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REVIEW

Amino Acid Transport in the Small Intestine

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The early developments in the understanding of intestinal transport of amino acids and the identification of separate mechanisms for transport of bipolar, cationic and anionic amino acids and of imino acids have been reviewed several times (Wilson 1962, Wiseman 1968, Munck 1981). The present state of knowledge is comprehensively described in recent reviews (Stevens 1992a, Ganapathy *et al.* 1994, Ferraris 1994). This overview will focus on transport across the brush border membrane of the mammalian small intestine using the transport function of the rabbit distal ileum as the system of reference.

Sodium dependent transporters

α-amino-monocarboxylic acids (bipolar amino acids) – system B

In the rabbit these amino acids are transported with high affinity by the sodium- and chloride-dependent β-alanine carrier (Munck 1985a, Andersen and Munck 1987, Munck and Munck 1994a). By lysine they are excluded from transport by the β-alanine carrier and by their sodium-dependent carrier (Munck 1985a, Munck and Munck 1994, 1994a). Therefore, the carrier of bipolar amino acids is characterized as being both sodium-dependent and chloride-independent. It is adequately described by its function at 140 mM NaCl under full inhibition by lysine.

Earlier comprehensive accounts of the specificity of this transport function (Schaeffer *et al.* 1973, Preston *et al.* 1974) were based on the assumption that one and only one carrier was responsible for all saturable transport with or without sodium present, neglecting evidence of a much more complicated situation (Munck and Schultz 1969a, 1969b). Nevertheless, the picture of its specificity and relative affinities which emerged from these studies has been confirmed (Sepulveda and Smith 1978, Paterson *et al.* 1979, 1980, 1981, Munck 1985a, Munck and Munck 1992b, 1994a). In the course of these studies it has also become clear (Munck and Munck 1994) that the carrier of bipolar amino acids is responsible for the lysine-resistant, sodium-dependent transport of phenylalanine and methionine. This transport had previously been ascribed to a separate carrier (Stevens *et al.* 1982, Del Castillo and Muniz 1991, Malo 1991). Thus with the exception of proline (Newey and Smyth 1964, Stevens and Wright 1985, Munck and Munck 1992c) this carrier transports exclusively bipolar amino acids with an affinity which increases for the aliphatic amino acids with increasing hydrophobicity of the side chain.

Comparably detailed descriptions do not exist for other species. It seems though, as reviewed before (Wilson 1962, Wiseman 1968, Munck 1981), that the patterns of specificity of a sodium-dependent carrier of neutral amino acids in rat (Munck and Rasmussen 1975, Munck 1983, Munck and Munck, unpublished), hamster (Wilson 1962), and guinea pig (Munck 1984b) are like those described for rabbit ileum and jejunum (Stevens *et al.* 1982, Munck 1985a, Munck *et al.* 1994, Munck and Munck 1994a). More recent studies describe similar patterns for mouse (Karasov *et al.* 1986), pig (Maenz and Patience 1992, Munck and Grøndahl 1994) and fetal human small intestine (Malo 1991).

Transport of glutamine and asparagine has not been extensively studied. Both sodium-dependent and sodium-independent glutamine transport into jejunal brush-border membrane vesicles of the rat, dog, and man is saturable (Bulus *et al.* 1989, Said *et al.* 1989, Van Voorhis *et al.* 1989). The sodium-dependent transport is electrogenic and is inhibited by serine and methionine (Bulus *et al.* 1989, Said *et al.* 1989). These

studies are not sufficient to associate glutamine and asparagine with any of the established transport systems. In a more elaborate inhibition study on the human colon cancer cell line Caco-2, sodium-dependent glutamine transport was inhibited by several neutral amino acids, but not by arginine and imino acids (Souba *et al.* 1992). For these cells alanine, cystine and serine were more efficient inhibitors than leucine suggesting transport by an ASC like system.

The β-alanine or $B^{0,+}$ carrier

The term "β-alanine carrier" was used to describe a sodium-dependent transporter of β-alanine in rabbit distal ileum, which has an even higher affinity for alanine, leucine and lysine (Munck 1985b, Andersen and Munck 1987). A transporter with similarly broad specificity was described in mouse blastocysts and termed $B^{0,+}$ (Van Winkle *et al.* 1985). Both transporters are now known to be chloride-dependent (Van Winkle *et al.* 1988b, Munck and Munck 1990, 1992a, 1994a). In addition, a sodium- and chloride-dependent carrier of taurine and β-alanine has been identified in several intestinal and renal epithelia (e.g. Hammerman and Sacktor 1978, Chesney *et al.* 1985, Turner 1986, Miyamoto *et al.* 1988, Wolff and Kinne 1988, Jessen *et al.* 1989, Benyajati and Johnson 1991, Munck 1994, Munck and Grøndahl 1994). It is therefore convenient to adopt the term $B^{0,+}$ to identify the "β-alanine carrier". In the rabbit the $B^{0,+}$ carrier is measurably present in the ileum only. It has not been demonstrable in rat, guinea pig (Munck and Munck 1994a, 1994b), man (Munck 1994) or hamster (Navab *et al.* 1984) small intestine. Its detection has not been the specific topic of studies in mouse intestine but the high degree of sodium-dependence of lysine transport (Karasov *et al.* 1986) suggests its presence in this species. Data obtained on the interaction between bipolar and cationic amino acids in confluent layers of Caco-2 cells (Hu and Borchardt 1992) and in *Xenopus laevis* oocytes injected with rat jejunal mRNA (McNamara *et al.* 1991) have been interpreted as representing the function of a $B^{0,+}$-like transporter. However, the description of specificity is insufficient for this conclusion, and information on chloride-dependence was not included. It is unclear how the $B^{0,+}$ carrier is related to the proton-coupled transport of alanine, MeAIB, and β-alanine demonstrated in human colonic cancer Caco-2 cells (Thwaites *et al.* 1993, 1994a, 1994b).

The imino acid carrier

A separate carrier of imino acids was first described in hamster small intestine (Hagihira *et al.* 1962) and later in the rat (Newey and Smyth 1964, Munck 1966b, Daniels *et al.* 1969a, 1969b), guinea pig (Munck 1983, 1984a), and rabbit (Stevens *et al.* 1982, Munck 1985b, Stevens and Wright 1985, 1987, Wright *et al.* 1985, Munck and Munck 1992c). In all species the imino acid carrier accepts both cyclic and aliphatic imino acids (Munck and Munck 1992c, Munck *et al.* 1994).

The rabbit small intestinal imino acid carrier has been most carefully examined by studies of alanine-resistant transport of proline (Stevens and Wright 1985, 1987) and by studies of MeAIB (2-methyl-amino-isobutyric acid), which seems to be transported exclusively by the imino acid carrier (Munck 1985b). The imino acid carrier can be completely inhibited by glycine, alanine, aminobutyric acid, and leucine with inhibitor constants between 300 mM and 23 mM, while β-alanine and lysine have no effect (Munck 1985b). It has not been possible to demonstrate a significant inhibitory effect of MeAIB on the transport of these neutral amino acids (Munck 1985b, Munck and Munck 1994a). This discrepancy is most likely caused by the relatively low affinities of these neutral amino acids for the imino acid carrier, which make its contribution to their total transport negligible. MeAIB is cotransported on the rabbit imino acid carrier with 2 sodium ions and 1 chloride ion (Munck and Munck 1990, Munck 1993).

The specificity and ion-dependency of the guinea pig imino acid carrier is the same as that of the rabbit (Munck 1984a, Munck and Munck 1994b). It is therefore evident that the mutual carrier of alanine and MeAIB described in isolated guinea pig enterocytes (Del Castillo and Muniz 1991) represents the function of the basolateral membrane of this epithelium.

The specificity of the rat imino acid carrier differs greatly from rabbit and guinea pig. Inhibition studies suggested that β- and γ-amino acids were transported by this carrier, while the inhibitory effects of neutral amino acids were uncertain (Daniels *et al.* 1969a, Munck 1977, 1981). It is now clear that the rat imino acid carrier besides transporting imino acids is (i) the only transporter of β-alanine and γ-amino-butyric acid; (ii) the principal carrier of taurine; (iii) that neutral amino acids with side chains in excess of one methyl group do not interact with the carrier, and (iv) that of these only glycine, D-alanine and AIB are transported (Munck *et al.* 1994). Therefore, β-alanine must be considered the best substrate for studies of the rat imino acid carrier. Very limited evidence suggests that the specificities of hamster (Hagihira *et al.* 1962) and mouse (Karasov *et al.* 1986) imino acid carriers are like those of the rabbit and guinea pig.

Anionic amino acids

Schultz *et al.* (1970) described sodium-dependent transport of anionic amino acids across the brush-border membrane of intact rabbit ileal epithelium. It appeared to have a 1 Na^+ : 1 amino acid transport stoichiometry, a transport capacity of 7 μmol

cm^{-2} h^{-1} and a $K_{1/2}$ of 8 mM. With brush-border membrane vesicles from rat (Corcelli *et al.* 1982, Corcelli and Storelli 1983), rabbit (Maenz *et al.* 1992), and man (Harig *et al.* 1987, Rajendran *et al.* 1987) a sodium-dependent, chloride-independent high affinity ($K_{1/2}$ < 0.1 mM), low capacity (3 pmol (mg protein)$^{-1}$ $(sec)^{-1}$) (Maenz *et al.* 1992), rheogenic transport (Kanai and Hediger 1992) has been described, which is stimulated by an outwardly directed potassium gradient (Corcelli and Storelli 1983, Kanai and Hediger 1992). With brush-border membrane vesicles from rabbit small intestine a second transport system was observed at pH 6.0 (Maenz *et al.* 1992). It could be distinguished from the former by being resistant to inhibition by D-aspartate, but fully inhibitable by phenylalanine, and by being absent at pH 8.0. It was further characterized by a low affinity ($K_{1/2}$ = 6 mM), a high capacity (J_{max} = 600 pmol (mg protein)$^{-1}$ $(sec)^{-1}$), and an inhibition specificity like the principal carrier of bipolar amino acids; that is a transporter very similar to the B carrier and to that described in intact rabbit ileal epithelium. It was assumed that at pH 6 the B carrier is made accessible to anionic amino acids by protonation. These results are best understood in the light of the inability to control pH of the unstirred layer outside the brush-border membrane of intact intestinal epithelia. They do, however, also indicate that the low affinity carrier must be dominant *in situ*. The fact that D-aspartate is transported exclusively by the carrier specific for anionic amino acids was used to demonstrate a cis-stimulation of this transporter by neutral amino acids (Maenz *et al.* 1993). A mechanistic model has not been proposed for cis-stimulation among amino acids. However, similar effects have been observed for leucine and methionine on influx of lysine and arginine across the brush border membrane of guinea pig small intestine (Robinson and Alvarado 1977, Munck 1984b) and of intracellular leucine on efflux of lysine across the basolateral membrane of rat and rabbit small intestine and the basolateral membrane of the hen colon (Munck 1989). Comparable studies on human jejunal brush-border membrane vesicles did not provide evidence of a second low affinity transporter (Harig *et al.* 1987, Rajendran *et al.* 1987), and studies do not exist for rat or guinea pig.

The β-amino acid carrier

A high affinity, low capacity, sodium- and chloride-dependent carrier specific for taurine and other β-amino acids has been demonstrated in all small intestinal (and renal) epithelia examined hitherto (Barnard *et al.* 1988, Moyer *et al.* 1988, Miyamoto *et al.* 1989, 1990b, Munck and Munck 1992b, 1994b, Munck and Grøndahl 1994), the adult cat being the only exception (Wolffram *et al.* 1991). Human Caco-2 cells also express this carrier (Tiruppathi *et al.* 1992). The initial inability to demonstrate this carrier in jejunal brush-border membrane vesicles (Stevens *et al.* 1982) and intact ileum (Munck 1985b), respectively, was overcome by using magnesium aggregation thereby avoiding calcium-induced inhibition (Miyamoto *et al.* 1990a) and by using a relevant concentration range (Munck and Munck 1992a, 1992b). The carrier is distinct from the β-alanine or $B^{0,+}$ carrier (Munck and Munck 1992a). The apparent affinity constants are between 20 and 40 μM and maximum transport rates are less than 1 % of the B carrier. The ion activation stoichiometry is ≥ 2 sodium : 1 chloride : 1 taurine (Miyamoto *et al.* 1989, 1990b, Munck and Munck 1994b). More elaborate studies on taurine and β-alanine transport in kidney proximal tubule brush-border membrane vesicles established an identical ion activation stoichiometry and provided substantial evidence for cotransport with chloride (Turner 1986, Wolff and Kinne 1988).

Taurine is transported both as a nutrient and as an osmolyte (Jones *et al.* 1993) and is transported by both the mucosal and the basolateral membrane (Benyajati and Bay 1994). In several cellular systems sodium- and chloride-dependent taurine transport takes part in volume regulation, and chloride channels activated in the process of regulatory volume decrease permit passage of organic solutes like inositol, betaine and taurine (Law 1991, Handler and Kwon 1993, Jackson and Strange 1993, Lambert and Hoffmann 1993). In isolated enterocytes from guinea pig small intestine activation of chloride pathways are necessary for volume regulation after amino acid and glucose uptake (MacLeod and Hamilton 1991). Whether passage through such pathways plays a role in transepithelial transport of nutrients is, however, presently not known.

Sodium-independent transport

It is the task of the small intestinal epithelium to extract nutrients from the gut lumen for delivery into the mucosal blood stream. This purpose is served by the preferential distribution of ion-coupled transporters to the brush-border membrane. Hence any ion-independent transport across this membrane will represent a leak, which will reduce the efficiency with which nutrients are cleared from the gut lumen. Nevertheless, uptake by and passage across intestinal epithelia in the nominal absence of sodium have always been seen as representing a sodium-independent carrier function, either by the carrier responsible for sodium-dependent transport, termed "slippage" (Stevens 1992b), or by separate carriers (Paterson *et al.* 1981, Stevens *et al.* 1982). The interpretation of data on sodium-independent transport is difficult. In the case of membrane vesicles because of possible contamination with basolateral membrane vesicles; and in the case of intact epithelia because of leakage of cytoplasmatic sodium to the unstirred layer outside the brush-border

membrane, but also because of the impossible task of distinguishing between very-low affinity transports and diffusion ahead of the space marker in the unstirred layer and through the paracellular route. With these caveats we will present our current understanding of sodium-independent transport in the rabbit ileum as a likely model.

Self-inhibition of proline influx was undetectable under sodium free conditions indicating that the imino acid carrier is strictly sodium-dependent and that diffusion alone accounts for proline transport under sodium-free conditions (Munck and Munck 1992c). Identical results have been obtained for the transport of β-alanine (Munck 1985b). Transport of glutamate under sodium free conditions at 0.02 and 1.0 mM (Munck and Munck, unpublished observations) correspond to permeabilities lower than those reported for proline and β-alanine rendering carrier mediated, sodium-independent transport of anionic amino acids very unlikely.

Sodium-independent transport of cationic and bipolar α-amino acids has consistently been reported (Munck and Schultz 1969a, Smith and Sepulveda 1979, Paterson *et al.* 1981, Stevens *et al.* 1982, Munck 1985a, Munck and Munck 1992b, 1994a). The nature of this transport has never been very clear. Thus, it was originally assumed to be by the same carrier as the sodium-dependent transport (Curran *et al.* 1967, Paterson *et al.* 1980). However, it is now clear that, except for the ileal β-alanine carrier, lysine inhibits alone a sodium-independent carrier of alanine (Paterson *et al.* 1981), alanine and leucine (Munck 1985a), glycine and phenylalanine (Munck and Munck 1994), that these bipolar amino acids have the same affinity for the lysine carrier in the presence as in the absence of sodium (Paterson *et al.* 1980, Munck 1985a), and that also the degree of inhibition is independent of the presence of sodium (Munck 1985a). It is therefore clear that the rabbit brush border membrane is without an equivalent of the y^+ system (Christensen 1990) and that the interaction between these two groups of amino acids corresponds to the description of system $b^{0,+}$ (Van Winkle *et al.* 1988a).

Mutual inhibition between lysine and leucine has been described in the rat (Munck 1984b). Transport of lysine is probably sodium-independent (Cassano *et al.* 1983) and the inhibitor constant for leucine against transport of lysine is sodium-independent (Munck and Munck, unpublished). This would be expected if the interaction was with a carrier with characteristics of the $b^{0,+}$ carrier. Interaction with the sodium-dependent carrier of bipolar amino acids can be excluded since methionine has a much higher affinity (K_i = 3 mM) for the latter than for the carrier shared with lysine (K_i = 11 mM) (Munck and Rasmussen 1975). Data from the mouse (Karasov *et al.* 1986) are consistent with those for rabbit and rat. It should, however, be noticed that the claim of a $b^{0,+}$ equivalent as responsible for these interactions can not yet be based on the application of the A-B-C test (Scriver and Wilson 1964). It should also be noticed that in the rat net transepithelial transport is seen only in the presence of sodium (Munck 1970), that sodium-dependent transport of lysine has been observed in brush-border membrane vesicles prepared from rats kept on a high-protein, low-carbohydrate diet (Wolffram and Scharrer 1984) and that injection of mRNA from rat small intestine into *Xenopus laevis* oocytes have led to the expression of sodium-dependent transport of lysine (Harvey *et al.* 1993).

Molecular biology of intestinal amino acid transport

The techniques of molecular biology have recently been used with magnificent results in the study of intestinal transport of hexoses (Wright *et al.* 1994). With these techniques it has also been possible to express intestinal amino acid transporters in *Xenopus laevis* oocytes. Oocytes injected with mRNA from rat small intestine expressed three types of lysine transport (Harvey *et al.* 1993): i) a sodium-dependent, leucine inhibitable transporter, probably responsible for lysine inhibitable transport of leucine; ii) a sodium-independent transport of lysine, sodium-independently inhibitable by leucine; iii) a sodium-independent transporter, sodium-dependently inhibitable by leucine. These transport functions correspond to the functions of systems $B^{0,+}$, $b^{0,+}$, and y^+. Of these $B^{0,+}$ seems to be present in rat intestine only under the conditions of a high-protein, low carbohydrate diet (Wolffram and Scharrer 1984), but y^+, as discussed above, is never present in the brush-border membrane.

When injected into oocytes mRNA from rabbit small intestine leads to expression of transport activities like those described for systems $b^{0,+}$ and y^+ (Magagnin *et al.* 1992). Of these $b^{0,+}$ is present in rabbit brush-border membrane, while y^+ is not. It seems likely that system y^+ is present in the basolateral membrane, since this membrane must serve nutritional needs of the enterocytes like those served by the whole membrane of non-polarized cells. Thus, not surprisingly intestinal mRNA turns the oocyte into an equivalent of an isolated enterocyte, which functionally is non-polarized (Del Castillo and Muniz 1991).

As a next step along the road indicated by the studies of hexose transporters the high affinity transporter of anionic amino acids has by expression cloning been characterized with respect to primary structure and function in oocytes (Kanai and Hediger 1992). Here its absolute requirement for sodium and its rheogenic function were confirmed (Kanai and Hediger 1992).

Table 1
Classification of amino acid carriers in the brush-border membrane of rabbit small intestine

Carriers	Substrates	Excluded substrates	Affinity ($K_{1/2}$)	Capacity (J_{max})	Na^+ dependence	Cl^- dependence	References
Neutral (B)	α-amino-monocarboxylic proline, phenylalanine, glutamate	MeAIB β-amino	0.2–20 mM	high	+	–	Hajjar and Curran 1970, Peterson *et al* 1970, Schultz *et al.* 1970, Preston *et al.* 1974, Paterson *et al.* 1981, Stevens *et al.* 1982, Munck 1985a, Maenz *et al.* 1992, Munck and Munck 1994, Nakanishi *et al.* 1994
IMINO	MeAIB proline	most other amino acids	0.3–3.6 mM	high	+	+	Stevens *et al.* 1982, Munck 1985b, 1993, Stevens and Wright 1985, Stevens and Wright 1987, Munck and Munck 1992c
β-amino	taurine β-alanine	non-β	14–46 μM	low	+	+	Miyamoto *et al.* 1989, 1990b, Munck and Munck 1992a, 1992b
β-alanine ($B^{0,+}$)	β-alanine lysine α-amino-monocarboxylic	taurine	0.1–2 mM	low	+	+	Munck 1985b, Andersen and Munck 1987, Munck and Munck 1992a, Munck 1994b
Cationic ($b^{0,+}$)	lysine α-amino monocarboxylic	β-amino imino	0.1–10 mM	low	–	–	Munck and Schultz 1969a, 1969b, Paterson *et al.* 1981, Stevens *et al.* 1982, Munck 1983, 1985a, Magagnin *et al.* 1992
Anionic (X_{AG})	glutamate aspartate	most other amino acids	12–60 μM	low	+	–	Berteloot 1984, Kanai and Hediger 1992, Maenz *et al.* 1992

Table 2
Classification of amino acid carriers in the brush-border membrane of guinea pig small intestine

Carriers	Substrates	Excluded substrates	Affinity ($K_{1/2}$)	Capacity (J_{max})	Na^+ dependence	Cl^- dependence	References
Neutral (B?)	α-amino-monocarboxylic proline	MeAIB β-amino cationic	2–7 mM	high	+	–	Munck 1983, 1984a, Del Castillo and Muniz 1991
IMINO	MeAIB proline	most other amino acids	0.7–1.2 mM	low	+	+	Hayashi *et al.* 1980, Munck 1984a, Del Castillo and Muniz 1991, Munck and Munck 1994b
β-amino	taurine β-alanine	non-β	37–155 μM	very low	+	+	Munck and Munck 1994b
Cationic ($b^{0,+}$?)	lysine α-amino monocarboxylic	β-amino imino	0.4 mM	low	–		Barry *et al.* 1961, Munck 1984b
Anionic (X_{AG})	glutamate aspartate	?	high	low	+	–	Munck and Munck, unpublished

Terminology

Instead of lengthy descriptive names for the multitude of cellular amino acid transporters a system of 1–3 letter symbols has been introduced (Christensen 1990). Of these systems A, L, y^+, ASC, β, and X_{AG} have been thought to be present in the brush border membrane of intestinal epithelia (Stevens 1992a, Kilberg *et al.* 1993). However, from the data reviewed here it is clear that the intestinal imino acid carrier is not equivalent to system A, that system L and y^+ are not present in the brush-border membrane, and that convincing evidence does not exist in support of a system ASC in any intestinal brush-border membrane. However, except for its chloride-dependence the intestinal taurine carrier is similar to the β-system, and X_{AG} seems an appropriate symbol for the high affinity carrier of anionic amino acids.

In an epithelium-like cellular system, the mouse blastocyst, transport systems have been described (Van Winkle *et al.* 1985, 1988a, 1988b), which are very similar to the sodium- and chloride-dependent β-alanine carrier in rabbit ileum and to the sodium-independent carrier of bipolar and cationic amino acids described in mouse rabbit and rat. For these two transporters it seems reasonable to adopt the terms $B^{0,+}$ and $b^{0,+}$, respectively. In analogy with these two terms the symbol "B" has been proposed for the sodium-dependent, lysine-resistant carrier of bipolar amino acids (Stevens 1992a). The present description of intestinal transport is summarized in Tables 1–3.

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Table 3
Classification of amino acid carriers in the brush-border membrane of rat small intestine

Carrier	Substrates	Excluded substrates	References
IMINO	small α-amino-monocarboxylic β, γ, D-amino	large α-amino-monocarboxylic cationic	Newey and Smyth 1964, Munck 1966b, 1983, Daniels *et al.* 1969a, Munck *et al.* 1994
Neutral (B)	α-amino-monocarboxylic proline methionine tryptophan	β-amino cationic	Newey and Smyth 1964, Munck 1966a, Daniels *et al.* 1969a, Baker and George 1971, Munck and Rasmussen 1975, Antonioli *et al.* 1978, Sepulveda and Robinson 1978, Wolffram and Scharrer 1984, Van Voorhis *et al.* 1990
β-amino	taurine	β-alanine non-β	Barnard *et al.* 1988, Moyer *et al.* 1988
Cationic ($b^{0,+}$)	cationic α-amino-monocarboxylic	all others	Robinson 1968, Munck and Rasmussen 1979, Cassano *et al.* 1983, Harvey *et al.* 1993, Wolfram *et al.* 1984
Anionic (X_{AG})	glutamate aspartate	most other amino acids	Corcelli *et al.* 1982

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