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Chloride-dependence of amino acid transport in rabbit ileum

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Chloride-dependence of influx across the brush-border membrane of distal rabbit ileum was examined for β-alanine, 2-methylaminoisobutyric acid (MeAIB), leucine, lysine, proline and D-glucose. Influx of leucine at 2 mM and of D-glucose at 0.5 mM was chloride-independent indicating that substitution of isethionate for chloride has no unspecific effect on sodium gradient driven transport processes. In contrast influx of β-alanine and MeAIB was totally dependent on the presence of chloride ions. In the absence of chloride, proline transport was reduced to 20% of its control level. This remaining transport can be accounted for by the function of the carrier of α-amino-monocarboxylic acids. Transport of leucine at 0.1 mM was reduced by absence of chloride. This is in accordance with the observation of leucine transport by the β-alanine carrier. The kinetics of chloride and sodium activation of transport of MeAIB were examined at 1 mM MeAIB. Chloride activation was characterized by a Hill coefficient of 1 and a $K_{1/2}$ of 23.5 mM, and sodium activation by a Hill coefficient of 2 and a $K_{1/2}$ of 51 mM. Thus cotransport of chloride with an imino acid would be compatible with the known rheogenic nature of this transport. This study adds the imino acid carrier and the β-alanine carrier to the group of chloride-dependent, epithelial amino acid transport systems.

Introduction

For some time chloride-dependent transport of amino acids has been known for red blood cells [1,2] and neuronal tissues [3]. More recently chloride dependence has been observed for transport of β-alanine [4], glycine [5] and taurine [6] in brush-border membrane vesicles (BBMV) from kidney proximal tubule and for taurine in intestinal epithelia [7]. In rat small intestine β-alanine is transported by the imino acid carrier and inhibition studies indicate that the imino acid carrier is also a transporter of taurine [8]. This coincidence with the transport function of the kidney proximal tubule, and the observation of a proline : sodium stoichiometry of 1 : 2 in rabbit jejunum [9] suggest that in general the imino acid carrier might be characterized by chloride dependence. This suggestion is supported by previous observations of partial chloride dependence of influx across the brush-border membrane, steady-state mucosal uptake and transepithelial net transport of proline in rat jejunum [10].

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In the present paper we examine the question of chloride dependence of the imino carrier in the rabbit ileum. In view of the evidence of a separate carrier of β-alanine, which judged from its specificity patterns [11] is different from the chloride-dependent carrier of taurine [4,7]. The chloride dependence of β-alanine transport is also briefly tested.

Materials and Methods

Female albino rabbits with a body weight of 2500–3000 g were kept with free access to food and water and killed by intravenous injection of pentobarbital sodium. The most distal 30 cm were excised, opened along the mesenterium, rinsed in chilled buffer solutions, and cut in halves A and B. These were then cut lengthwise in segments A_1, A_2, B_1 and B_2 allowing a total of 16 measurements on each rabbit.

All solutions were made from a phosphate buffer with a pH of 7.4 and the composition of (mM): Na, 140; K, 8; Ca, 2.6; Mg, 1; Cl, 140; phosphate, 8; SO_4, 1. The solutions contained 5 mM D-glucose except when otherwise stated. The concentrations of sodium or chloride were varied by substitution with *N*-methyl-D-glucamine and isethionate, respectively.

^{14}C-labeled β-alanine, 2-methylaminoisobutyric acid (MeAIB), leucine, lysine, proline and D-glucose (Glc),

and ^{3}H-labeled polyethyleneglycol (mol. wt. 4000) (^{3}H-PEG) were purchased from Du Pont, NEN Research Product.

Using the technique described for rabbit ileum [15] influx across the brush-border membrane of a substance 'A' (J_{mc}^{A}) was measured at the concentrations of amino acids stated and at different concentrations of chloride or sodium ($[Cl^-]_m$, $[Na^+]_m$) in the mucosa-bathing solutions. Chamber and procedure have previously been described in detail. A mucosal surface of 0.62 cm^2 is exposed in the bottom of a well, which allows vigorous stirring and aeration by a stream of O_2 at 37° C. The tissues are preincubated for 25 min at the concentrations of chloride and sodium to be used in the test solutions. Before injecting the test solution, which contains substance 'A' with its ^{14}C-tracer and ^{3}H-PEG as a marker of extracellular contamination, the well and mucosal surface is gently wiped with soft paper to remove adherent preincubation solution. After 0.5 min the incubation is terminated by injection of a large volume of chilled, isotonic mannitol solution. The tissues are excised, briefly washed in chilled mannitol solution, and extracted overnight in 0.1 M HNO_3. The extract is analyzed for ^{14}C and ^{3}H activity. These activities are used for calculation of J_{mc}^{A}.

The kinetics of chloride and sodium activation of J_{mc}^{MeAIB} were estimated by non-linear, least square fitting to

$$J_{mc}^{MeAIB} = \frac{J_{max} \cdot [Cl^- \text{ or } Na^+]_m^n}{K_{1/2}^n + [Cl^- \text{ or } Na^+]_m^n} \quad (1)$$

where J_{max} is J_{mc}^{MeAIB} at 1 mM under maximum activation by chloride or sodium; $K_{1/2}$ is the concentration of chloride, respectively, sodium, at which half-maximum activation is obtained. $[Cl^- \text{ or } Na^+]_m^n$ is the concentration of the variable activator ion and n is the Hill coefficient. J_{mc} is in $\mu mol \cdot cm^{-2} \cdot h^{-1}$ and concentrations are in mM. The errors on the estimates of activation kinetics are S.D. The data are evaluated by Student's t-test with P-values less than 0.05 as the limit of statistical significance.

Results

Chloride-dependence of amino acid influx across the brush-border membrane

The chloride-dependence of J_{mc} of β-alanine (1 mM), MeAIB (1 mM), leucine (2.0 or 0.1 mM), proline (1 mM), and D-glucose (0.5 mM) was surveyed in paired experiments at 140 mM or 0 mM chloride. In these experiments segments A_1 and B_1 were used to measure transport in the presence of chloride while segments A_2 and B_2 were used with chloride free solutions.

$J_{mc}^{\beta\text{-Ala}}$ was examined for chloride-dependence because in the rat small intestine it is transported by the imino acid carrier [8], in dog kidney proximal tubules by a chloride-dependent carrier [4] and in the rabbit small intestine by a very low-capacity, very high-affinity transport mechanism for taurine [7] in addition to a medium-capacity, so called β-alanine carrier [11]. MeAIB was chosen because it is an almost ideal substrate for the imino acid carrier [11]. Leucine was chosen as a substrate for the carrier of neutral amino acids [12], and as a possible test substance for unspecific effects of substituting isethionate for chloride. It was also tested at 0.1 mM because at this concentration it has been possible to demonstrate a contribution by the β-alanine carrier to transport of leucine [11]. Proline we examined as a substrate for the imino acid carrier and for the carrier of neutral amino acids [13] and because a Hill coefficient of two had been reported for sodium-activation of its uptake by rabbit jejunal brush-border membrane vesicles [9]. Similarly a Hill coefficient of two had been attributed to sodium activation of rabbit jejunal brush-border membrane transport of D-glucose [14]. Therefore, glucose was included in this series of screening experiments and meassured in both jejunum and distal ileum.

The results of this series of experiments are shown in Fig. 1. It is seen that under nominally chloride-free conditions $J_{mc}^{\beta\text{-Ala}}$ and J_{mc}^{MeAIB} are reduced to levels which do not differ significantly from the estimates of diffusive transport usually associated with the present technique [11]. Also J_{mc}^{Pro} is greatly reduced in the absence of chloride. The J_{mc}^{Pro} seen under chloride-free conditions can be accounted for by proline transport by the carrier of neutral amino acids [13], which by the chloride-independence of J_{mc}^{Leu} at 2 mM leucine is demonstrated to be chloride-independent. The partial chloride-dependence of J_{mc}^{Leu} at 0.1 mM leucine agrees with the chloride-dependence of the β-alanine carrier. The chloride-independence of J_{mc}^{Glu} and J_{mc}^{Leu} at 2 mM leucine indicate that substitution of isethionate for chloride does not affect sodium-dependent transport of sugars or amino acids nonspecifically.

Kinetics of chloride and sodium activation of J_{mc}^{MeAIB} measured at 1 mM MeAIB

The data of Fig. 1 on J_{mc} of MeAIB and proline demonstrate chloride dependence of the imino acid carrier of the rabbit ileum. Having once demonstrated the usefulness of MeAIB as a test substance for the imino acid carrier [11] we examined the kinetics of sodium and chloride activation of the imino acid carrier at 1 mM MeAIB.

Chloride activation of J_{mc}^{MeAIB} was examined in paired experiments at 0, 8, 17, 35, 70 and 140 mM chloride. The activation is demonstrated by Fig. 2. The best fit of Eqn. 1 to these data is described as

$$J_{mc}^{MeAIB} = \frac{(0.93 \pm 0.03) \cdot [Cl^-]_m}{(23.5 \pm 2.0) + [Cl^-]_m} \quad (2)$$

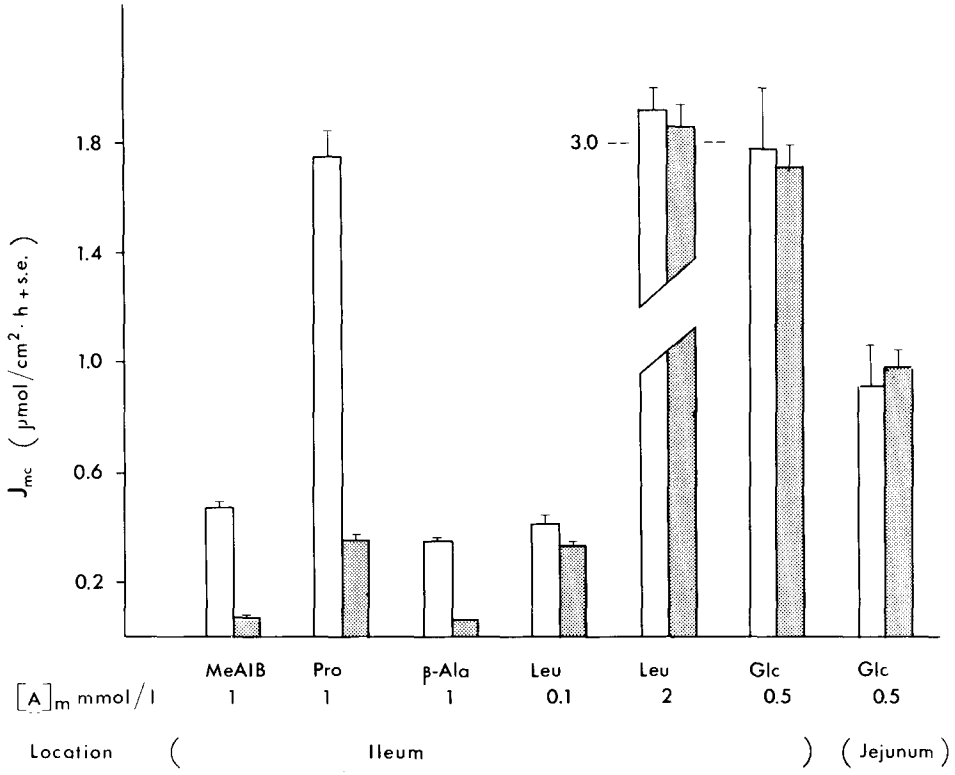

Fig. 1. The chloride-dependence of amino acid influx (J_{mc}) measured in the pressence of 5 mM D-glucose, and of D-glucose in jejunum and ileum. Each bar represents the mean ± S.E. of eight observations. Open columns: 140 mM chloride; closed columns: 0 mM chloride.

Sodium activation af J_{mc} was examined in paired measurements of J_{mc} at 0, 6, 12, 23, 35, 46, 93 and 140 mM sodium after 25 min preincubation at these concentrations. The results are shown in Fig. 3. The best fit of Eqn. 1 to these data is described as

$$J_{mc}^{MeAIB} = \frac{(1.34 \pm 0.08) \cdot [Na^+]_m^2}{(51.4 \pm 2.6)^2 + [Na^+]_m^2} \quad (3)$$

assuming a diffusive contribution to J_{mc}^{MeAIB} of 0.05 $\mu mol \cdot cm^{-2} \cdot h^{-1}$. The sigmoidal shape of the activation curve is consistent with a Hill coefficient of two. Acceptable fits could not be derived assuming Hill coefficients of one or three.

Discussion

The survey of chloride dependence (Fig. 1) provides four pieces of information. First, the chloride independence of leucine and glucose transport gives the crucial information that substitution of isethionate for chloride is without unspecific effect on sodium-coupled transport of sugars and amino acids. Second, the absolute chloride dependence of J_{mc}^{MeAIB} and the reduction of

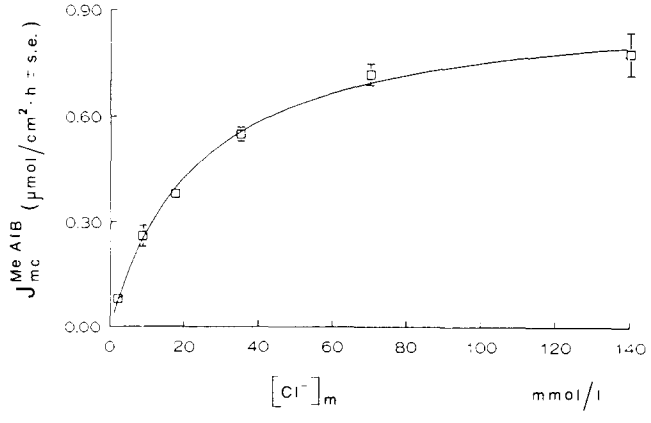

Fig. 2. Chloride activation of J_{mc}^{MeAIB} in rabbit ileum. Each point represents the mean ± S.E. of seven observations. The curve is described by Eqn. 2.

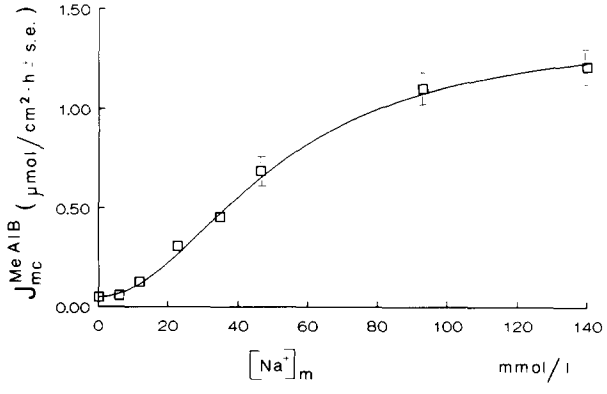

Fig. 3. Sodium activation of J_{mc}^{MeAIB} in rabbit ileum. Each point represents the mean ± S.E. of seven observations. The curve is described by Eqn. 3.

J_{mc}^{Pro} to a value, which can be accounted for by the carrier of neutral amino acids [16] demonstrate that the presence of chloride ions is essential for transport by the imino acid carrier. Third, the complete chloride dependence of $J_{mc}^{\beta\text{-ala}}$ demonstrates that transport by the β-alanine carrier is chloride dependent. By its inhibition profile, low K_i for α-aminoisobutyrate (AIB), leucine and alanine [11] the β-alanine carrier can be distinguished from the chloride-dependent taurine carrier, which is not inhibited by AIB, alanine or leucine [7]. The present results thus increase the number of chloride-dependent intestinal systems by two to a total of three. Finally, although the Hill coefficient of two for sodium activation af imino acid carrier uptake of proline by rabbit brush-border vesicles can be seen as heralding its chloride dependence, the chloride independence of J_{mc}^{Glu} demonstrates that this will not always be the case.

The kinetic details of chloride- and sodium-activation of the imino acid carrier are important for considering the possibility of imino acid/chloride cotransport, as this must be done in the light of the demonstrated rheogenic nature of the imino acid/sodium cotransport [17].

The data of Fig. 2 as described by Eqn. 2 confirm the chloride dependence of J_{mc}^{MeAIB}. The Hill coefficient of one agrees with results for β-alanine transport by kidney proximal tubule [4] and for taurine transport by rabbit jejunal brush-border vesicles [7]. The $K_{1/2}$ of 23.5 mM is similar to the estimate of 32 mM for β-alanine transport by dog kidney proximal tubule brush-border vesicles [4].

Fig. 3 and Eqn. 3 describe sodium activation of J_{mc}^{MeAIB}. The Hill coefficient of two indicates that more than one sodium ion are cotransported with each molecule of MeAIB and demonstrates that rheogenic sodium/MeAIB cotransport is compatible with MeAIB/chloride cotransport. It is noteworthy that the present estimate of a $K_{1/2}$ for sodium is identical with the estimate reached for sodium activation of proline uptake by brush-border membrane vesicles [9].

The present study has demonstrated that the imino acid carrier and the β-alanine carrier of rabbit ileum are chloride dependent. The kinetics of activation of J_{mc}^{MeAIB} by sodium and chloride are consistent with a transport stoichiometry of 1 MeAIB: 2 Na: 1 Cl and therefore, with both cotransport of MeAIB and chloride, and rheogenic, sodium-dependent transport of this imino acid. The chloride independence of J_{mc}^{Glu} demonstrates that a Hill coefficient of two for sodium activation should not be equated with chloride dependence of the transport being studied.

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References

1 Imler, J.R. and Vidaver, G.A. (1972) Biochim. Biophys. Acta 288, 153–165.
2 King, P.A. and Gunn, R.B. (1989) J. Gen. Physiol. 93, 321–342.
3 Kanner, B.I. and Schuldiner, S. (1987) CRC Crit. Rev. Biochem. 22, 1–38.
4 Turner, R.J. (1986) J. Biol. Chem. 261, 16060–16066.
5 Scalera, V., Corcelli, A., Frassanito, A. and Storelli, C. (1987) Biochim. Biophys. Acta 903, 1–10.
6 Zelikovic, I., Stejskal-Lorenz, E., Lohstroh, P., Budreau, A. and Chesney, R.W. (1989) Am. J. Physiol. 256, F646–F655.
7 Miyamoto, Y., Tiruppathi, C., Ganapathy, V. and Leibach, F.H. (1989) Am. J. Physiol. 257, G65–G72.
8 Munck, B.G. (1984) in Intestinal Transport (Gilles-Baillien, M. and Gilles, R., eds.), p. 260, Springer Verlag, Berlin.
9 Stevens, B.R. and Wright, E.M. (1987) J. Biol. Chem. 262, 6546–6551.
10 Munck, B.G. (1972) J. Physiol. 223, 699–717.
11 Munck, B.G. (1985) J. Membr. Biol. 83, 15–24.
12 Munck, B.G. (1985) J. Membr. Biol. 83, 1–13.
13 Stevens, B.R. and Wright, E.M. (1985) J. Membr. Biol. 87, 27–34.
14 Kaunitz, J.D., Gunther, R. and Wright, E.M. (1982) Proc. Natl. Acad. Sci. USA 79, 2315–2318.
15 Schultz, S.G., Curran, P.F., Chez, R.A. and Fuisz, R.E. (1967) J. Gen. Physiol. 50, 1241–1260.
16 Munck, L.K. and Munck, B.G. (1989) Z. Gastroenterol. 27, 297.
17 Wright, E.M., Schell, R.E. and Stevens, B.R. (1985) Biochim. Biophys. Acta 818, 271–274.