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H^+-coupled α-methylaminoisobutyric acid transport in human intestinal Caco-2 cells

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Abstract

Transepithelial apical-to-basal transport and cellular uptake of the non-metabolisable amino acid α-methylaminoisobutyric acid (MeAIB) across confluent monolayers of the human intestinal epithelial cell line Caco-2 are enhanced by a transepithelial pH gradient (apical pH 6.0, basolateral pH 7.4). In Na^+-free conditions (apical pH 7.4, basolateral pH 7.4), net absorption (120 ± 58 pmol/cm^2 per h, $n = 13$) and uptake across the apical membrane (cell/medium ratio 0.56 ± 0.06, $n = 13$) are low. However, in Na^+-free conditions with apical pH 6.0, net absorption (685 ± 95 pmol/cm^2 per h, $n = 15$) and intracellular accumulation (cell/medium ratio 3.63 ± 0.29, $n = 14$) were marked. Continuous monitoring of intracellular pH (pH_i) in BCECF (2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein)-loaded Caco-2 cell monolayers indicated that apical addition of MeAIB (20 mM) was associated with H^+-flow across the apical membrane in both Na^+ and Na^+-free conditions. This transport process is rheogenic in Na^+-free media, stimulating an inward short-circuit current in voltage-clamped Caco-2 cell monolayers. On the basis of competition for MeAIB accumulation and pH_i experiments, L-proline, glycine, L-alanine and β-alanine are also substrates for H^+-linked transport at the apical membrane of Caco-2 cells but L-valine, L-leucine and L-phenylalanine are not. These data are consistent with the expression, in the apical brush-border membrane of Caco-2 cells, of a H^+-coupled, Na^+-independent MeAIB carrier.

Keywords: Proton/amino acid transport; Brush-border membrane; Intestine; Epithelium; Caco-2 cell; Intracellular pH

1. Introduction

α-Methylaminoisobutyric acid (MeAIB) is a non-metabolisable amino acid analogue which has been used widely as a model substrate in the study of amino acid transport both in vivo [1] and in vitro [2]. Generally, this substrate is transported via the Na^+-dependent system A but is excluded by system ASC [3]. Thus, MeAIB has been used as a key substrate in distinguishing between neutral amino acid transport via these two carriers in both Ehrlich cells [4] and Chinese hamster ovary cells [2,5]. MeAIB transport has also been demonstrated in a number of epithelia (both intact tissues and membrane vesicle preparations) including rabbit ileal mucosa [6,7], rabbit jejunal brush-border membrane vesicles [8], rat small intestine [9], guinea-pig isolated small intestinal cells [10], mouse jejunal everted sleeves [11], rabbit kidney brush-border membrane vesicles [12], and rat placental microvillous membrane vesicles [13]. Transepithelial absorption of amino acids in the intestine involves movement across both the apical and basolateral membranes of the intestinal enterocyte. At the apical membrane MeAIB interacts with the Na^+-dependent Imino system which it shares with proline (Pro) and hydroxyproline [8]. System A is absent from the apical membrane of the intestinal epithelium and is confined to the basolateral membrane [3,14].

Historically, the coupling of solute transfer secondarily to metabolic energy in mammalian cells has been attributed to the transmembrane Na^+ electrochemical gradient [15]. However, it is now accepted that the transintestinal absorption of certain solutes (e.g., dipeptides) is energised primarily by the H^+ electrochemical gradient and is not coupled directly to Na^+ transport [16]. We have used intact monolayers of the human colon carcinoma cell line Caco-2 as a model for intestinal epithelial absorption of a number of solutes [17–22]. Caco-2 cells express a number of transport systems characteristic of the native intestine including H^+-linked dipeptide transport [17], and Na^+-dependent solute transporters including those for glucose

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[23], bile acids [24] and amino acids [25,26]. We have provided evidence that β-alanine [19], proline [21] and L-alanine [20] transport across intact human intestinal cultured epithelia (Caco-2) may be energised by the proton electrochemical gradient. Amino acid/proton symport was confirmed directly using Caco-2 cells loaded with the pH-sensitive fluorescent dye BCECF [19–21], transport of β-alanine, proline and L-alanine being associated with rapid intracellular acidification.

The purpose of this study was 2-fold. Firstly, to investigate the transport of a non-metabolisable amino acid in Caco-2 cells since the results of many studies of amino acid transport have been difficult to interpret due to metabolism of the substrate under investigation. Secondly, to study the role of H^+/amino acid symport in the transepithelial transport of MeAIB. It has been assumed previously that MeAIB was exclusively a substrate for the Na^+-dependent systems Imino and A. Extensive use of MeAIB as a key substrate in amino acid transport studies is based on this assumption.

2. Materials and methods

2.1. Materials

α-[1-^{14}C]Methylaminoisobutyric acid (specific activity 55 mCi/mmol) and D-[1(n)-^{3}H]mannitol (specific activity 26.4 Ci/mmol) were obtained from NEN. L-[4,5-^{3}H]Leucine (specific activity 194 Ci/mmol) and L-[2,3,4,5,6-^{3}H]phenylalanine (specific activity 131 Ci/mmol) were from Amersham. α-(Methylamino)isobutyric acid and other amino acids were from Sigma. All other chemicals were from Merck. Cell culture consumables were from Life Technologies. All amino acids were the L-isomer unless stated otherwise.

2.2. Cell culture

Caco-2 cells (passage number 111–121) were cultured as described previously [17]. Cell monolayers were prepared by seeding at high density ($(4.4–5.0) \cdot 10^5$ cells/cm^2) onto 12 or 24.5 mm diameter tissue culture inserts (Transwell polycarbonate filters (Costar)) [17]. Cell monolayers were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air. Cell confluence was estimated by microscopy and determination of transepithelial resistance. Radiolabelled flux, pH_i and conductance experiments for MeAIB were performed 18–37 days after seeding and 18–24 h after feeding.

2.3. Amino acid intracellular accumulation

Measurements were performed essentially as described previously [17,19]. Briefly, cell monolayers were extensively washed (four times in 500 ml of modified Krebs buffer (of composition (all mmol/l), NaCl 140, KCl 5.4, $CaCl_2$ 2.8, $MgSO_4$ 1.2, NaH_2PO_4 0.3, KH_2PO_4 0.3, Hepes 10, glucose 10 (pH to 7.4 at 37°C with Tris-base))) or Na^+-free Krebs buffer where appropriate (as above but choline Cl replacing NaCl and NaH_2PO_4 omitted), and placed in fresh 6-well plates, each well containing 2 ml of prewarmed modified Krebs or Na^+-free Krebs buffer (pH 7.4). Aliquots of fresh Krebs buffer or Na^+-free Krebs (pH 7.4) were then placed in the apical chamber. Choline media did not contain detectable Na^+ (< 0.1 mM). In addition, in experimental media following incubation with cell layers no Na^+ could be detected by flame-photometry. Where the apical pH was held at 6.0, 10 mM Mes was used to replace Hepes. Radiolabelled MeAIB was used at tracer concentrations (0.2 μCi/ml) with MeAIB added to give a final concentration of 100 μM (or as stated). [^{3}H]Mannitol (0.2 μCi/ml) was included in experiments to assess the passive component of MeAIB transepithelial transport and cellular accumulation. At the end of the incubation period (60 min) cell monolayers were washed by sequentially transferring tissue culture inserts through 4 beakers each containing 500 ml of ice-cold Krebs (or Na^+-free Krebs) buffer (pH 7.4) to remove any extracellular radiolabel, and removed from the insert. Cell monolayer-associated radiolabel was determined by scintillation counting. Cellular accumulation of MeAIB is expressed as μM or mM (or as a cell to medium (C/M) ratio). Cell height was determined by confocal imaging of intact cell layers and this value was used to estimate intracellular water [22]. No allowance was made for osmotically inactive space, thus intracellular concentrations are likely to be underestimated. [^{3}H]Leucine and [^{3}H]phenylalanine were used to determine cellular uptake of leucine and phenylalanine across the apical membrane of Caco-2 cell monolayers. In all experiments dual-label scintillation counting was performed, appropriate corrections being made for channel spillover and quenching.

2.4. Intracellular pH measurements

For pH_i measurements [17,18], Caco-2 cells (on 12 mm diameter Snapwell (Costar) polycarbonate filters) were loaded by incubation with BCECF-AM (5 μM), in both apical and basal chambers, for 40 min at 37°C. After loading, the inserts were placed in a 24 mm diameter perfusion chamber mounted on the stage of an inverted fluorescence microscope (Nikon Diaphot). Perfusion of the apical and basal chambers was accomplished as described previously [17]. All solutions were preheated to 37°C. Intracellular H^+ concentration was quantified by fluorescence (excitation at 440/490 nm and emission at 520 nm) from a small group of cells (5–10) using a photon counting system (Newcastle Photometric Systems). Intracellular BCECF fluorescence was converted to pH_i by comparison with values from an intracellular calibration curve using nigericin (10 μM) and high K^+ solutions [27]. Results are

expressed as pH_i or as ΔpH_i/min (mean ± S.E., n). The rate of change of intracellular pH (ΔpH_i/min) was calculated by linear regression using Photon Counter System 4.7 (Newcastle Photometric Systems). Changes in ΔpH_i/min (due to a change in the composition of the superfusate) were determined by linear regression, by comparison of the linear portions of the trace over 30–50 s (15–25 data points) periods before and after the change in composition.

2.5. Electrophysiological determinations

Measurements of short-circuit current (I_{sc}) were made essentially as described previously [19,22]. Cultured epithelial layers (on 12 mm diameter Snapwell (Costar) polycarbonate filters) were mounted in Ussing type chambers (Precision Instrument Design) maintained at 37°C, connected to an automatic voltage current clamp (WPI DVC 1000) via KCl/agar salt-bridges and reversible electrodes (Ag/AgCl for current passage, calomel for voltage sensing). Measurements of open-circuit electrical p.d., transepithelial resistance (R_T) and short-circuit current (I_{sc}) were made in modified and Na^+-free Krebs solutions (see above). The chemical flux equivalent of the I_{sc} ($I_{sc} = JzF$, where J is the chemical flux, z the valence and F the Faraday constant) is 1 μamp/cm^2 = 36 nmol/cm^2 per h for a monovalent ion.

2.6. Statistics

Statistical comparison of mean values were made using one-way analysis of variance (ANOVA) where multiple comparisons were made with a Dunnett's multiple comparison test. Constants for Michaelis-Menten kinetics were calculated by non-linear regression with the method of least-squares (FIG-P, Biosoft).

3. Results

3.1. Transepithelial transport and intracellular accumulation

In Na^+-containing media, with both apical and basolateral pH 7.4, net absorptive transport of MeAIB (Fig. 1) is low (19 ± 58 pmol/cm^2 per h, $n = 11$) although this amino acid is accumulated within the cell layer across the apical membrane (cell/medium (C/M) ratio 4.7 ± 1.5, $n = 12$). The Na^+-dependency of MeAIB uptake across both apical and basolateral membranes (apical pH 7.4, basolateral pH 7.4) is demonstrated in Fig. 1. In the absence of extracellular Na^+ apical loading is reduced to 0.56 ± 0.05 (C/M ratio, $n = 13$) and basolateral loading from 1.7 ± 0.4 (C/M ratio, $n = 12$) to 0.01 ± 0.02 (C/M ratio, $n = 13$). Net absorption of MeAIB is markedly increased upon apical acidification to pH 6.0 (Fig. 1) in both Na^+-containing (1112 ± 89 pmol/cm^2 per h, $n = 13$)

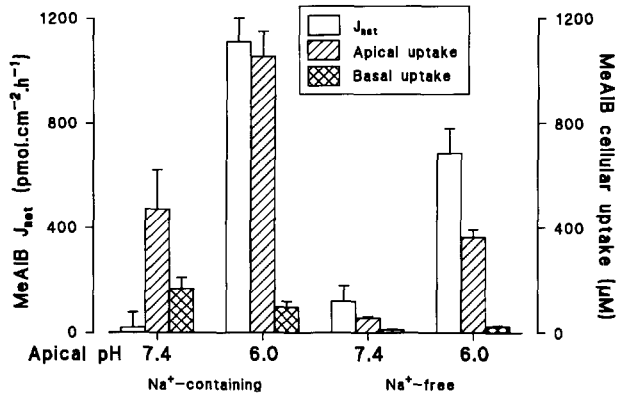

Fig. 1. The effects of extracellular Na^+ and pH on transepithelial transport and cellular uptake of MeAIB. Transepithelial transport (J_{net}, open columns) and cellular uptake of MeAIB across both apical (hatched-columns) and basolateral (cross-hatched columns) membranes were determined. Experiments were performed in Na^+-containing and Na^+-free conditions, with apical pH 7.4 or pH 6.0. Basolateral pH was 7.4 in all experiments. MeAIB was present in both apical and basolateral compartments at 100 μM. Results are expressed as mean ± S.E. ($n = 11$–15).

and Na^+-free conditions (685 ± 95 pmol/cm^2 per h, $n = 15$). Under these conditions (apical pH 6.0, basolateral pH 7.4) accumulation across the apical membrane is increased to 10.6 ± 1.0 (C/M ratio, $n = 14$) in Na^+-containing media (Fig. 1) suggesting that the transmembrane electrochemical gradient for Na^+ alone may not adequately account for accumulative transport in these conditions. When Na^+ was completely replaced by choline, upon apical acidification (pH 6.0) an increase in cellular accumulation of MeAIB (C/M ratio increased from 0.56 ± 0.05 ($n = 13$) to 3.6 ± 0.3 ($n = 14$)) was observed which displayed saturation kinetics (Fig. 2). The Michaelis-Menten fit for intracellular accumulation gave an apparent K_m of 11.5 ± 2.1 mM. In contrast cellular uptake of MeAIB across the basal surface, measured under identical experimental conditions, shows no accumulation and displays a linear dependence upon MeAIB concentration (Fig. 2). In contrast to MeAIB, accumulation of phenylalanine and leucine across the apical membrane of Caco-2 cells was not stimulated by apical acidity. In Na^+-free conditions, in the absence of a transepithelial pH gradient (apical pH 7.4, basolateral pH 7.4) uptake of phenylalanine (C/M ratio 0.67 ± 0.03, $n = 9$) and leucine (C/M ratio 0.82 ± 0.04, $n = 6$) were low. This uptake across the apical membrane was not significantly increased ($P > 0.05$) by apical acidity (C/M ratio for phenylalanine 0.74 ± 0.05 ($n = 9$) and leucine 0.91 ± 0.05 ($n = 6$)).

3.2. Inhibition of MeAIB accumulation

In Na^+-free conditions, apical acidity (apical pH 6.0, basolateral pH 7.4) stimulates intracellular accumulation of MeAIB across the apical surface. The Na^+-independent, pH-stimulated intracellular accumulation of MeAIB across

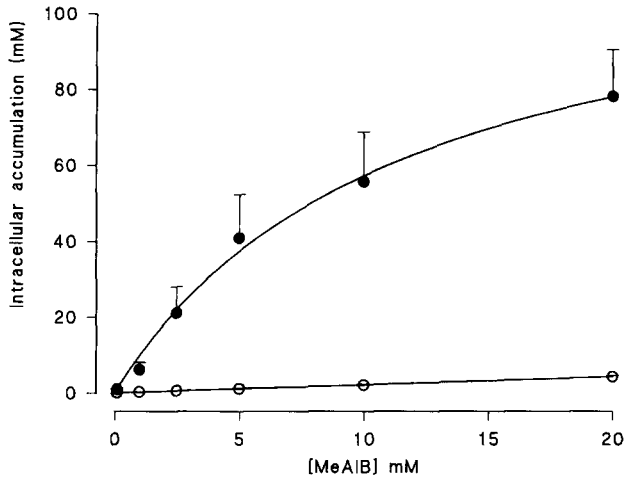

Fig. 2. Concentration dependence of MeAIB cellular accumulation in Caco-2 epithelia. Cellular accumulations of MeAIB were determined in Na^+-free media with apical pH 6.0, basolateral pH 7.4. MeAIB concentrations were equal in both apical and basal solutions. Results are mean ± S.E., $n = 6$ cell layers per concentration. Cellular accumulation from basolateral (○) and apical (●) surfaces. The rectangular hyperbola shown for apical accumulation is the Michaelis-Menten fit for the data (see text).

the apical membrane was significantly reduced in the presence of 20 mM cold MeAIB, L-alanine, β-alanine, L-proline and glycine (all $P < 0.01$) whereas accumulation in the presence of L-phenylalanine, L-leucine or L-valine (Fig. 3) was not reduced (all $P > 0.05$).

3.3. Intracellular pH measurements

If the pH-dependence of MeAIB transport across the apical membrane is due to coupling of amino acid influx to protons, transport should be associated with intracellular

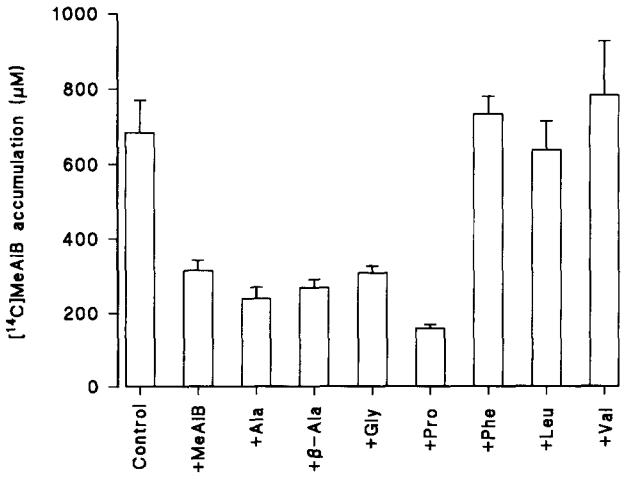

Fig. 3. Selective inhibition of intracellular accumulation of MeAIB by various amino acids. MeAIB accumulation was determined in Na^+-free media with the apical pH held at 6.0. MeAIB was present at 100 μM whilst competing amino acids were all at 20 mM. All data are the mean ± S.E. of four separate epithelial layers. Other details as for Fig. 2.

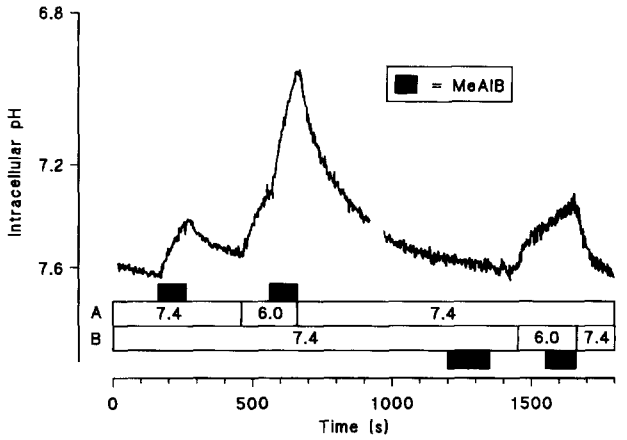

Fig. 4. Coupling of MeAIB influx to proton influx across the apical membrane. Intracellular pH was measured in monolayers of Caco-2 cells loaded with the pH sensitive fluorochrome BCECF. Experiments were performed in Na^+-containing buffers. From left to right, the effect of apical exposure to 20 mM MeAIB with apical pH at 7.4 or 6.0 (basolateral pH 7.4). The effect of basal exposure to 20 mM MeAIB with basal pH at 7.4 or 6.0 (apical pH 7.4). A single trace representative of four others.

acidification. Fig. 4 shows a typical trace of pH_i, measured in epithelial layers of Caco-2 cells loaded with the intracellular pH-sensitive fluorochrome BCECF. At pH 7.4, a rapid intracellular acidification was observed upon apical addition of MeAIB (20 mM), which was partially reversed upon removal of amino acid from the apical solution (Fig. 4). Alteration of apical pH from 7.4 to 6.0 caused intracellular acidification. A rapid further acidification was produced upon addition of MeAIB (20 mM) to the apical bathing medium (Fig. 4). This acidification was reversed upon removal of the pH gradient and MeAIB from the apical surface (Fig. 4). In contrast, addition of MeAIB (20 mM) to the basolateral chamber had no effect on pH_i at pH 7.4 or 6.0 (Fig. 4). The H^+-linked carrier for MeAIB is, therefore, expressed solely at the brush-border membrane.

As noted with the pH-stimulated increase in MeAIB accumulation across the apical membrane, MeAIB-associated intracellular acidification across the apical membrane of Caco-2 cells can be demonstrated in both Na^+-containing and Na^+-free conditions (Fig. 5). When Na^+ is removed from both apical and basolateral perfusates a small intracellular acidification is observed (Fig. 5). Acidification of the apical chamber to pH 6.0 produces a similar excursion in pH_i in Na^+-containing and Na^+-free conditions. When the MeAIB/pH 6.0 (Na^+-free) buffer is replaced at the apical surface by Na^+-free pH 7.4 buffer the pH_i recovers but at a slower rate than in the presence of Na^+ (Fig. 5). Addition of extracellular Na^+ to the basolateral chamber only has no effect on the rate of recovery. However, when Na^+ is returned to the apical chamber recovery is more rapid (Fig. 5) suggesting that an apical Na^+/H^+ exchange mechanism is important in pH_i homeostasis during H^+-coupled amino acid absorption.

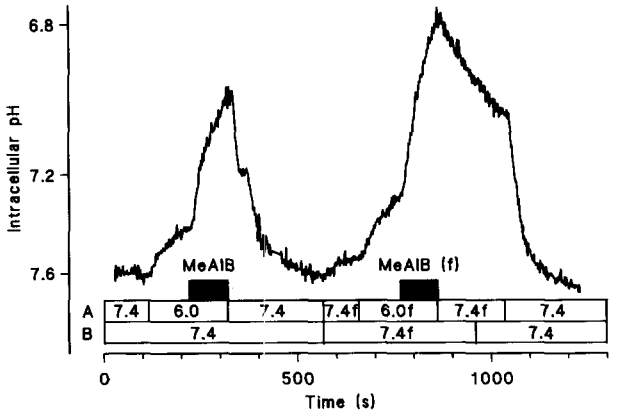

Fig. 5. The effect on intracellular pH of MeAIB in both Na^+-containing and Na^+-free conditions. MeAIB (20 mM) was superfused across the apical surface of Caco-2 cell monolayers at pH 6.0. f denotes Na^+-free buffers. Note that after apical exposure to MeAIB in Na^+-free media the Na^+ is returned sequentially to the basolateral then apical superfusates. A single trace representative of three separate experiments.

Fig. 6 compares the effect of MeAIB with L-leucine and L-phenylalanine (all 20 mM, apical pH 6.0). It is clear that the rate of intracellular acidification with leucine or phenylalanine is slower than that seen with MeAIB. Addition of 20 mM MeAIB to the apical surface of Caco-2 cells significantly ($P < 0.001$) increased the rate of acidification from 0.060 ± 0.018 ΔpH_i/min ($n = 4$) with pH 6.0 alone to 0.312 ± 0.056 ΔpH_i/min ($n = 4$). In contrast, phenylalanine (0.054 ± 0.010 ΔpH_i/min, $n = 4$) and leucine (0.065 ± 0.012 ΔpH_i/min, $n = 4$) did not significantly ($P > 0.05$) effect (increase or decrease) the rate of acidification measured with pH 6.0 alone (0.045 ± 0.010 ($n = 4$) and 0.059 ± 0.016 ($n = 4$) measured before the additions of phenylalanine and leucine, respectively). Nor did phenylalanine or leucine effect pH_i when superfused across the apical membrane at pH 7.4 (not shown). Thus, the ability of amino acids to lower pH_i is related to their inhibitory activity on MeAIB absorption.

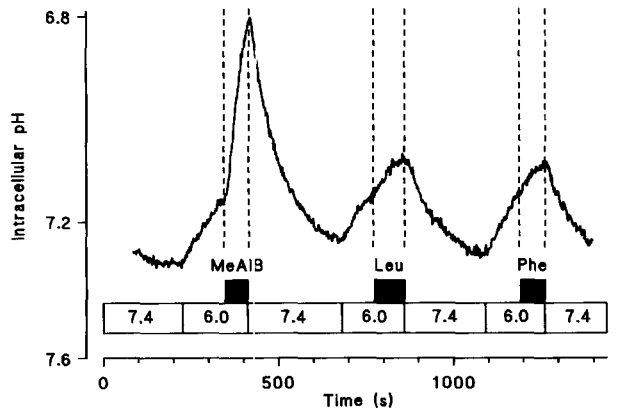

Fig. 6. Comparison of the effect on intracellular pH of MeAIB, leucine (Leu) and phenylalanine (Phe), all at 20 mM. Amino acids were superfused across the apical membrane at pH 6.0 in Na^+-containing media. A single trace representative of four others.

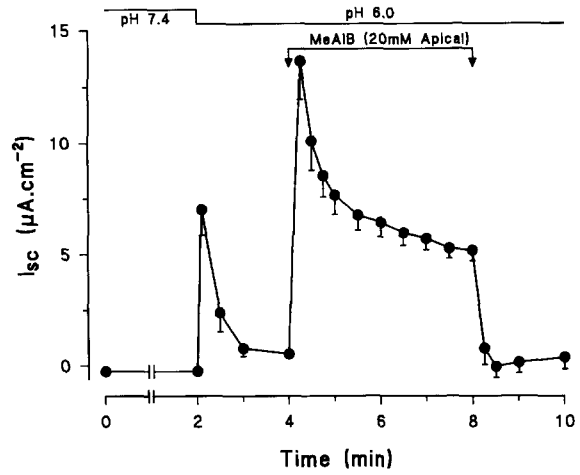

Fig. 7. MeAIB transport in short-circuited Caco-2 epithelial monolayers is rheogenic. Epithelial monolayers of Caco-2 cells were continuously short-circuited in Na^+-free media and the short-circuit current (I_{sc}) response to 20 mM MeAIB in the apical solution determined. Data are the mean ± S.E. of four epithelial preparations.

3.4. Amino acid dependent I_{sc} in voltage-clamped epithelial layers

The electrogenicity of proton/MeAIB transport was examined using voltage-clamped epithelial layers of Caco-2 cells in Ussing chambers. Na^+-free conditions (apical pH 6.0) were chosen to eliminate any Na^+-dependent absorptive flux. MeAIB (20 mM) induced a rapid and reversible inward short-circuit current (I_{sc}) (Fig. 7), consistent with proton/MeAIB symport. This MeAIB induced increase in I_{sc} was saturable (Fig. 8) with half-maximal activation of I_{sc} observed at 6.2 ± 0.5 mM. The maximal increase in I_{sc} was 297 ± 7 nmol/cm^2 per h.

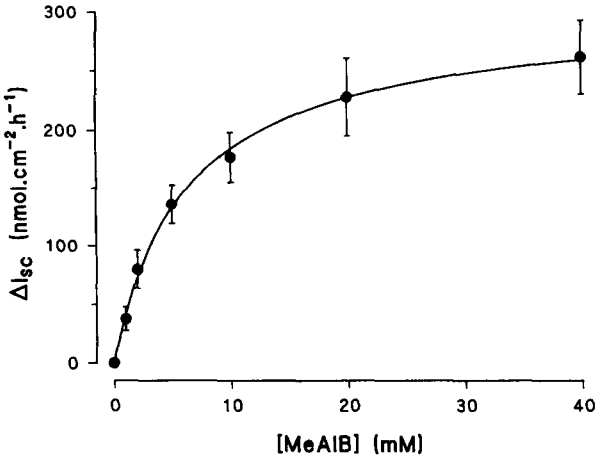

Fig. 8. Concentration dependence of the change in I_{sc} after 2–3 min of exposure to MeAIB, compared to control values, in Na^+-free media at pH 6.0. I_{sc} is expressed as the chemical equivalent (see Methods). Data are the mean ± S.E., $n = 4$. Solid line is the Michaelis-Menten fit of the data.

4. Discussion

Although H^+-linked solute transport in bacteria [28] is accepted, and sequence homology between bacterial and mammalian solute (sugar) transporters has been described [28,29], evidence for H^+-linked solute transport in mammalian tissues is greeted with some scepticism. The only precedent for the H^+, rather than Na^+, electrochemical gradient being the major driving force for intestinal solute absorption is dipeptide transport [16,30,31]. In experiments using intestinal brush-border membrane vesicles dipeptide uptake is independent of Na^+, driven by proton gradients, and is electrogenic [16,30,31]. Monolayers of the intestinal epithelial cell line Caco-2 express the proton-coupled dipeptide transporter [17]. Glycylsarcosine transport is accelerated by apical acidity [17], and is associated with intracellular acidification [17,18] and stimulation of inward I_{sc} in voltage-clamped Caco-2 cell monolayers [22]. Thus, three independent measures (flux, pH_i and I_{sc}) demonstrate H^+-coupled dipeptide transport in intact Caco-2 cell monolayers. In the human small intestine an area of low pH adjacent to the apical membrane (the acid microclimate) has been demonstrated both in vivo [32] and in vitro [33]. Studies of intestinal dipeptide transport emphasise the potential importance of the acid microclimate [32,33] as a driving force in normal intestinal absorptive function. The purpose of this investigation was to determine the role of H^+ in amino acid transport using the non-metabolisable amino acid analogue MeAIB and the model intestinal cell line Caco-2.

Absorptive transport of amino acids across the intestinal enterocyte involves a number of Na^+-dependent and Na^+-independent transport systems arranged in series at the apical and basolateral cell membranes [1,3,14,34]. The non-metabolisable amino acid analogue MeAIB has been used extensively to define the transport of amino acids via system A [35] which is expressed solely at the basolateral membrane in the intestine [3,14]. At the intestinal brush-border, MeAIB transport via a Na^+-dependent route is restricted to the Imino transport system [3,14]. The present observations of MeAIB accumulation across apical and basolateral borders are consistent with the expression of Na^+-dependent transporters for MeAIB at both apical and basolateral membranes of Caco-2 cells. In addition to Na^+-dependent transport of MeAIB, it is apparent that MeAIB transport at the apical brush-border membrane may be energised by the proton electrochemical gradient (Fig. 1). Acidification of the apical medium is associated with increased net absorption and cellular accumulation of MeAIB in both Na^+ and Na^+-free conditions. Transport of MeAIB is associated with an increased flow of protons across the apical membrane (Fig. 4), in a similar fashion to that described previously for β-alanine [19], L-proline [21] and L-alanine [20]. The cellular acidification is observed with addition of MeAIB to the apical membrane only (Fig. 4). This effect is independent of extracellular Na^+ (Fig. 5). At the pH values used in the present study MeAIB will exist predominately as a zwitterion so the final evidence that proton/MeAIB symport activity exists, and is likely to be electrogenic, is the observation that under Na^+-free conditions MeAIB generates a saturable inward I_{sc} (Fig. 8) similar to that reported previously for β-alanine [19]. The difference between the magnitude of kinetic constants in amino acid intracellular accumulation (11.5 mM) and I_{sc} (6.2 mM) reflect the dependence of these parameters on separate transport and homeostatic mechanisms at each epithelial border. Thus, the results of three independent measurements (intracellular accumulation, intracellular acidification and stimulation of inward I_{sc}) show that MeAIB is transported across the apical membrane of this model intestinal epithelium (Caco-2) by a H^+-coupled symporter.

Metabolism and the existence of multiple carriers with overlapping specificity complicate analysis of amino acid transport [34]. However, results from this study suggest that the specificity of the H^+-dependent, Na^+-independent system described here for the non-metabolisable analogue MeAIB, appears novel since any relationship to an intestinal carrier described previously is unclear. Although MeAIB, Gly, L-Ala, and L-Pro are all transported by system A this transporter is absent from the intestinal apical membrane and its activity is thought to decrease (rather than increase) at lower pH values [3]. L-Leu and L-Phe failed to inhibit MeAIB transport suggesting that Na^+-independent system L and Na^+-dependent systems Phe or B are also not involved [3,14,34]. At lower pH values the specificity of system L broadens to include substrates for system A [3]. However, unless system L excludes Leu at lower pH values, MeAIB transport described here is unlikely to be mediated via system L. The specificity of the carrier is unlike the Na^+-dependent systems B, ASC or β as MeAIB is excluded from each of these carriers [3,34]. The transporter showing the greatest degree in overlap in specificity is the Na^+-dependent Imino carrier which shows species variation in its Cl^--dependency [7,9,36]. In the rat small intestine [9,36], the Imino carrier is Cl^--independent and transports MeAIB, β-Ala and L-Pro. These are all substrates for H^+-coupled transport in Caco-2 cells [19,21]. The Na^+-dependent uptake of β-Ala in rat intestine was inhibited by Gly, L-Ala and α-aminoisobutyrate (AIB) but not by L-Leu [9]. However, transport of L-Ala was not inhibited by 40 mM β-Ala [9]. In Caco-2 cells [20], pH-stimulated L-Ala transport was inhibited by β-Ala, L-Pro, AIB and Gly (all 20 mM). Therefore, there are both similarities and differences in the specificities of the H^+-coupled carrier in Caco-2 cells and the Na^+-dependent Imino carrier in rat small intestine. These results also suggest that MeAIB may not always be the most appropriate substrate for characterising transport by the Na^+-dependent systems Imino and A.

In addition to MeAIB, those amino acids displaying H^+- stimulated transport in Na^+-free media include β-

alanine [19], L-alanine [20], and L-proline [21]. The present data show that H^+-driven MeAIB accumulation across the apical membrane is inhibited by β-alanine, L-alanine and L-proline. It is possible that since MeAIB, β-alanine, L-proline and L-alanine all produce intracellular acidification, particularly when apical pH is held at pH 6.0, that cross inhibition may occur via a decrease in the proton-motive force rather than by a common carrier. For instance we have recently noted that glycine may inhibit H^+-stimulated dipeptide (Gly-Sar) transport in Na-free media [37], despite the evidence that amino-acids are not substrates for the cloned PepT1 transporter expressed in oocytes [38]. Ultimately the expression of the cloned transporter(s) responsible for H^+/amino acid cotransport in *Xenopus* oocytes will be necessary to establish the specificity of such transporters in an unambiguous way [38]. Most recently [39], apical-to-basal D-cycloserine transport across Caco-2 cell monolayers has been demonstrated which is pH-dependent (weakly Na^+-dependent) and inhibited by a range of amino acids including proline, L-alanine and β-alanine. However, MeAIB did not inhibit D-cycloserine transport [37] suggesting that more than one transporter may be responsible for H^+-coupled amino acid transport in this human intestinal cell line.

In renal brush-border membrane vesicles prepared from the rabbit proximal convoluted tubule, H^+-dependent glycine, proline and AIB transport have been demonstrated [40–42]. H^+-gradient dependent AIB uptake was inhibited by L-Pro, L-Ala and Gly but not by L-Ser and L-Phe [40]. Whether the transport system described in renal vesicles is identical in molecular terms to the intestinal transport identified in this study will require cloning and sequencing of the carrier(s) involved.

In summary, the current demonstration of a proton/amino acid transport system for MeAIB (in Na^+-free conditions), in human intestinal Caco-2 cells, reinforces our previous data [19–21] for H^+-linked transport of the natural α-amino acid (L-alanine), a non-α-amino acid (β-alanine) and an imino acid (L-proline). Taken together these data suggest that the H^+-electrochemical gradient may play a more extensive role in intestinal absorption of solutes than was previously imagined.

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References

[1] Christensen, H.N. (1990) Physiol. Rev. 70, 43–77.
[2] Shotwell, M.A., Jayme, D.W., Kilberg, M.S. and Oxender, D.L. (1981) J. Biol. Chem. 256, 5422–5427.
[3] Barker, G.A. and Ellory, J.C. (1990) Exp. Physiol. 75, 3–26.
[4] Christensen, H.N., Liang, M. and Archer, E.G. (1967) J. Biol. Chem. 242, 5237–5246.
[5] Bass, R., Hedegaard, H.B., Dillehay, L., Moffet, J. and Englesberg, E. (1981) J. Biol. Chem. 256, 10259–10266.
[6] Munck, B.G. (1985) J. Membr. Biol. 83, 15–24.
[7] Munck, L.K. (1993) Am. J. Physiol. 265, G979–G986.
[8] Stevens, B.R., Ross, H.J. and Wright, E.M. (1982) J. Membr. Biol. 66, 213–225.
[9] Munck, B.G., Munck, L.K., Rasmussen, S.N. and Polache, A. (1994) Am. J. Physiol. 266, R1154–R1161.
[10] Del Castillo, J.R. and Muniz, R. (1991) Am. J. Physiol. 261, G1030–G1036.
[11] Karasov, W., Solberg, D., Carter, S., Hughes, M., Phan, D., Zollman, F. and Diamond, J. (1986) Am. J. Physiol. 251, G501–G508.
[12] Mircheff, A.K., Kippen, I., Hirayama, B. and Wright, E.M. (1982) J. Membr. Biol. 64, 113–122.
[13] Alonso-Torre, S.R., Serrano, M.A., Medina, J.M. and Alvarado, F. (1992) Biochem. J. 288, 47–53.
[14] Stevens, B.R., Kaunitz, J.D. and Wright, E.M. (1984) Annu. Rev. Physiol. 46, 417–433.
[15] Crane, R.K., Miller, D. and Bihler, I. (1961) in Membrane Transport and Metabolism (Kleinzeller, A. and Kotyk, A., eds.), pp. 439–449, Czech. Acad. Sci., Prague.
[16] Ganapathy, V. and Leibach, F.H. (1985) Am. J. Physiol. 249, G153–G160.
[17] Thwaites, D.T., Brown, C.D.A., Hirst, B.H. and Simmons, N.L. (1993) J. Biol. Chem. 268, 7640–7642.
[18] Thwaites, D.T., Brown, C.D.A., Hirst, B.H. and Simmons, N.L. (1993) Biochim. Biophys. Acta 1151, 237–245.
[19] Thwaites, D.T., McEwan, G.T.A., Brown, C.D.A., Hirst, B.H. and Simmons, N.L. (1993) J. Biol. Chem. 268, 18438–18441.
[20] Thwaites, D.T., McEwan, G.T.A., Brown, C.D.A., Hirst, B.H. and Simmons, N.L. (1994) J. Membr. Biol. 140, 143–151.
[21] Thwaites, D.T., McEwan, G.T.A., Cook, M.J., Hirst, B.H. and Simmons, N.L. (1993) FEBS Lett. 333, 78–82.
[22] Thwaites, D.T., McEwan, G.T.A., Hirst, B.H. and Simmons, N.L. (1993) Pflügers Arch. 425, 178–180.
[23] Blais, A., Bissonnette, P. and Berteloot, A. (1987) J. Membr. Biol. 99, 113–125.
[24] Hidalgo, I.J. and Borchardt, R.T. (1990) Biochim. Biophys. Acta 1035, 97–103.
[25] Hu, M. and Borchardt, R.T. (1992) Biochim. Biophys. Acta 1135, 233–244.
[26] Nicklin, P.L., Irwin, W.J., Hassan, I.F. and Mackay, M. (1992) Biochim. Biophys. Acta 1104, 283–292.
[27] Thomas, J.A., Bushbaum, R.N., Zimniak, A. and Racker, E. (1979) Biochemistry 18, 2210–2218.
[28] Henderson, P.J.F. (1990) J. Bioenerg. Biomembr. 22, 525–570.
[29] Maiden, M.C.J., Davis, E.O., Baldwin, S.A., Moore, D.C.M. and Henderson, P.J.F. (1987) Nature 325, 641–643.
[30] Ganapathy, V., Burckhardt, G. and Leibach, F.H. (1984) J. Biol. Chem. 259, 8954–8959.
[31] Ganapathy, V. and Leibach, F.H. (1983) J. Biol. Chem. 258, 14189–14192.
[32] Rawlings, J.M., Lucas, M.L. and Russel, R.I. (1987) Scand. J. Gastroenterol. 22, 377–384.
[33] Lucas, M.L., Cooper, B.T., Lei, F.H., Johnson, I.T., Holmes, G.K.T., Blair, J.A. and Cooke, W.T. (1978) Gut 19, 735–742.

[34] Hopfer, U. (1987) in Physiology of the Gastrointestinal Tract, 2nd Edn. (Johnson,, L.R., ed.), pp. 1499–1526, Raven Press, New York.
[35] McGiven, J.D. and Pastor-Anglada, M. (1994) Biochem. J. 299, 321–334.
[36] Munck, L.K. and Munck, B.G. (1994) Am. J. Physiol. 266, R997–R1007.
[37] Thwaites, D.T., Hirst, B.H. and Simmons, N.L. (1994) Br. J. Pharmacol., in press.
[38] Fei, Y.J., Kanai, Y., Nussberger, S., Ganapathy, V., Leibach, F.H., Romero, M.F., Singh, S.K., Boron, W., and Hediger, M.A. (1994) Nature, 368, 563–566.
[39] Ranaldi, G., Islam, K. and Sambuy, Y. (1994) Antimicrob. Agents Chemother. 38, 1239–1245.
[40] Jessen, H., Vorum, H., Jorgensen, K.E. and Sheikh, M.I. (1991) J. Physiol. 436, 149–167.
[41] Rajendran, V.M., Barry, J.A., Kleinman, J.G. and Ramaswamy, K. (1987) J. Biol. Chem. 262, 14974–14977.
[42] Roigaard-Petersen, H., Jacobsen, C. and Sheikh, M.I. (1987) Am. J. Physiol. 253, F15–F20.