq. (1):

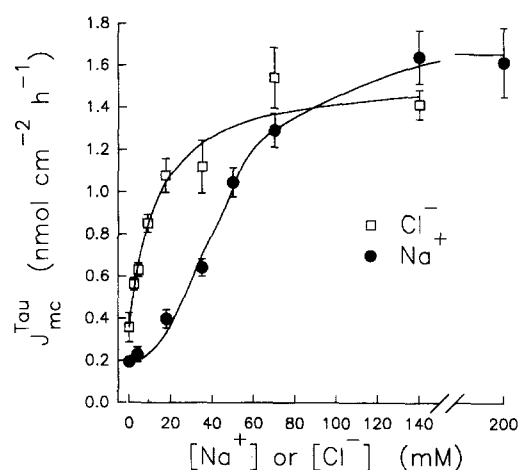$$J_{mc}^{Tau} = 0.034 \pm 0.001[Tau] \, nmol \, cm^{-2} \, h^{-1} \qquad (9)$$



Fig. 2. Sodium- and chloride-activation of taurine influx across the brush-border membrane of pig small intestine. Taurine influx was measured at 5 μM taurine in the presence of 140 mM chloride and 0–200 mM sodium (●) and in the presence of 140 mM sodium and 0–140 mM chloride (□). Data are means ± S.E. of 6 paired observations. The lines are described by Eqs. (6) and (7).
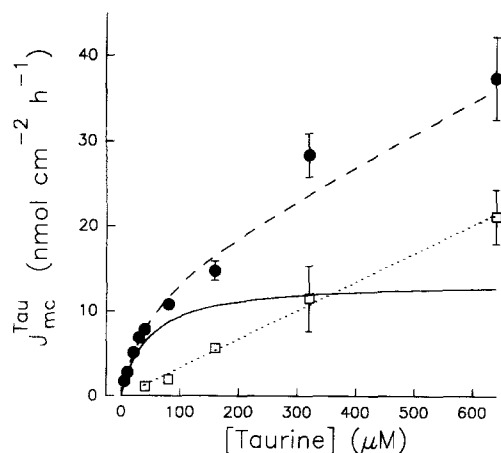
Fig. 3. The kinetics of taurine influx across the brush-border membrane of pig small intestine. Taurine influx was determined in paired experiments at 140 mM NaCl and 5–640 $\mu$M taurine ($\bullet$; $n = 7$–14) and at 0 mM sodium and 40–640 $\mu$M taurine ($\square$; $n = 4$). The data are means $\pm$ S.E. The sodium-dependent fluxes could be fitted to Eq. (1) (Eq. (8) and broken line) and the saturable term of Eq. (8) is shown as the full line. The sodium-independent fluxes could only be fitted to the linear part of Eq. (1) and is shown as the dotted line (Eq. (9)).

Thus, the estimates of linear, presumably diffusive influx were almost identical whether obtained from the fluxes measured in the presence or absence of sodium as illustrated by the parallel lines at saturating taurine concentrations in Fig. 3. This indicates the existence of only one carrier of taurine in the concentration range examined.

Influx of $\beta$-alanine was measured at eight different concentrations of $\beta$-alanine between 9 and 640 $\mu$M in the presence of 140 mM NaCl (Fig. 4). The best fit (Chi
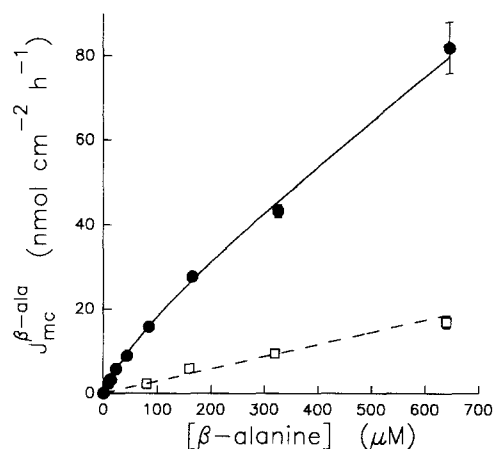


Fig. 4. The kinetics of $\beta$-alanine influx across the brush-border membrane of pig small intestine. $\beta$-Alanine influx was determined in paired experiments at 140 mM NaCl and 9–640 $\mu$M $\beta$-alanine ($\bullet$; $n = 6$–8). The full line is the best fit of these data to Eq. (1) and is described by Eq. (10). $\beta$-Alanine influx measured at 0 mM sodium and 80–640 $\mu$M taurine ($\square$; $n = 4$) could only be fitted to the linear term of Eq. (1) and the broken line describes this fit given in Eq. (11). The data are means $\pm$ S.E.

Table 3
A (taurine)-B ($\beta$-alanine)-C (hypotaurine) test of taurine influx in pig small intestine

| $Y$ | | $K^i$ ($Y \to X$) ($\mu$M) | |
| --- | --- | --- | --- |
| | $X$: | taurine | $\beta$-alanine |
| Taurine | | $44 \pm 13$ (S.D.) | $41 \pm 5$ ($n = 5$) |
| $\beta$-Alanine | | $160 \pm 22$ ($n = 7$) | $97 \pm 23$ (S.D.) |
| Hypotaurine | | $43 \pm 4$ ($n = 7$) | $22 \pm 5$ ($n = 7$) |

The apparent affinity constants for taurine and $\beta$-alanine from Eqs. (8) and (10) were used. Fluxes were corrected for the non-saturable contribution before calculating $K_i$ assuming one saturable process (Eq. (12)). $\beta$-Alanine influx was determined at five different taurine concentrations, and $K_i^{\text{Tau}}$ against $J_{\text{mc}}^{\beta\text{-Ala}}$ is the means $\pm$ S.E. of the $K_i$ values determined at each concentration. The other $K_i$ values were calculated likewise.

square $= 1.284$; d$f = 5$; $P = 0.94$) of these data to Eq. (1) was

$$J_{\text{mc}}^{\beta\text{-Ala}} = \frac{(15.9 \pm 5.6)[\beta\text{-Ala}]}{(96.8 \pm 22.7) + [\beta\text{-Ala}]}$$
$$+ 0.102 \pm 0.010[\beta\text{-Ala}] \, \text{nmol cm}^{-2} \, \text{h}^{-1} \quad (10)$$

$\beta$-Alanine influx was also measured at 80 to 640 $\mu$M $\beta$-alanine in the absence of sodium (Fig. 3). These sodium-independent fluxes could only be fitted to a linear function:

$$J_{\text{mc}}^{\beta\text{-Ala}} = 0.029 \pm 0.001[\beta\text{-Ala}] \quad (11)$$

The measured sodium-independent influx of $\beta$-alanine and taurine are very similar. The discrepancy between the measured sodium-independent $\beta$-alanine influx and the estimated non-saturable component of Eq. (10) could be an indication of the existence of a second carrier of $\beta$-alanine with a lower affinity [32]. This question was addressed in two ways.

First, an ABC test [33] was performed using taurine as substrate A, $\beta$-alanine as substrate B and hypotaurine as substrate C (Table 3). (A) Taurine influx was measured in paired experiments with 5 $\mu$M taurine in the presence of 0, 10, 20, 40, 80, 160, 320 and 640 $\mu$M $\beta$-alanine ($n = 4$ observations at each inhibitor concentration). (B) Similarly, $\beta$-alanine influx was determined using 5 $\mu$M $\beta$-alanine as substrate with the addition 0, 10, 20, 30, 40, and 80 $\mu$M taurine ($n = 6$). (C) Finally, influx of both taurine and $\beta$-alanine (5 $\mu$M) was measured after adding 0, 10, 20, 30, 40, 80, 160, and 320 $\mu$M hypotaurine ($n = 5$). All fluxes were corrected for the non-saturable component using the diffusive permeabilities from Eqs. (8) and (10), and the apparent affinity constants from these equations were used to calculate the $K_i$ values:

$$\frac{J_1}{J_0} = \frac{K_{1/2}^A + [A]}{K_{1/2}^A + [A] + \left( K_{1/2}^A [I] \right)/K_i} \quad (12)$$

where $J_1$ is the influx of taurine, respectively $\beta$-alanine (A) in the presence of the inhibitor in the concentration [I] and $J_0$ is influx in the absence of inhibitor. Table 3
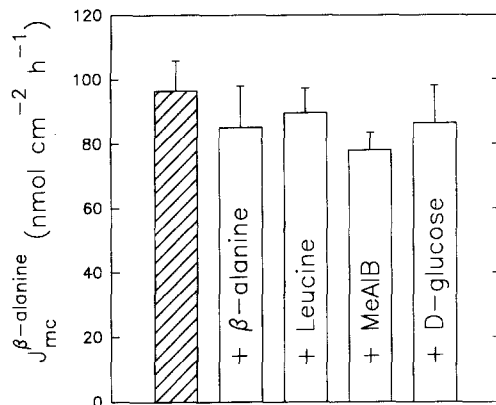
Fig. 5. Screening for a low affinity carrier of β-alanine in the brush-border membrane of pig small intestine. Influx of β-alanine was determined in paired experiments at 140 mM NaCl and 1 mM β-alanine in the presence of 40 mM D-mannitol (single crossed bar) or 40 mM of the inhibitors stated. Results are means + S.E. of 6 observations. All differences are NS by one-way ANOVA.

demonstrates that both the apparent affinity constants for taurine and β-alanine, respectively, and their inhibitory constants against transport of the other are the same. Furthermore, the inhibitory constant of hypotaurine is similar for the two. Thus, taurine and β-alanine compete for transport by one high affinity carrier of β-amino acids.

Second, paired measurements were made of β-alanine influx at 5 $\mu$M β-alanine: $J_{mc}^{\beta\text{-Ala}}$ was $2.04 \pm 0.09$ and $1.71 \pm 0.08$ nmol cm$^{-2}$ h$^{-1}$ in the absence and presence of 1 mM leucine as inhibitor, respectively ($P = 0.03$; $n = 8$). $J_{mc}^{\beta\text{-Ala}}$ was $1.74 \pm 0.13$ and $1.57 \pm 0.09$ nmol cm$^{-2}$ h$^{-1}$ in the absence and presence of 1 mM lysine as inhibitor, respectively (NS; $n = 8$). Leucine inhibition of taurine influx measured at 5 $\mu$M taurine was similar to the effect on β-alanine influx but statistically insignificant; $J_{mc}^{Tau}$ was $2.06 \pm 0.17$ in the absence of inhibitor and $1.74 \pm 0.17$ nmol cm$^{-2}$ h$^{-1}$ in the presence of 1 mM leucine ($P = 0.07$; $n = 8$). Since a second possible carrier of β-alanine would be a low affinity carrier $J_{mc}^{\beta\text{-Ala}}$ was measured at 1 mM β-alanine in the presence of the usual substrates for the known carriers as inhibitors (Fig. 5). A second carrier was not identified. Furthermore, the lack of self-inhibition (Fig. 5) seems to exclude the possibility of a second, low affinity carrier of β-alanine.

## 4. Discussion

This study demonstrates substrate influx across the brush-border membrane of pig small intestine in vitro. The tissue exhibits saturable and sodium-dependent, phloridzin inhibitable influx of D-glucose and saturable, sodium-dependent influx of amino acids. The apparent affinity constants were 1.3 mM for leucine and 2.4 mM for D-glucose, approximately the same as estimates obtained with brush-border membrane vesicles [12,34] and with the everted

sleeve technique [35]. Thus, luminal stirring was efficient even though the mucosal area was increased from 0.62 to 1.00 cm$^2$.

Small differences in transport capacity along the small intestine with an insignificant minimum in the distal part was reported for sodium-dependent transport of leucine and proline in 20 day piglets using the everted sleeve technique [15], which is very similar to the influx technique. The inverse gradient of that described by Table 1 – maximal rates of transport in proximal small intestine and minimal rates in distal ileum – was reported for total D-glucose uptake in four week old piglets using 50 mM D-glucose [35]. Since carrier mediated transport across the brush-border membrane varies with age and diet in addition to anatomical localization [36,37] it is possible that the gradient changes from 4 to 6–8 weeks. However, the discrepancy is more likely due to difficulty identifying the carrier mediated transport when using substrate concentrations more than ten times the apparent affinity constant. Furthermore, the proximal small intestine is more leaky than the distal part. The regional variation in amino acid transport in the pig reported here is similar to that reported for the guinea pig, a herbivore, and the rat, an omnivore [25].

Lysine is used in the rabbit to exclude leucine from transport on a sodium-independent $b^{0,+}$ carrier of cationic and bipolar amino acids [28,30,38]. Since our data on the regional distribution of lysine influx demonstrated only a small rate of transport we did not use lysine inhibition. However, the existence of a $b^{0,+}$ carrier in the pig was suggested by the demonstration of a lysine-inhibitable fraction of sodium-independent leucine influx. The influx of MeAIB, an almost ideal substrate for the rabbit imino acid carrier [39,40], is very small. Yet, a chloride-dependent carrier is present (Table 2).

Using the ABC test [33] we have characterized a chloride-dependent, high affinity, low capacity carrier of β-amino acids (Eqs. (8) and (10); Tables 2 and 3). It is the only carrier of taurine (Eqs. (8) and (9); Fig. 3). The rate of non-saturable β-alanine influx exceeds the sodium-independent influx suggesting the existence of a second carrier of β-alanine (Eqs. (10) and (11); Fig. 4). Such a carrier, the $B^{0,+}$ carrier, exists in rabbit distal ileum [27,39]. It is chloride-and sodium-dependent and accepts bipolar and cationic amino acids [27,39]. The absence of chloride-dependent transport of glycine and lysine studied in relevant $\mu$M concentrations (Table 2) and the absence of lysine inhibitable β-alanine transport demonstrates that a $B^{0,+}$ carrier is not present in the pig mid intestine. A small inhibitory effect of leucine on both β-alanine and taurine influx measured in the $\mu$M range was observed. Leucine might interact with the β-amino acid carrier, although such an interaction has not been observed in other species [16]. The lack of self-inhibition observed at 1 mM β-alanine is a strong argument against a second carrier of β-alanine. In addition, none of the substrates chosen to represent the

Table 4
The high affinity β-amino acid carrier in the brush border membrane of mammalian small intestine

| | Substrate | $K_{1/2}$ ($\mu$M) | $J_{max}$ [a,b,c] | $K_{1/2}^{Na}$ (mM) | $n_H^{Na}$ | $K_{1/2}^{Cl}$ (mM) | $n_H^{Cl}$ | $K_i^{\beta-Ala}/K_i^{Tau}$ ($\mu$M) | References |
|---|---|---|---|---|---|---|---|---|---|
| Rat [a] | | | | | | | | | |
| suckling | Tau | 75 | 3.2 | + | | + | | 87 | [41] |
| 6–8 week | | 25 | 8.6 | + | | + | | | [25,42] |
| Rabbit | | | | | | | | | |
| jejunum | Tau [a] | 14 | 3.7 | 145 | 3 | 50 | 1 | 150 [c] | [43] |
| | Tau [b] | 34 | 11.6 | 121 | 2.1 | + | | | [25,28] |
| | β-Ala [a] | 46 | 7.2 | 140 | 2.7 | 80 | 1 | 151 [c] | [44] |
| ileum | Tau [b] | 41 | 24.4 | | | | | 126 | [16] |
| Guinea pig | | | | | | | | | |
| jejunum [b] | Tau | 37 | 5.2 | 121 | 2.1 | + | | 99 | [25] |
| | β-Ala | 155 | 10.9 | + | | + | | | |
| Swine | | | | | | | | | |
| jejunum [b] | Tau | 44 | 13.6 | 46 | 2.4 | 13 | 1 | 160 | [45] and present study |
| | β-Ala | 97 | 15.9 | + | | + | | 41 | |
| Human | | | | | | | | | |
| duodenum [d] | Tau | | | + | | + | | | [17] |
| | β-Ala | | | + | | + | | | |

[a] Brush-border membrane vesicles; $J_{max}$ given as nmol mg protein$^{-1}$ h$^{-1}$.
[b] Influx; $J_{max}$ given as nmol cm$^{-2}$ h$^{-1}$. + denotes that transport is ion-dependent. $n_H^{Na}$ and $n_H^{Cl}$ are the Hill coefficients obtained by ([b]) nonlinear regression analysis of ion activation studies or ([a]) by interpolation of curves.
[c] Determined from measurements at one inhibitor concentration.
[d] Steady-state mucosal uptake into biopsies.

known carriers described in other species inhibited the influx of β-alanine. If a second carrier of β-alanine is present in the pig small intestine we have not been able to identify it. We have not found other reports of β-amino acid transport in pig small intestine. The characteristics of the β-amino acid carrier reported here are very similar to that of the β-amino acid carriers described in the small intestine of other species (Table 4).

The present study demonstrates that the brush-border membrane of pig small intestine possesses carriers of amino acids and D-glucose, that the characteristics of the β-amino acid carrier is similar to those described in other species and that the B$^{0,+}$ carrier is not present. Future research will characterize transport of neutral amino acids in the pig small intestine and through comparison with transport systems present in the human small intestine reveal whether the pig is a suitable animal model for studies of nutrient absorption and its regulation.

## References

[1] Powell, D.W. (1987) in Physiology of the gastrointestinal tract (Johnson, L.R., ed.), pp. 1267–1305, Raven Press, New York.

[2] Tumbleson, M. (1986) Swine in biomedical research, Plenum Press, New York.
[3] Daniels, V.G. (1972) Comp. Biochem. Physiol. A Comp. Physiol. 42, 689–692.
[4] Smith, M.W. and James, P.S. (1976) Biochim. Biophys. Acta 419, 391–394.
[5] Smith, M.W. (1988) Comp. Biochem. Physiol. A Comp. Physiol. 90, 577–582.
[6] Smith, M.W. (1981) Experientia 37, 868–870.
[7] Burton, K.A., James, P.S., Smith, M.W. and Young, J.D. (1980) J. Physiol. 308, 451–464.
[8] Dauncey, M.J., Ingram, D.L., James, P.S. and Smith, M.W. (1983) J. Physiol. 341, 441–452.
[9] James, P.S. and Smith, M.W. (1976) J. Physiol. 262, 151–168.
[10] Sepulveda, F.V. and Smith, M.W. (1979) J. Physiol. 286, 479–490.
[11] Wolffram, S., Berger, B., Grenacher, B. and Scharrer, E. (1989) J. Nutr. 119, 706–712.
[12] Wolffram, S., Eggenberger, E. and Scharrer, E. (1986) Comp. Biochem. Physiol. A Comp. Physiol. 84, 589–593.
[13] Maenz, D.D. and Patience, J.F. (1992) J. Biol. Chem. 267, 22079–22086.
[14] Rhoads, J.M., MacLeod, R.J. and Hamilton, J.R. (1989) J. Pediatr. Gastroenterol. Nutr. 9, 225–231.
[15] Vega, Y.M., Puchal, A.A. and Buddington, R.K. (1992) J. Nutr. 122, 2430–2439.
[16] Munck, L.K. and Munck, B.G. (1992) Am. J. Physiol. 262, G609–G615.
[17] Munck, L.K. (1995) Gut 36, 215–219.
[18] Miyamoto, Y., Balkovetz, D.F., Leibach, F.H., Mahesh, V.B. and Ganapathy, V. (1988) FEBS Lett. 231, 263–267.
[19] Jessen, H. and Sheikh, M.I. (1991) Biochim. Biophys. Acta 1064, 189–198.
[20] Turner, R.J. (1986) J. Biol. Chem. 261, 16060–16066.
[21] Hammerman, M. and Sacktor, B. (1978) Biochim. Biophys. Acta 509, 338–347.
[22] Wolff, N.A. and Kinne, R. (1988) J. Membr. Biol. 102, 131–139.
[23] Benyajati, S. and Johnson, J.L. (1991) Am. J. Physiol. 260, R879–R888.

[24] Benyajati, S. and Bay, S.M. (1992) Pflugers Archiv. Eur. J. Physiol. 421, 168–175.

[25] Munck, L.K. and Munck, B.G. (1994) Am. J. Physiol. 266, R997–R1007.

[26] Thomson, A.B. (1979) J. Membr. Biol. 50, 141–163.

[27] Munck, L.K. and Munck, B.G. (1995) Biochim. Biophys. Acta 1235, 93–99.

[28] Munck, L.K. and Munck, B.G. (1992) Biochim. Biophys. Acta 1116, 83–90.

[29] Schultz, S.G., Curran, P.F., Chez, R.A. and Fuisz, R.E. (1967) J. Gen. Physiol. 50, 1241–1260.

[30] Ganapathy, V., Brandsch, M. and Leibach, F.H. (1994) in Physiology of the Gastrointestinal Tract (Johnson, L.R., ed.), pp. 1773–1794, Raven Press, New York.

[31] Munck, L.K. and Munck, B.G. (1990) Biochim. Biophys. Acta 1027, 17–20.

[32] Christensen, H.N. (1989) Methods Enzymol. 173, 576–616.

[33] Scriver, C.R. and Wilson, O.H. (1964) Nature 202, 92–93.

[34] Keljo, D.J., MacLeod, R.J., Perdue, M.H., Butler, D.G. and Hamilton, J.R. (1985) Am. J. Physiol. 249, G751–G760.

[35] Puchal, A.A. and Buddington, R.K. (1992) Am. J. Physiol. 262, G895–G902.

[36] Buddington, R.K. (1992) Am. J. Physiol. 263, R503–R509.

[37] Ferraris, R.P., Lee, P.P. and Diamond, J.M. (1989) Am. J. Physiol. 257, G689–G697.

[38] Munck, B.G. (1985) J. Membr. Biol. 83, 1–13.

[39] Munck, B.G. (1985) J. Membr. Biol. 83, 15–24.

[40] Stevens, B.R. and Wright, E.M. (1985) J. Membr. Biol. 87, 27–34.

[41] Moyer, M.S., Goodrich, A.L., Rolfes, M.M. and Suchy, F.J. (1988) Am. J. Physiol. 254, G870–G877.

[42] Barnard, J.A., Thaxter, S., Kikuchi, K. and Ghishan, F.K. (1988) Am. J. Physiol. 254, G334–G338.

[43] Miyamoto, Y., Tiruppathi, C., Ganapathy, V. and Leibach, F.H. (1989) Am. J. Physiol. 257, G65–G72.

[44] Miyamoto, Y., Nakamura, H., Hoshi, T., Ganapathy, V. and Leibach, F.H. (1990) Am. J. Physiol. 259, G372–G379.

[45] Wolffram, S., Hagemann, C. and Scharrer, E. (1991) Am. J. Physiol. 261, R1089–R1095.