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COMPETITIVE INTERACTIONS BETWEEN L-ALANINE AND L-PHENYLALANINE IN RABBIT ILEUM

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SUMMARY

Studies of the interactions between the influxes of L-alanine and L-phenylalanine across the brush border of rabbit ileum indicate that for both amino acids the "inhibitor constant" (K'_I) is approximately twice the "transport constant" (K_A). These findings are consistent with the presence of separate influx mechanisms for alanine and phenylalanine, such that (i) both amino acids can interact (bind) with each mechanism and (ii) interaction with the inhibitory amino acid results in a complex that cannot translocate across the membrane.

The unidirectional influxes of L-alanine and L-phenylalanine across the brush border of rabbit ileum have been studied extensively in this and other laboratories. For each amino acid, the relation between influx and the amino acid concentration in the mucosal solution is consistent with a single saturable process that conforms to Michaelis–Menten kinetics. In three separate studies, carried out by different groups of investigators, the concentration of alanine needed to elicit a half-maximal influx was 9 mM[1–3]. The concentration of phenylalanine needed to elicit a half-maximal influx determined in this laboratory is 3.5 mM[3] in good agreement with the value of 2.7 ± 0.7 reported by HAJJAR AND CURRAN[4] for the same preparation. However, the maximal influx of alanine has been found consistently to be significantly greater than that of phenylalanine[3], suggesting that these two neutral amino acids may not share the same influx mechanism. To explore this problem further we have examined competitive interactions between alanine and phenylalanine influxes. The reasoning behind this approach is as follows:

If two amino acids interact with the same influx mechanism in a strictly competitive manner, the unidirectional influx of one amino acid (A) in the presence of the competing amino acid (I) is given by:

$$J_{A'} = \frac{[A]J_A^{max}}{[A] + K_A + K_A[I]/K_I'}$$

where J_A^{max} is the maximal influx of A, K_A is the concentration of A needed to elicit a half-maximal influx and the terms in brackets are the concentrations of A and I in the mucosal solution[1]. K_I' is the inhibitor constant which, for a strictly competitive

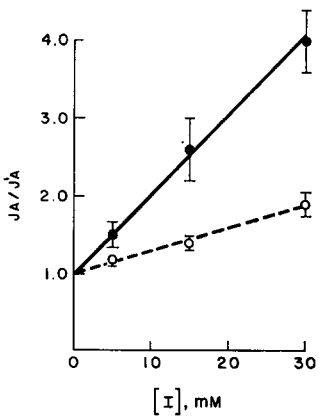
interaction, should equal the concentration of I needed to elicit a half-maximal influx of I. It follows that at constant $[A]$

$$J_A/J_A' = 1 + K_A[I]/K_I'([A] + K_A)$$

where J_A is the unidirectional influx of A when $[I] = 0$ and J_A' is the influx of A in the presence of I. Thus, J_A/J_A' should be a linear function of $[I]$ with an intercept (when $[I] = 0$) of 1.0 and a slope equal to K_A/K_I' $([A] + K_A)$. Knowing K_A and $[A]$ the value of K_I' can be calculated readily.

In these experiments we determined the unidirectional influxes of alanine from mucosal solutions containing 3 mM alanine and either 0, 5, 15 or 30 mM phenylalanine. In addition, the unidirectional influxes of phenylalanine were determined from mucosal solutions containing 3 mM phenylalanine and either 0, 5, 15 or 30 mM alanine. The methods for determination of the unidirectional influxes across the brush border of rabbit ileum have been described in detail[5]. All mucosal solutions were isoosmotic through the addition of appropriate concentrations of mannitol. The results are illustrated in Fig. 1; the solid circles designate the effects of varying concentrations of phenylalanine on the influx of alanine and the open circles designate the effects of alanine on the influx of phenylalanine. In both instances there is a linear relation between J_A/J_A' and $[I]$ that intercepts the ordinate at unity. However, the calculated K_I' for phenylalanine is 7.5 mM; a value that is 2.1 times the directly measured concentration needed to elicit a half-maximal influx of phenylalanine. Similarly, the value of K_I' for alanine is 22 mM, a value that is 2.4 times the concentration needed to elicit a half-maximal influx of alanine. In both instances, the inhibitor constant, K_I' is approximately twice the directly determined K_A.

A possible explanation for these observations is that two different carrier mechanisms are responsible for the influxes of alanine and phenylalanine across the brush border and that each amino acid can interact with the membrane component responsible for the influx of the other but the resulting complex is incapable of translocating across the membrane. A formal description of this model is illustrated in



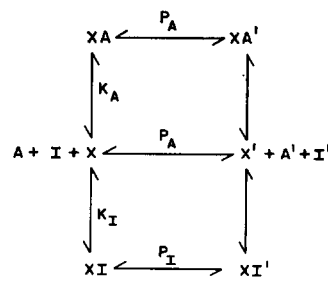


Fig. 1. Effects of varying concentrations of phenylalanine on alanine influx (●), and effects of varying concentrations of alanine on phenylalanine influx (○). Each point is the average of 8 determinations ± S.E.

Fig. 2. Kinetic model for the interaction of amino acids A and I with a membrane component, X. The primes (') designate the intracellular compartment.

Fig. 2. We assume that the brush border contains a component X that is capable of binding A to form XA with an apparent dissocation constant* K_A. X can also bind I to form the complex XI having an apparent dissociation constant K_I. P_A and P_I are the rate constants for translocation of XA and XI, respectively, across the brush border. The assumptions underlying this model have been discussed in detail previously[1]. The unidirectional influx of A in the presence of I (assuming that $[A']$ and $[I']$ are negligible) is given by:

$$J_{A'} = \frac{[A]J_A^{\max}}{[A] + K_A + (1 + P_I/P_A)\,(K_A[I]/2K_I)}$$

Clearly, the inhibitor constant is given by:

$$K_{I'} = 2K_I/(1 + P_I/P_A)$$

Thus, $K_{I'} = K_I$ only when $P_I = P_A$. When $P_I = 0$ (*i.e.* when the complex XI is incapable of translocating across the membrane) $K_{I'} = 2K_I$.

Thus, the observation that the inhibitor constants for the mutual interactions between alanine and phenylalanine influxes are approximately twice the concentrations of these amino acids needed to elicit a half-maximal influx is consistent with a model in which: (a) alanine and phenylalanine influxes are mediated by two different mechanisms; (b) these mechanisms do not differ with respect to their abilities to bind these two neutral amino acids (*i.e.* alanine can bind equally well with the phenylalanine influx mechanism as with the alanine influx mechanism); however, (c) the binding of alanine with the phenylalanine influx mechanism or the binding of phenylalanine to the alanine influx mechanism is non-productive inasmuch as the resulting complexes are incapable of translocation across the brush border.

CHRISTENSEN[6] has amassed a large body of evidence suggesting that several agencies with overlapping specificities are involved in the transport of neutral amino acids in a variety of cell systems. In general, neutral amino acids with large apolar side chains (*e.g.* phenylalanine) are transported predominantly by one mechanism, whereas amino acids such as alanine are transported predominantly by another. The present results suggest that this concept may apply to rabbit small intestine. Further, there are two examples of competitive inhibitions of intestinal sugar transport by substances that do not appear to enter the intestinal cell, *i.e.* phlorizin[7] and L-fucose[8]. CASPARY *et al.*[8] have suggested that L-fucose binds to the carrier mechanism responsible for sugar transport but that the resultant complex is incapable of translocation. The present results lend support to this notion that binding and translocation are distinct processes with separate structural prerequisites and are analogous to the binding and catalytic steps that characterize enzymic reactions. Finally these results indicate that the demonstration of kinetics consistent with classical competitive inhibition does not imply that the inhibitor constant is identical with the transport constant or that the inhibitor is necessarily transported by the agency it inhibits.

* The influxes of alanine and phenylalanine are Na^+-coupled processes. The "apparent dissociation constant" is a phenomenologic constant that includes the interaction between the amino acid and the membrane component as well as the interaction with Na^+, and should not be construed to be a true dissociation constant. The solution of the more complex model that includes interactions with Na^+ leads to the same conclusion as that deduced from the simpler construct illustrated in Fig. 2.

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