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Transport of the α-amino-mono-carboxylic acid L-alanine by the β-alanine carrier of the rabbit ileum

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The proposal that the β-alanine carrier of the rabbit ileum is a high affinity carrier of the neutral amino acids was examined by means of measurements of influx across the brush border membrane of the intact epithelium using L-alanine as a representative of the neutral amino acids. Confirming the proposal, evidence was provided for mutual competitive inhibition between β-alanine and L-alanine; and it was also demonstrated that a process contributes to the influx of L-alanine, which is characterized by a maximum rate of transport equal to that of β-alanine and a K_t, which is equal to the K_i of L-alanine against the influx of β-alanine. In the concentration range 0.01 to 0.125 mM the influx of L-alanine was found to be linearly related to the concentration indicating a significant unstirred layer influence on present and previous estimates of the K_t values for influx of amino acids across the brush-border membrane of intact intestinal epithelia.

We have recently [1–3] interpreted data on the transport of α-amino-monocarboxylic acids (neutral amino acids), cationic amino acids, non-α-amino-monocarboxylic acids, and imino acids across the brush-border membrane of the rabbit ileum in terms of five separate, saturable transport systems (carriers). Of these a 2-methylaminoisobutyric acid carrier, with many characteristics in common with the imino acid carrier of the hamster [3] and rat [4] small intestine as well as with the imino acid carrier of the rabbit jejenum [5,6], and the low-affinity carrier of cationic amino acids appear to be low-affinity carriers of neutral amino acids, which have previously been lumped into one low-affinity transport [7] (K_i^{Ala} 80–150 mM); a medium-affinity carrier specific for neutral amino acids, most extensively characterized by Preston, Schaeffer and Curran (1974) [8], and the high-affinity carrier of cationic amino acids both appear to be medium-affinity carriers of neutral amino acids (K_i^{Ala} 7–10 mM). Finally the carrier of non-α-amino-monocarboxylic acids, the β-alanine carrier, appears to be a high-affinity carrier of neutral and cationic amino acids (K_i^{Ala} 0.1 mM).

In spite of the low capacity of this β-alanine carrier (0.8 μmol/cm² per h) its high-affinity for the neutral amino acids indicates that, located at the threshold to the coecum, it could play a major role as a conservator of amino acids for the animal.

We have now made an attempt to provide more direct evidence for the participation of the β-alanine carrier in the transport of neutral amino

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acids. For this purpose we have chosen alanine, because its K_i against β-alanine and presumably its K_t for transport by the β-alanine carrier is close to two orders of magnitude lower than the next lowest K_i^{Ala}, 8 mM against the high-affinity carrier of lysine.

Ideally it should be proven [9] that alanine is transported by a carrier with a K_t equal to its K_i against β-alanine(A), that β-alanine has a K_i against the transport of alanine equal to its K_t(B), and that inhibitors exist with identical K_i values against alanine and β-alanine(C), the ABC-test. However, because alanine is transported by several carriers from which it cannot be competitively excluded without also excluding it from transport by the β-alanine carrier, the last step of the ABC-step cannot be successfully taken. For the same reason it is not possible to demonstrate that β-alanine has a K_i against the transport of alanine equal to its K_t of 2 mM.

Confronted with these obstacles we have attempted to demonstrate that there is a contribution to the influx of alanine across the brush-border membrane, J_{mc}^{Ala}, with a K_t similar to the K_i of 0.1 mM, that alanine is a competitive inhibitor of $J_{mc}^{\beta\text{-Ala}}$, and that β-alanine is a competitive inhibitor of J_{mc}^{Ala}.

The distal 30 cm of the ileum from 2.5–3 kg, female, white rabbits were prepared for mounting in the previously described influx chambers [10,11]. In these chambers the tissues were preincubated for 25 min at (mM) 140 Na, 8 K, 2.6 Ca, 1 Mg, 140 Cl, 8 PO_4, 1 SO_4, and 5 D-glucose at pH 7.4 and 37 °C, under aeration with 100% O_2. Uptake was measured in 0.5 min incubations with this solution supplemented with alanine and/or β-alanine as described below. ^{14}C-labelled amino acids were used and contamination with the incubation fluid was determined by ^{3}H-labelled polyethylene glycol (mol. wt. 4000). The radioactively labelled substances were purchased from New England Nuclear Co. The kinetic analyses of the results were performed by non-linear fitting to a model of 1 or 2 Michaelis-Menten processes with or without a linear, supposedly diffusive contribution:

$$J_{mc} = \frac{J'_{max}\cdot[A]}{K'_t+[A]} + \frac{J''_{max}\cdot[A]}{K''_t+[A]} + P[A] \qquad (1)$$

The estimates of the parameters are stated ± S.D. The errors of the J_{mc} data are S.E.

The influx of β-alanine, $J_{mc}^{\beta\text{-Ala}}$, was measured in paired experiments at 1, 2, 3, 4, 5, 8, 10, 16 and 20 mM β-alanine in the presence of 0.5 mM L-alanine, and at 1 mM β-alanine without L-alanine. Were L-alanine a competitive inhibitor of $J_{mc}^{\beta\text{-Ala}}$ with a K_i of 0.1 mM, then 0.5 mM L-alanine should raise the apparent K_t of $J_{mc}^{\beta\text{-Ala}}$ from the previously reported 2 mM to 12 mM without affecting the $J_{max}^{\beta\text{-Ala}}$. The results (Fig. 1) were best described as

$$J_{mc}^{\beta\text{-Ala}} = \frac{(0.62\pm0.97)[\beta\text{-Ala}]}{(6.87\pm11.01)+[\beta\text{-Ala}]} + (0.06\pm0.03)[\beta\text{-Ala}] \qquad (2)$$

which according to the chi-square tests fits the data with a P value of 0.45. In the absence of L-alanine $J_{mc}^{\beta\text{-Ala}}$ was 0.38 ± 0.04 μmol/cm² per h (n = 8), which is within the range of the previously reported results. Thus these results are consistent with L-alanine being a competitive inhibitor of the transport of β-alanine.

The influx of L-alanine, J_{mc}^{Ala}, was measured at 0.125, 0.25, 0.5, 1.0, 1.5, 2, 3, 4, and 5 mM L-alanine. As shown in Fig. 2 the results of these experiments clearly demonstrated the existence of a high-affinity, low-capacity contribution to J_{mc}^{Ala} in addition to the combined contributions by lower-to-low affinity carriers and diffusion. With a P value of 0.95 by the chi-square test the best

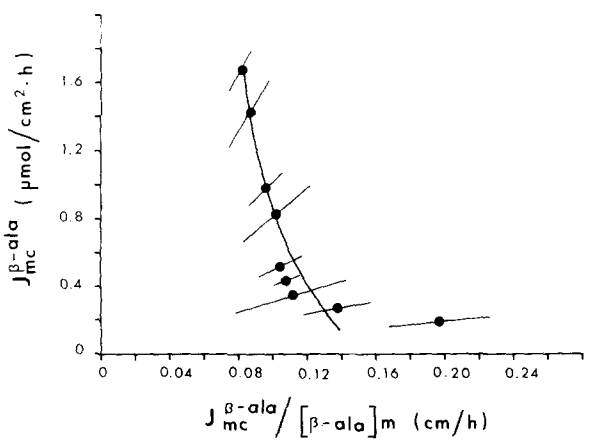

Fig. 1. Influx of β-alanine across the brush-border membrane of the distal rabbit ileum. The curve represents the Eadie-Hofstee transformation of Eqn. 2. Errors are ± S.E.

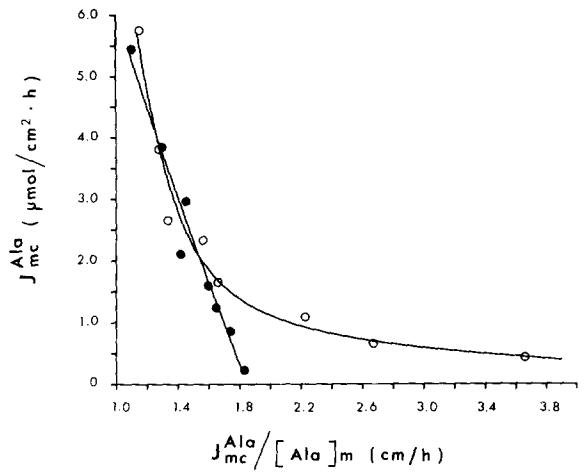

Fig. 2. Influx of L-alanine across the brush-border membrane of the distal rabbit ileum. Open circles indicate data obtained in the absence of β-alanine. Closed circles indicate data obtained in the presence of 80 mM β-alanine. The curves represent Eadie-Hofstee transformations of Eqns. 3 and 4. Errors are ± S.E.

fit to the result is described as

$$J^{Ala}_{mc} = \frac{(0.49 \pm 0.15)[Ala]}{(0.09 \pm 0.06) + [Ala]} + \frac{(30 \pm 14)[Ala]}{(24 \pm 15) + [Ala]} \quad (3)$$

The J_{max} and K_t of the first term of this equation agree well with the present and the previously reported value of 0.8 for $J^{\beta\text{-Ala}}_{max}$ and with the previous estimate of 0.1 mM as K_i^{Ala} against $J^{\beta\text{-Ala}}_{mc}$. According to Eqn. 3 at 90 mM L-alanine J^{Ala}_{mc} should be 24.2 μmol/cm² per h. This corresponds well with the previously [1] reported value of 20.5 ± 0.7 μmol/cm² per h ($n = 8$). Under close to maximum inhibition by lysine this value could be reduced to 14.8 ± 0.3 μmol/cm² per h ($n = 8$), which by the further addition of 100 mM leucine was reduced to a value of 5.4 ± 0.2 μmol/cm² per h ($n = 6$). Similarily at 1 mM alanine sequential inhibition by lysine and alanine demonstrated [1] that the second term of Eqn. 2 must describe the combined contributions of at least two other saturable transport processes in addition to any diffusive contribution.

With a K_t of 0.1 and 2 mM for, respectively, alanine and β-alanine, 80 mM β-alanine should increase the apparent K_t of alanine for the β-alanine carrier to the level previously observed [1] for transport of alanine by the carrier of neutral amino acids and by the high-affinity carrier of lysine. Accordingly, in an Eadie-Hofstee plot, 80 mM β-alanine should eliminate the indication of a high-affinity contribution to the transport of alanine. In consequence of these considerations J^{Ala}_{mc} was measured in paired experiments at 0.125, 0.5, 0.75, 1.0, 1.5, 2, 3, 5 and 10 mM alanine all with 80 mM β-alanine.

The results of these experiments are shown in Fig. 2, from which it is seen that the consequences of adding 80 mM β-alanine were as expected. Under these conditions the transport of alanine is best described as

$$J^{Ala}_{mc} = \frac{(9.5 \pm 3.1)[Ala]}{(5.8 \pm 1.5)[Ala]} + (0.20 \pm 0.16)[Ala] \quad (4)$$

which by the chi-square test fits the results with a P value of 0.95.

We have previously [1] provided evidence that $J^{\beta\text{-Ala}}_{mc}$ like J^{Ala}_{mc} is sodium-dependent and, therefore, presumably rheogenic. Interpreting the data of fig. 2 we have neglected the possible effect on J^{Ala}_{mc} of a rheogenic $J^{\beta\text{-Ala}}_{mc}$. This is justified by the low $J^{\beta\text{-Ala}}_{max}$, by the presence of 5 mM D-glucose as previously discussed [1], and by the previous demonstration [1] that even at 90 mM, alanine, with a J_{max} more than ten times that of β-alanine, did not significantly affect the transport of galactose as measured at 1 mM D-galactose.

The data for J^{Ala}_{mc} at 90 mM alanine as well as the ability to competitively reduce $J^{\beta\text{-Ala}}_{mc}$ to 0.01–0.03 μmol/cm² per h at 1 mM β-alanine [2] indicate that any diffusive contribution to J^{Ala}_{mc} must be less than 0.06 μmol/cm² per h per mM. Therefore the second term of Eqn. 4 in all likelihood includes a contribution by a low-affinity carrier. To verify this interpretation J^{Ala}_{mc} was measured in paired experiments at 1 mM alanine, at 1 mM alanine + 300 mM mannitol, and at 1 mM alanine + 100 mM lysine-HCl + 100 mM leucine and 10 mM proline. In μmol/cm² per h ± S.E. J^{Ala}_{mc} were, respectively, 2.22 ± 0.15 ($n = 9$), 2.07 ± 0.17 ($n = 10$), and 0.087 ± 0.005 ($n = 9$). These results demonstrate that J^{Ala}_{mc} is not significantly affected by the osmotic effect of the inhibitors, and in view of the fact that in spite of the high concentrations of inhibitors one third of the remaining J^{Ala}_{mc} will be by the carrier of neutral amino acids and by the low-affinity carrier of

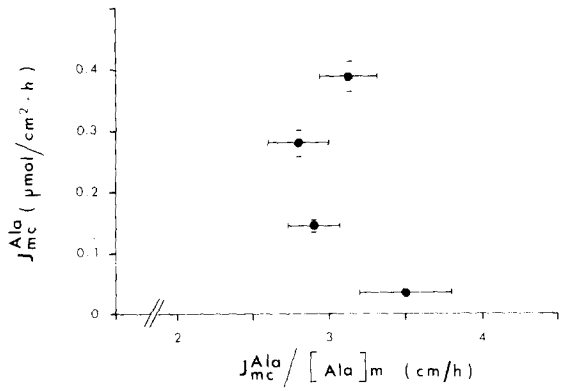

Fig. 3. Influx of L-alanine across the brush-border membrane of the distal rabbit ileum. Errors are ±S.E.

lysine these data confirm the above interpretation of the second term of Eqn. 4.

In a previous discussion [1] of the significance of the unstirred layer for the results obtained with the present technique it was observed that the apparent absence of a high-affinity contribution to the transport of D-glucose might argue for a significant effect, whereas the very similar estimates of the K_t or K_i of proline for the imino acid carrier of the rabbit small intestine, 0.8 mM with the present technique [2] and 0.6 mM by the microvesicle technique [12] argued against such an effect. However, more recent estimates [6] of the K_t of proline by the microvesicle technique, 0.2–0.3 mM, indicate that in our experiments the unstirred layer may play a significant role. Thus to get a better perspective on the present results we measured J^{Ala}_{mc} in paired experiments at 0.010, 0.050, 0.100 and 0.125 mM alanine. As shown in Fig. 3 the results demonstrate that at these three concentrations the values of J^{Ala}_{mc}/[Ala] do not differ significantly from each other indicating that the unstirred layer has been the rate limiting barrier, and that in Eqn. 3 the K_t of alanine for the β-alanine carrier is overestimated.

In summary we have demonstrated a contribution to J^{Ala}_{mc} with the kinetic constants expected for the transport of alanine by the carrier of β-alanine; and we have provided evidence for mutual competitive inhibition between alanine and β-alanine to a degree consistent with their sharing the carrier of β-alanine. We can, therefore, conclude that, as previously proposed, the carrier of β-alanine is a high-affinity carrier of neutral amino acids, and by analogy of cationic amino acids. As previous investigators]5,7,8,12] we have been unable by kinetic analysis to resolve the intestinal transport of an amino acid into all the contributing processes. Our results once again demonstrate the need to supplement the purely kinetic analysis with the use of a variety of competitive inhibitors. Finally, the influence of the unstirred layer has been reemphasized. In this respect the present technique is inferior to that of the microvesicle [6]; but in contrast to the latter the present technique is not haunted by significant rheogenic effects, most likely because the presence of 5 mM D-glucose and hence a high rate of sodium cotransport ensures that the basolateral pump-leak regulation [13–15] of the membrane potential is activated already during the period of preincubation.

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