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Review

Deciphering the mechanisms of intestinal imino (and amino) acid transport: The redemption of SLC36A1

David T. Thwaites *, Catriona M.H. Anderson

Epithelial Research Group, Institute for Cell and Molecular Biosciences, Faculty of Medical Sciences, Framlington Place, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4HH, UK

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Abstract

The absorption of zwitterionic imino and amino acids, and related drugs, is an essential function of the small intestinal epithelium. This review focuses on the physiological roles of transporters recently identified at the molecular level, in particular SLC36A1, by identifying how they relate to the classical epithelial imino and amino acid transporters characterised in mammalian small intestine in the 1960s–1990s. SLC36A1 transports a number of D- and L-imino and amino acids, β- and γ-amino acids and orally-active neuromodulatory and antibacterial agents. SLC36A1 (or PAT1) functions as a proton-coupled imino and amino acid symporter in cooperation with the Na^+/H^+ exchanger NHE3 (SLC9A3) to produce the *imino acid carrier* identified in rat small intestine in the 1960s but subsequently ignored because of confusion with the *IMINO* transporter. However, it is the sodium/imino and amino acid cotransporter SLC6A20 which corresponds to the *betaine carrier* (identified in hamster, 1960s) and *IMINO* transporter (identified in rabbit and guinea pig, 1980s). This review summarises evidence for expression of SLC36A1 and SLC6A20 in human small intestine, highlights the differences in functional characteristics of the *imino acid carrier* and *IMINO* transporter, and explains the confusion surrounding these two distinct transport systems.

Keywords: Amino acid transport; Imino acid transport; Epithelia; Intestinal absorption; Ion/solute cotransport; Na^+/H^+ exchange

Contents

Abbreviations: ABA, aminobutyric acid; AIB, aminoisobutyric acid; ACPC, 1-aminocyclopropanecarboxylic acid; AHA, 4-amino-5-hexynoic acid or γ-acetylenic GABA; APSA, 3-amino-1-propanesulfonic acid or homotaurine; BCECF, 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein; BBMV, brush-border membrane vesicles; CHLP, cis-4-hydroxy-L-proline; HRPE, human retinal pigment epithelial cells; HUGO, Human Genome Organisation; MeAIB, α(methyl) aminoisobutyric acid; OMIM, Online Mendelian Inheritance in Man; I_{sc}, short-circuit current; TACA, trans-4-aminocrotonic acid

* Corresponding author. Tel.: +44 191 222 8559; fax: +44 191 222 7424.
E-mail address: d.t.thwaites@ncl.ac.uk (D.T. Thwaites).

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1. A short history of intestinal zwitterionic imino and amino acid absorption through the 20th century

The study of intestinal amino acid absorption has been constrained by dogma, of one sort or another, for much of the last 100 years. The first half of the 20th Century was dominated by the view that amino acid absorption from the gut was a purely passive or diffusive process (for review see Wiseman [1]). However, as long ago as 1937, Höber and Höber demonstrated that the percentage absorption of amino acids such as glycine and alanine from the rat small intestine was greatest at low concentrations suggesting a saturable process [2]. They called this saturable process *an accelerating factor* [2]. [Please note that throughout this review the names given to the various transport systems, as characterised functionally in epithelial tissues, are highlighted in italics]. In the early 1950s, Wiseman [3] demonstrated that the absorption of neutral or zwitterionic (dipolar) amino acids by the rat small intestine in vitro was active in the sense that, when a racemic mixture of amino acids was placed in equal concentrations on either side of the intestine, the L-enantiomer was transported in the absorptive direction against a concentration gradient. Agar et al. [4] demonstrated that the absorptive movement of L-histidine across rat jejunum in vitro was active as it was inhibited by the metabolic inhibitors cyanide and dinitrophenol. Thus in 1955, Wiseman [5] concluded that a single *common special mechanism* was responsible for the absorption of L-forms of neutral amino acids and that this mechanism excluded D-forms. When the literature on intestinal amino acid absorption was reviewed in 1968 (including studies published up to 1965) [1] the prevailing view that L-, but not D-, amino acids were transported by active or uphill transport had changed little except that four distinct pathways for neutral amino acids had been classified thus superseding the proposal that absorption was via a single common mechanism.

The earliest evidence for a role of extracellular Na^+ in intestinal transport of neutral amino acids demonstrated that transport of monoiodotyrosine [6], tyrosine and phenylalanine [7] were reduced when extracellular Na^+ was replaced either by K^+ or Li^+. Christensen and colleagues [8] had earlier suggested that glycine uptake in Ehrlich mouse ascites carcinoma cells could occur in the form of a complex between the carrier, glycine and the sodium ion and that uptake "could be wholly driven by the energy inherent in the asymmetry of cellular alkali metal distribution". Thus the Na^+ gradient hypothesis, which was originally proposed to account for the uphill accumulation of sugars, was extended so that Na^+ movement down its concentration gradient was considered the driving force for the active uphill accumulation of solutes such as amino acids [9–11]. In 1967, Curran, Schultz and colleagues [12,13] measured unidirectional Na^+ and alanine influx across the mucosal surface of rabbit ileum and provided the first clear demonstration of coupled intestinal uptake of amino acid and Na^+. In 1975, following the advent of brush-border membrane vesicle (BBMV) technology, the luminal localisation of a Na^+/amino acid cotransporter was demonstrated unequivocally by measurement of a Na^+-dependent L-alanine overshoot in BBMV prepared from rat small intestine [14].

Thus, by the mid 1960s, the principle that neutral amino acid transport across the luminal membrane of the small intestinal epithelium was limited to uptake of L-amino acids via Na^+-coupled cotransporters was firmly established. Unfortunately, this canon has remained embodied in most reviews of amino acid transport and in almost all current textbooks of physiology. We say, unfortunately, because although the Na^+ gradient hypothesis is as applicable today as it was in the 1960s to describe movement through many transporters of neutral amino acids there has, for many years, been a large body of evidence to suggest that other amino acids (e.g. D-amino acids) are absorbed by concentrative or uphill transport mechanisms and that these mechanisms may not be linked directly to Na^+ cotransport.

Despite H^+-coupled amino acid transport being widespread in plants, fungi and bacteria [15–18] there has been relatively little evidence for such a coupling in mammalian tissues. A role for the H^+-electrochemical gradient as a driving force in dipolar amino acid transport across the mammalian small intestinal wall

has not been ignored completely [19]. However, support for this mode of transport has generally been in the minority despite compelling evidence for the existence of the so called "acid microclimate" (an area of low pH approximately pH 6.1–6.8) at the luminal surface of the mammalian small intestine [20,21].

The purpose of this review is to encapsulate the evidence from the 1950s to the present date for the existence of a transport system which fits neither the substrate selectivity nor ion-coupling of the dogma describing neutral amino acid transport across the luminal surface of the mammalian small intestine: in other words we will summarise here the evidence for a H^+-coupled transporter of L- and D-zwitterionic amino and imino acids. This transport system is known currently as PAT1 (or proton-coupled amino acid transporter 1) or SLC36A1 (which is the first member of solute carrier family 36) [22–26]. In addition we will explain the reasons for the lack of clarity in the literature relating to the intestinal absorption of neutral amino and imino acids which should help to eliminate confusion in this research field in the future.

2. The 1950s–1960s: transport of D-amino acids

The first dogma to be addressed here is the belief, which originated in the 1950s, that only L-enantiomers of amino acids are absorbed from the small intestine. Contrary to this view, the majority of pre-1950 studies suggested that both L- and D-forms of amino acids were absorbed at similar rates (for review see Wiseman [1]). However, when the literature was reviewed in the late 1960s those earlier studies (pre-1950) were dismissed as being unreliable due to the methodologies used [1]. This may or may not have been a reasonable assumption to make. Here we will focus on the evidence produced in the post-1950 literature. The initial evidence for "absorption" came from measurements of the rate of disappearance of a range of amino acids from the intestinal lumen of the rat ileum in vivo [27]. Amino acids were presented as racemic mixtures and in each case the disappearance of the L-form was more rapid than the D-form. No measurements were made of the transported material (either that taken up into the mucosa or that passing into the serosal compartment). Unfortunately, the L- and D-forms of any particular amino acid were never examined separately so it was not possible to discount competition for a single transport site with greater affinity for L-amino acids. Similar observations were made in human ileum (in vivo) [28] where disappearance of the L-amino acids from a racemic mixture was more rapid than the D-amino acids. Wiseman [3] also compared L- and D-amino acid movement across rat small intestine in vitro where an increase in the appearance of L-amino acids in the serosal solutions matched the decrease observed in the luminal solutions. No such change was observed with the D-amino acids although only racemic mixtures were used (see above). A consistent observation can be made from these three studies [3,27,28], that is, the ability of the apparent transport site to distinguish between stereoisomers varies depending upon the amino acid under investigation suggesting that not all D-amino acids are excluded to the same degree. From studies where L- and D-amino acids were investigated independently, and the nature of the material crossing the intestinal wall determined, the difference between "absorption" of L- and D-amino acids becomes less marked. Matthews and Smyth [29] found that when a racemic mixture of alanine was added to the small intestine of the cat more L-alanine than D-alanine appeared in the portal blood. However, D-alanine was absorbed as indicated by its appearance in the blood (although very high luminal concentrations were used). In the same study [29], D-alanine transfer across the mucosal wall was detected in vitro and the transfer at 37 °C was 4-fold greater than at 18 °C suggesting non-passive movement. Smyth and Taylor [30] used an in vitro preparation of rat small intestine and compared material in mucosal and serosal solutions after a 1 h incubation. L-Alanine was accumulated in the serosal solution 4-fold above luminal levels whereas the concentration of D-alanine was similar in both luminal and serosal solutions suggesting that D-alanine was absorbed but at a lower rate than L-alanine. Importantly the rate of transepithelial transfer of D-alanine was greater than a number of solutes (urea, ascorbic acid and fructose) now recognised to undergo carrier-mediated transport across the small intestinal wall. Jervis and Smyth [31] measured disappearance of amino acids from rat small intestine and found that when introduced separately L-histidine was absorbed 9.9-fold greater than D-histidine. In contrast, although L-methionine was absorbed as rapidly as L-histidine it was absorbed only 1.5-fold greater than D-methionine suggesting that some D-amino acids were absorbed more rapidly than others. Furthermore, cross-competition between the D- and L-amino acids led to the conclusion that the movement of D-amino acids must involve processes other than passive diffusion [31]. The same authors [32] later demonstrated that D-methionine was transported across the rat intestine in vitro against a concentration gradient and this transfer was inhibited by metabolic inhibitors and L-methionine. These observations [32] forced a reinterpretation of Wiseman's studies [1,3,5,27] and suggested that D-methionine transport had not been observed earlier because of the presence of L-methionine in the racemic mixture. Jervis and Smyth [32] concluded, therefore, that Wiseman's *common* mechanism was not absolutely stereospecific although active transfer of D-methionine would not necessarily mean that all D-amino acids were transported actively [32]. Similarly Finch and Hird [33] found that D-alanine uptake (over 4 min) into rat small intestinal segments was via a rapid and saturable process (following Michaelis–Menten kinetics) when uptake was compared at 1 and 10 mM. In addition, D-Serine was concentrated in the serosal solution 2-fold above luminal levels when measurements were made using everted sacs of rat small intestine [34,35].

Thus, the view that only L-amino acids were absorbed across the intestinal wall was only really valid for the period between 1951 and 1959. It is probably correct to conclude that most L-amino acids are absorbed more rapidly than most D-amino acids but, in the absence of a detailed study where all amino acids were investigated individually and evidence for cross-competition determined, the theory that all D-amino acids are excluded from any transport site should not have been made quite so forcefully. In addition, a greater rate of absorption of any

particular L-amino acid over its D-form should not necessarily have led to the conclusion that D-amino acids were not absorbed at all. It is clear that as long ago as 1959 there were serious doubts about this concept and by 1964 there was enough evidence to suggest that D-serine, D-alanine and D-methionine were absorbed. It should be noted, as will be discussed below, that both D-serine and D-alanine are substrates for PAT1 (SLC36A1) [22,23,26].

3. The 1960s–1970s: evidence for multiple imino acid transporters in the small intestine and kidney

3.1. The imino acid carrier in the rat small intestine

While the studies in the 1950s were focused on identifying which neutral amino acids were substrates for carrier-mediated absorption, studies in the 1960s provided evidence for multiple carriers [36,37]. The first clear evidence for the existence of more than one neutral amino acid transporter in rat small intestine came from a study by Newey and Smyth [37]. Transepithelial transfer (and tissue accumulation) of amino acids across rat small intestine were determined in vitro and observations confirmed in vivo [37]. Evidence for two neutral amino acid carriers was produced. The first had a high affinity for methionine and a much lower affinity for glycine and proline. The second carrier transported glycine and proline but had no affinity for methionine or leucine. They named the second transporter the *glycine–proline carrier* [37] but subsequently changed the name to *sarcosine system* [38,39] or *sarcosine carrier* [40,41] (Table 1). Two other groups also characterised this transporter using everted sacs of rat small intestine in vitro and they used the terms *imino acid carrier* [42] and *methionine-insensitive sarcosine–glycine–proline system* [43] (Table 1). This transport system corresponds to *system 4* in Wiseman's five-system classification for intestinal amino acid transporters [1]. We shall use the term *imino acid carrier* [42] to describe the characteristics of this transport system in

Table 1
Chronological summary of the names used to describe the imino acid carrier (SLC36A1) and the IMINO transporter (SLC6A20)

	SLC36A1		SLC6A20	
1960s	glycine–proline carrier	Newey and Smyth [37]	betaine carrier	Lin et al. [36] Hagihara et al. [49]
	imino acid carrier	Munck [42]	transport system for N-substituted amino acids	Hagihara et al. [49]
	"imino acid carrier" of the small intestine of the rat	Munck [42]	"imino acid carrier" of the small intestine of the hamster	Munck, [42]
	system 4	Wiseman [1]	system 3	Wiseman, [1]
	sarcosine system	De la Noüe et al. [38]		
	sarcosine carrier	Daniels et al. [40]		
1970s	methionine-insensitive 'sarcosine–glycine–proline' system	Thompson et al. [43]		
	β	Miller et al. [58]		
	imino acid transport system	Lerner and Karcher [59]		
1980s	N-methylamino acid or sarcosine carrier	Munck [62]	PRO/MeAIB pathway	Stevens et al. [64]
	H^+-L-proline cotransport	Røigaard-Petersen et al. [95]	**IMINO**	Stevens et al. [65]
	H^+ glycine co-transport system	Rajendran et al. [94]	IMINO transporter	Stevens and Wright [66]
			proline/sodium (IMINO) cotransporter	Stevens and Wright [177]
			Imino carrier	Stevens and Wright [67]
			system 5	Munck [69]
			Imino, imino system, imino acid transport pathway (IMINO system)	Satoh et al. [77]
1990s	*proline–glycine–betaine pathway*	Wunz and Wright [103]	*imino pathway*	Wunz and Wright [103]
	system 5	Wunz and Wright [103] (using Mircheff et al. nomenclature [105])	*system 6*	Wunz and Wright [103] (using Mircheff et al. nomenclature [105])
	proton/amino acid transport, proton/amino acid symport, system PAT	Thwaites et al. [46,109]	rabbit jejunal 'imino carrier', rabbit ileal 'imino acid carrier'	Munck and Munck [72]
	rat imino acid carrier, rat IMINO	Munck and Munck [80]	rabbit imino acid carrier, guinea pig imino acid carrier, rabbit IMINO, guinea pig IMINO	Munck and Munck [80]
2000s	LYAAT-1	Sagné et al. [140]	SIT1	Takanaga et al. [152]
	PAT1	Boll et al. [22] Chen et al. [23]	XT3s1	Kowalczuk et al. [153]
	Tramdorin 3	Bermingham et al. [178]		

Transporter names and references given in italics in this table correspond to observations made using renal tissues.

rat small intestine (Table 1). Overall, this series of studies identified that rat small intestine possessed a transport system for glycine, L- and D-alanine, β-alanine, L- and D-proline, L- and D-hydroxyproline, sarcosine, betaine, β-aminobutyric acid (β-ABA), γ-aminobutyric acid (GABA), L- and D-azetidine-2-carboxylate, L- and D-pipecolic acid, with weaker evidence for D-serine and α-aminoisobutyric acid (AIB) [37–44]. It should be noted that these are all substrates for PAT1 (SLC36A1) [22,23,26,45–47] (see Table 2).

Table 2
Tissue distribution and functional characteristics of the imino acid carrier (SLC36A1)

Tissue distribution	
mRNA	Mammalian: small intestine (duodenum, jejunum, ileum), oesophagus, stomach, caecum, colon, rectum, kidney, placenta, liver, pancreas, lung, heart, brain (neurones but not glial cells), skeletal muscle, testes, spleen [22,23,25,26,57,140,141,146]
Protein	Brush-border membrane of the small intestine (human, rat, Caco-2 cells); in neurones primarily lysosomal but also plasma-membrane and other sub-cellular organelles (rat) [23,26,140,146,179]
Function	Brush-border membrane of human Caco-2 cell monolayers, human duodenal biopsies, rat small intestinal mucosal sheets, rat small intestinal BBMV, chicken small intestine, eel intestinal enterocytes, brush-border of lizard small intestine, rabbit renal (pars convoluta) BBMV, plasma-membrane of dissociated rat hippocampal neurones [26,46,57–59,93–103,107–116,128–130,141,169]
Functional characteristics	
Ion coupling	H^+-coupled (stoichiometry 1:1 H^+:amino acid) [22,109] In intact intestinal epithelia is partially Na^+-dependent due to functional coupling with the Na^+/H^+ exchanger NHE3 [26,46,56,109–111,114]
Electrogenicity	Electrogenic transport of amino and imino acids [22,26,45,46,107–111,114,116,143] Electroneutral transport of short-chain fatty acids [143]
General substrate characteristics	Small unbranched, apolar, zwitterionic, α-, β-, γ-amino and imino acids; D- and L-amino and imino acids; heterocyclic amino and imino acids containing 4- to 6-membered rings; N-methylated amino and imino acids
Substrates	glycine, D- and L-alanine, D- and L-proline, D-serine, D-cysteine, D- and L-cycloserine, trans-4-hydroxy-L-proline, β-alanine, taurine, MeAIB, AIB, N-methyl-alanine, sarcosine, dimethylglycine, betaine, GABA, β-ABA, isonipecotic acid, nipecotic acid, D- and L-pipecolic acid, D- and L-azetidine-2-carboxylic acid, APSA, ACPC, AHA, TACA, vigabatrin, guvacine, CHLP, cis-4-hydroxy-D-proline (CHDP), 3,4-dehydroproline, thiaproline, acetate, propionate, butyrate, pentanoate, hexanoate [23,24,26,37–43,46,47,61,62,81,107–116,143]
Non-transported inhibitors	serotonin, L-tryptophan, 5-hydroxy-L-tryptophan, tryptamine [144]

Thompson et al. [43] found that decreasing bulk luminal pH from pH 7.3 to pH 6.3 increased uptake of glycine, L-proline, sarcosine and β-alanine in both phosphate and bicarbonate buffered salines (whereas L-methionine uptake was unaffected) which suggested that a pH-dependent transporter was present at the mucosal surface of the rat small intestine. The idea that a physiological pH gradient exists across the luminal surface of the mammalian small intestine is supported by experiments using ion-selective microelectrodes to demonstrate the "acid microclimate" (an area of low pH adjacent to the mucosal surface). In rat jejunum in vitro this microclimate has been measured between approximately pH 6.1–6.8 [20,21]. Clearly any manoeuvre which increases the H^+ concentration at the mucosal surface will likely stimulate transport via a pH-dependent mechanism. Inclusion of glucose in the incubation buffers bathing the mucosal surface of rat small intestine in vitro causes a decrease in the surface pH [48] although the mechanism responsible for this acidification is unclear. It is interesting to note that in the studies of the *imino acid carrier* discussed here, when glucose was included in the buffers bathing the mucosal surface of the everted sacs of rat small intestine in vitro there was an increase in uptake of glycine, L-proline, β-alanine, sarcosine, D- and L-alanine (all PAT1 substrates) whereas L-methionine (which is excluded from PAT1) uptake was unaffected [38,41,44]. Thus, by 1970, it was apparent that there existed a transporter of both L- and D-amino and imino acids that was driven by decreasing extracellular pH. None of these studies investigated the Na^+-dependence of amino/imino acid uptake. It should be noted that PAT1 (SLC36A1) is a pH-dependent transporter of both L- and D-amino and imino acids [22,23,26].

3.2. The betaine carrier in the hamster small intestine

At the same time that the *imino acid carrier* was being characterised in rat small intestine (see Section 3.1 above) similar studies were identifying the characteristics of amino and imino acid transport across the small intestine of the hamster [36,49,50]. As observed using rat small intestine, neutral amino and imino acid uptake across the hamster small intestine was via more than one transporter. A Na^+-dependent transporter was identified and named as the *betaine carrier* [36,49] or *transport system for N-substituted amino acids* [49] (see Table 1). This transporter has some similarities in substrate specificity to the rat *imino acid carrier* as it accepts betaine, dimethylglycine, sarcosine, proline, hydroxyproline and pipecolic acid but, crucially, it excludes the *imino acid carrier* substrate glycine [36,49,50]. The possibility that these differences in substrate specificity of the rat *imino acid carrier* [37,42] and the hamster *betaine carrier* [36,49] could be due to species differences was raised by both Newey and Smyth [37] and Munck [42] in the mid-1960s. Overall it is apparent that no evidence was produced from these studies in hamster small intestine to support the role of a transport system with identity to the rat *imino acid carrier*. Indeed Wiseman [1] identified the two transporters as being distinct with the *betaine carrier* corresponding to *system 3* (as stated above the *imino acid carrier* corresponds to *system 4*) in

his five-system classification for intestinal amino acid transporters [1] (see Table 1). Unfortunately this distinction, and the possibility that there may be species-specific variation in the complement of amino acid transport systems expressed at the intestinal brush-border membrane, was seemingly forgotten for much of the following 30–40 years.

3.3. The imino acid carrier in the kidney

Munck [42] observed the similarities between the rat intestinal *imino acid carrier* and a transporter of proline, hydroxyproline and glycine identified in the renal tubular epithelium during investigation of hyperaminoacidurias [51,52]. In some patients with familial hyperprolinemia (characterised by renal loss of glycine, proline and hydroxyproline), prolinuria was observed only when the plasma proline concentration was greater than 0.8 mM (normal levels are less than 0.3 mM) [52]. However, when plasma proline was greater than 0.8 mM there was also an increase in excretion of hydroxyproline and glycine (despite plasma levels of both remaining normal) suggesting the existence of a low-affinity transport system shared by proline, glycine and hydroxyproline [52] (indicative of transport via an *imino acid carrier*). At lower plasma concentrations, the imino acids and glycine were reabsorbed by separate high-affinity transport systems [52], one of which could be the *betaine carrier*. A genetic defect in a shared low-affinity transport system seems likely to be responsible for the autosomal recessive disorder iminoglycinuria (Online Mendelian Inheritance in Man (OMIM) database, OMIM 242600, at www.ncbi.nlm.nih.gov) [51,53,54]. Mohyuddin and Scriver [55] characterised imino acid and glycine transport in rat kidney and identified a series of transport systems with characteristics that could account for the reabsorptive processes identified in man [52,53]. These included separate high affinity [Michaelis constant (K_m), 0.1 mM] transport systems for glycine and proline, and a low affinity transport system (glycine K_m, 2.7 mM; proline K_m, 5 mM) which was shared by the *imino acid carrier* (and PAT1) substrates proline, hydroxyproline, glycine, alanine and AIB [55]. Interestingly, unlike many neutral amino acid transporters, the low-affinity (*imino acid carrier*-like) transport system did not show absolute dependence on extracellular Na^+ as proline accumulation in rat kidney cortex slices was reduced by only 31% (at a proline concentration of 3 mM) following extracellular Na^+ removal [55]. The characteristic of partial Na^+-dependence in intact epithelial preparations [26,56,57] is consistent with PAT1 (SLC36A1) function at the brush-border of the proximal tubule.

3.4. Conclusions from studies during the 1960s–1970s

By the early 1970s, evidence from studies in human and rat intestinal and renal tissues suggested the existence of a low affinity transport system for imino and amino acids (the *imino acid carrier*) that: (i) showed weak stereospecificity; (ii) was pH-dependent, being stimulated by low pH; and (iii) was only partially Na^+-dependent. These are all characteristics that are now associated with the function of PAT1 (SLC36A1) in intact epithelial tissues [26,56,57]. Evidence from studies using hamster small intestine failed to provide any evidence for an *imino acid carrier*-like transport system but instead demonstrated the presence of a transporter (the *betaine carrier*) with a distinct substrate selectivity.

4. The 1970s–1990s: species differences in intestinal imino (amino) acid transport

4.1. The imino acid carrier in rat and chicken small intestine

Multiple amino and imino acid transporters have been identified in chicken small intestine [58]. Although there are some inconsistencies in the studies, one of these transporters, named as *β* or as an *imino acid transport system* [58,59], (Table 1) can be identified as a low affinity (K_i 3–8 mM for proline, β-alanine and GABA) carrier of proline, β-alanine, GABA, D- and L-imino acids, taurine, sarcosine, AIB, D- and L-alanine, and L-azetidine-2-carboxylic acid [58–60]. Over the substrate range investigated this selectivity is identical to observations of mucosal influx via the *imino acid carrier* in rat small intestine [61,62]. In addition to those substrates identified in the chicken, in the rat small intestine the heterocyclic piperidine carboxylic acids pipecolic, nipecotic and isonipecotic acid were identified as substrates for this carrier along with D-serine and D-alanine [61,62]. When reviewing the literature in 1981, Munck [62] concluded that amino acid uptake across the intestinal brush-border membrane was via four carriers, one of which transported imino acids and amino acids containing the amino group in the β- or γ-positions. He described this carrier as the *imino acid carrier* or *N-methylamino acid or sarcosine carrier* (Table 1) although suggested that alternative names might be more appropriate once the range of transported substrates had been identified. We could append here that the transporter name might need revisiting once the mechanism of ion-coupling is identified. The observation of D-alanine transport via the *imino acid carrier* is interesting as even in the original demonstration of Na^+/L-alanine cotransport using rat intestinal BBMV it was also observed that D-alanine uptake, although not being Na^+-dependent (as demonstrated by the absence of a Na^+-dependent overshoot), was carrier-mediated and reduced in the presence of glycine [14], characteristics consistent with PAT1 (SLC36A1) function even in the absence of a pH gradient.

4.2. The Na^+-dependent IMINO transporter in rabbit and guinea pig small intestine

From the late 1960s onwards, the rabbit became a popular choice for investigations of intestinal amino acid uptake either via influx into intact mucosal sheets or BBMV [12,13,63–73]. Few early studies investigated uptake of imino acids although a small component (5%) of mucosal uptake of glycine was inhibited by proline suggesting low level expression of a shared transporter in rabbit ileum [63]. In the 1980s, imino acid uptake was investigated intensively using rabbit jejunal BBMV in a series of studies by Stevens, Wright and colleagues [64–67].

They identified three separate brush-border Na^+-dependent transporters for neutral amino acids and named one of these transporters sequentially the *Pro/MeAIB Pathway* [64], *IMINO transporter* [65,66] or *Imino carrier* [67] (Table 1). This transporter has been generally called the *IMINO* transporter over recent years and we will use that name in this review. Although the *IMINO* transporter has some similarities with the *imino acid carrier* described earlier in rat small intestine there are clear differences in both substrate selectivity and ion dependency (see below and Section 4.3). The rabbit *IMINO* transporter has a higher affinity for proline than the rat *imino acid carrier* with a K_m of approximately 200–300 μM [66,67]. Like the rat *imino acid carrier*, the rabbit *IMINO* transporter accepts heterocyclic compounds (e.g. proline and pipecolic acid) and N-substituted amino acids [e.g. sarcosine, betaine, α(methyl)aminoisobutyric acid (MeAIB)] but is stereoselective (with a preference for L- over D-enantiomers), and excludes a number of substrates for the rat *imino acid carrier* including β-alanine, GABA, glycine, L-alanine, AIB and taurine [66,69,73]. The jejunal BBMV used in those studies [64–67] were prepared using a Ca^{2+}-precipitation technique which produced a BBMV population devoid of any β-alanine transport [74]. However, the method of preparation does not appear to effect the functioning of the *IMINO* transporter (nor is it the reason for the lack of effect of β-alanine on the *IMINO* transporter) in those studies as a similar substrate specificity was identified when uptake was measured by influx across intact mucosal sheets of rabbit ileum [69]. Influx via the *IMINO* transporter is equivalent to the 5th system of the five transporters identified at the rabbit ileal brush-border membrane by Munck [69].

Similar observations to those described in rabbit small intestine were made in guinea pig small intestine using either BBMV or intact mucosal sheets [75–77]. The *IMINO* transporter in the guinea pig was initially incorrectly attributed to a *system A*-like transporter (due mainly to the incorrect, but very common, assumption that MeAIB is a specific *system A* substrate) [75]. It should be noted that *system A* is found exclusively at the basolateral membrane of intestinal enterocytes [64,65,78]. The substrate selectivity of this guinea pig *IMINO* transporter is identical to the rabbit *IMINO* transporter and the hamster *betaine carrier* (see section above) suggesting that the *IMINO* transporter and the earlier-identified *betaine carrier* represent functional activity of the same transport system (Table 1). The *IMINO* carrier is defined as the Na^+-dependent component of proline uptake that is insensitive to L-alanine [66,67].

4.3. Comparative studies of the imino (amino) acid transporters in rat, rabbit and guinea pig small intestine

The summary of the experimental evidence given above suggests that the transporters of imino and amino acids at the brush-border membranes of different species are distinct with an *imino acid carrier*-like transporter being expressed predominantly in rat and chicken and an *IMINO*-like transporter being primarily responsible for uptake in rabbit, guinea pig and hamster small intestine [36–43,49,50,58–62,64–69,75–77]. These differences in both substrate selectivity and ion dependency were the focus of a number of studies by Munck and colleagues in the early 1990s [71–73,79–81]. A key difference in substrate specificity between species is that in the rat (*imino acid carrier*) there is either no discrimination between L- and D-forms of amino acids (e.g. proline, hydroxyproline) or a preference for the D-form as is the case with serine and alanine [38–41,61,62,68,81]. In contrast the rabbit and guinea pig *IMINO* transporter prefers proline and hydroxyproline in the L-form [66,68,69,76]. Two groups of substrates in particular display the key differences in the selectivity of the *imino acid carrier* and *IMINO* transporters (Fig. 1). The rat *imino acid carrier* has a clear preference for aminobutyric acid when the amino group is in the β-(β-ABA) or γ-(GABA) position and has little affinity when the amino group is in the α-(α-ABA) position (Fig. 1) [39,61,62,68,80,81]. In the rabbit and guinea pig this preference is reversed [66,68,69,76]. These differences are emphasised by the fact that the β amino acids β-alanine and taurine are substrates for the rat *imino acid carrier* but are excluded by the rabbit and guinea pig *IMINO* transporters [38,39,41,61,62,66,68,73,76,80,81]. The second group of compounds that distinguish the transport sites of these carriers are the heterocyclic piperidine carboxylates where there is a relatively poor discrimination by the rat *imino acid carrier* but a slight preference when the amino group is in the γ-(isonipecotic acid) or β-(nipecotic acid) positions compared to the α-(pipecolic acid) position (Fig. 1) [61,62,68]. In contrast, the rabbit and guinea pig *IMINO* transporter has a clear preference for pipecolic over nipecotic over isonipecotic acid (Fig. 1) [66,68,69,76].

The ion dependency of the rat *imino acid carrier* and rabbit and guinea pig *IMINO* transporter are also distinct. Studies using BBMV demonstrate unequivocally that the *IMINO* transporter is Na^+-dependent in both rabbit jejunum [64,67] and guinea pig ileum [75,77]. In contrast, it is surprising that there are no published studies of Na^+-dependent *imino acid carrier*- (or even *IMINO*-) like transport of, for example, proline or MeAIB in studies using rat intestinal BBMV. When the Na^+-dependency of these transporters was investigated by rapid influx using mucosal sheets it was clear that MeAIB uptake was completely Na^+-dependent in rabbit ileum and guinea pig jejunum but was only partially Na^+-dependent (approximately 40% of uptake was detected even in the absence of Na^+) in rat jejunum [71,79], as observed previously in rat renal cortex [55]. In addition, *IMINO* transport activity in the rabbit ileum and guinea pig jejunum was Cl^--dependent whereas *imino acid carrier* function in the rat jejunum was Cl^--independent [71,79,82]. The rat *imino acid carrier* is pH-dependent [43]. We can find no information about the pH dependence of the *IMINO* transporter from studies using BBMV or intact tissues.

4.4. Conclusions from intestinal studies during the 1970s–1990s

With the benefit of hindsight it seems clear that there are at least two major intestinal transporters of imino (and amino) acids namely the *imino acid carrier* [37,42,61,62] and the

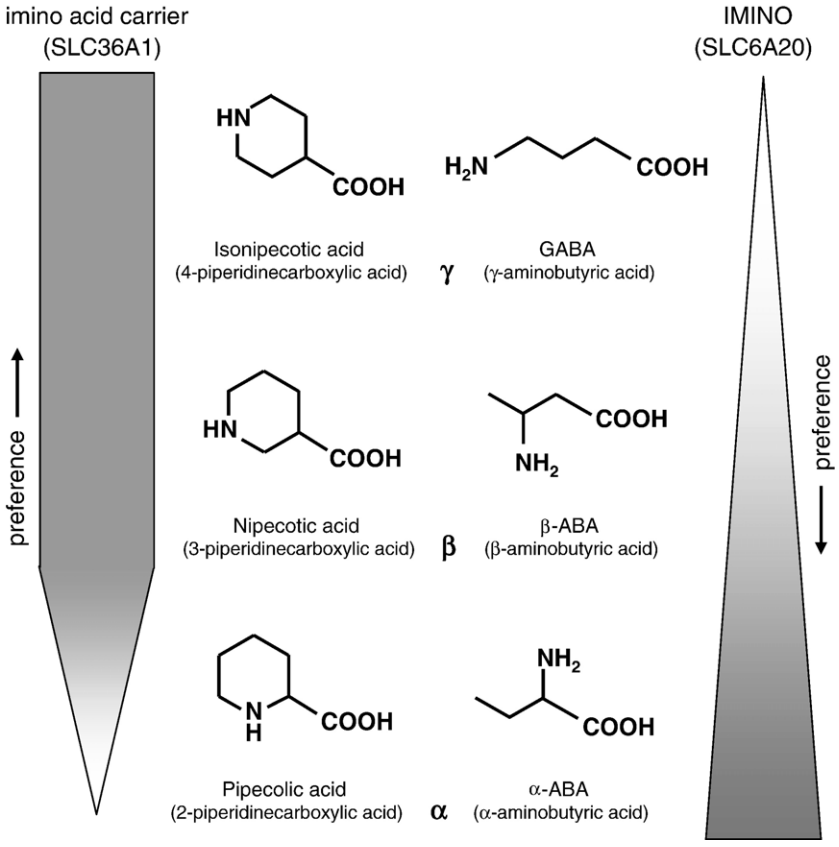

Fig. 1. Schematic representation of the order of preference of the *imino acid carrier* (SLC36A1) and the *IMINO* transporter (SLC6A20) for two groups of compounds where the amino (or imino) group is in the α-, β- or γ-position.

IMINO transporter (*betaine carrier*) [36,65,66,69] (Table 1). There are some similarities between these two carriers notably in substrate selectivity but also clear differences both in substrate selectivity and ion coupling [61,62,65,66,68,69,79–81]. The relative levels of expression of these two carriers at the intestinal luminal surface appear to vary between species and may vary along the length of the small intestine. However, from the 1980s onwards most reviews and papers of amino and imino acid transport (with the notable exception of those by Munck and Munck) include evidence for, or discussion of, the *IMINO* transporter but not the *imino acid carrier*. There is probably no single reason for this but a few possibilities are suggested here.

Firstly, as described above and raised by some investigators of intestinal amino acid transport, species differences in terms of amino acid transport do occur but are often ignored. This omission has been exacerbated by the increased use of the rabbit as the main model of investigation from the 1960s onwards. Obviously any transport system not expressed, to any great degree, in the rabbit small intestine will cease to be the focus of much research activity or the centre of attention when discussed in review (no matter how well characterised that transporter may have been, and important it may have been considered, in the 1960s and 1970s) [1,37–43,61]. This is emphasised as the classification system used to name and identify intestinal amino acid transporters from the 1980s to the present day is primarily influenced by studies using rabbit small intestine [65,70,80,84–87]. Apart from Munck and colleagues [68,80] few reviews describe the results of studies using rat tissue (and the *imino acid carrier*) or those aimed at identifying species differences.

Secondly, and this point is closely related to the first, it is probably true that most people (again with some exceptions [79–81,83]) who were aware of the existence of studies of both the *imino acid carrier* and *IMINO* transporter may have considered that these two transport systems simply represented species-specific congeners of the same carrier, even though the two transport systems were recognised as long ago as the 1960s as being two separate entities [1].

Thirdly, much of the work on intestinal amino and imino acid transport from the 1980s onwards has used BBMV (prepared predominantly from rabbit small intestine) as the main experimental tool. The introduction of rapid measurements of amino acid influx across the mucosal surface had earlier allowed identification of the substrate specificity and ion-coupling of the amino acid transporters at the brush-border membrane of the rabbit small intestine. These observations were confirmed and expanded greatly by the use of rabbit intestinal BBMV in the 1980s [64–67]. In the rat small intestine, evidence for the *imino acid carrier* provided from studies in the 1960s, using, for example, everted sacs, was supported by the rapid mucosal influx measurements made during the 1970s–1990s [61,62,68,81]. However, these observations may have been ignored subsequently because they could not be confirmed using rat intestinal BBMV. The reason for this apparent lack of consistency could be that investigators were attempting to

identify a Na^+-dependent transporter either because of the Na^+-coupled dogma or perhaps an assumption that the rat *imino acid carrier* should have a similar ionic-coupling to the rabbit *IMINO* transporter (see Section 4.3). In hindsight there is no real evidence to suggest that the *imino acid carrier* is truly Na^+-coupled.

Fourthly, the confusion regarding the names given to these two transport systems is enough to bewilder even the most discerning reader (Table 1). Even Munck and Munck, who were the most persistent investigators involved in characterising these different transporters and highlighting differences in function, used both '*imino carrier*' and '*imino acid carrier*' terms to describe the rabbit *IMINO* transporter when comparing amino acid uptake in rabbit jejunum and ileum [72]. Similarly both *IMINO* transporter and *imino acid carrier* terms were used to describe both transporters in their review in 1994 [80]. However, the chaotic nomenclature is demonstrated most "clearly" by the fact that, within the same UK Physiology Department over a period of 3 years, three separate research groups published articles on the same rat *imino acid carrier* but gave it a multitude of different names (Table 1) [1,38–40,43]. If departmental colleagues could not agree on a single name it is not surprising that the rest of the scientific community became confused!

Thus, the result of these studies in the 1970s–1990s is that, despite extensive evidence for two separate amino and imino acid transporters, only the *IMINO* transporter name has remained in the literature and generally the studies cited are those that describe observations made using rabbit small intestine. This focus is emphasised if we consider published reviews over this time-frame. Since 1984 there have been a number of excellent reviews on amino and imino acid transport some of which have been focused on intestinal mechanisms while others have included sections on, or at least discussion of, the transporters expressed in the small intestine [65,84–90]. This list is by no means exhaustive but does point to the reason why the *imino acid carrier* name and data have disappeared from the consciousness of the current research community. A Web of Knowledge search [91] as of June 2006, demonstrates that these reviews [65,84–88,90] are influential, widely-read and quoted, attracting 1339 citations between them. Notably these reviews all identify the *IMINO* transporter and associated studies but none include any reference to the *imino acid carrier* and the studies in rat small intestine. In contrast, the most recent reviews (1994–1995) [80,92] to describe the *imino acid carrier* and underline the species differences between rabbit, guinea pig and rat have attracted only 53 citations. Earlier reviews (1968–1983) [1,61,62,68] that included details of the *imino acid carrier* have been cited 267 times in total but very infrequently over the last 20 years. Similarly, if we look at the original data for the two transporters, although the original studies of the *imino acid carrier* by Newey and Smyth [37], and Munck [42] attracted 158 citations, later studies [39–41,43,79,81] average only 21 citations per paper. In contrast, the original *IMINO* (and *betaine carrier*) papers by Lin et al. [36] and Stevens et al. [64] have attracted 444 citations whereas the later studies [66,67,69] average 60 citations per paper. Thus, it is clear, that we, the scientific community have concluded erroneously that the *IMINO* transporter and *imino acid carrier* are one and the same and subsequently ignored most comparative studies. Thus, most people in the field are aware of the *IMINO* transporter but few are aware of the functional characteristics (substrate specificity and ion coupling) of the *imino acid carrier*. The question is then: is there any recent evidence in the literature for an *imino acid carrier*-like transporter and what role might it play?

5. The 1980s–1990s: the pH-dependent, Na^+-independent, amino and imino acid transporter in rabbit renal brush-border membrane vesicles

Studies using BBMV prepared from the rabbit renal pars convoluta identified a low affinity transport system which had several characteristics (substrate affinity, substrate specificity and pH dependence) similar to those described for the *imino acid carrier* in rat small intestine [93–103]. However, the link between the *imino acid carrier* and the pH-dependent, Na^+-independent, renal transporter was not made and although many of these intestinal and renal studies were published over a similar time-frame it is important to appreciate that there was no cross-referencing between these studies of intestinal and renal imino and amino acid transport [37–43,61,62,68,69,76,79–81,93–103]. In the rabbit renal BBMV, transport of D- and L-imino and amino acids was rheogenic (even in the absence of Na^+), was increased by lowering extravesicular pH and was pH gradient-dependent (reduced in the presence of the H^+ ionophore FCCP). These data suggested that transport occurred via a H^+/amino acid symport mechanism [93–103]. A Na^+-independent, pH-dependent, rheogenic uptake of proline, hydroxyproline, glycine, β-alanine, L-alanine, taurine, betaine and AIB (all *imino acid carrier* and PAT1 substrates) was detected [93–103] and the relative low affinity (K_m, 2.8–9.7 mM) was similar to that determined either using rat kidney slices in vitro or in vivo in humans [52,55]. Cross-competition experiments demonstrated that this low affinity transport system accepts sarcosine, and L- and D-forms of alanine, proline and hydroxyproline [94,96,98,99].

In addition to the pH-dependent, Na^+-independent, *imino acid carrier*-like transporter described above a Na^+-dependent, high-affinity (K_m 160–260 μM for proline and hydroxyproline), *IMINO*-like transporter was identified in BBMV prepared from rabbit renal pars recta or total cortex which also transported D-imino acids and betaine [93,96,103,104]. This *IMINO* function probably corresponds to *system 6*, of the six Na^+-dependent amino acid transport systems identified earlier by Wright and colleagues, suggested as being defective in prolinuria [103,105]. Recently, Miyauchi et al. [57] demonstrated functions consistent with expression of both *imino acid carrier* and *IMINO*-like transporters in BBMV prepared from rabbit kidney.

6. The 1990s–2000s: system PAT in the human intestinal epithelial cell line Caco-2

6.1. H^+-coupled, pH-dependent, Na^+-independent amino and imino acid transport

In 1994, Munck and colleagues [81] considered the species differences described above and posed the following question: "… it becomes a question of more general and clinical interest of which species, guinea pig, rabbit, or rat, is the best model for the function of the human small intestine". Clearly the best model of the human small intestine is the human small intestine. However, mechanistic investigation of membrane transport across the human small intestine is difficult due to the lack of available viable human tissue samples. The Caco-2 cell line has been used extensively over the last 15 years as a model system for transport studies across the human small intestinal epithelium. This model system has a number of key advantages as the cells are of human origin, allow easy access to both the apical and basolateral surfaces of the enterocyte, express a small intestinal phenotype and consequently provide a useful model for transcellular measurements [106]. These advantages must be considered alongside the fact that the cells originate from a colonic cancer and this colonic origin influences the high resistance nature of the epithelium and paracellular pathway [106]. Despite these potential limitations, Caco-2 cell monolayers grown on permeable filters probably represent the best available model for studies of transcellular movement across the human small intestinal epithelium.

Thwaites and colleagues used confluent monolayers of the human intestinal epithelial cell line Caco-2, grown on permeable filters, to demonstrate that the brush-border membrane of intestinal cells possessed a H^+-coupled, pH-dependent, rheogenic imino and amino acid transport system which functioned even in the absence of extracellular Na^+ [46,107–114]. This transport system was named *system PAT* [46,109] (Table 1). Direct evidence for H^+/amino and imino acid symport was produced using Caco-2 cell monolayers loaded with the pH-sensitive dye 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF) [107,108]. Transport of these zwitterionic substrates was shown to be rheogenic, even in the absence of extracellular Na^+, by inward short-circuit current (I_{sc}) measurements in Caco-2 cell monolayers under voltage-clamped conditions [107,108]. This series of studies demonstrated pH-dependent uptake of the *imino acid carrier* substrates β-alanine, L-proline, L-alanine, MeAIB, glycine, AIB, taurine, GABA and betaine [45,46,107–112,114]. In contrast, uptake of amino acids excluded from the *imino acid carrier* such as leucine [111], phenylalanine [46], glutamic acid [112] and lysine [112] was not stimulated following a decrease in apical pH. A combination of cross-competition experiments, measurements of amino acid-stimulated decrease in intracellular pH, and measurements of amino acid-induced inward I_{sc} in Na^+-free conditions demonstrated that this human *system PAT*, like the rat *imino acid carrier*, mediated transport of D- and L-proline, D- and L-alanine, D-serine, D-cycloserine, trans-4-hydroxy-L-proline, β-alanine, taurine, glycine, MeAIB, AIB, sarcosine, dimethylglycine, betaine, GABA, β-ABA, isonipecotic acid, nipecotic acid, D- and L-pipecolic acid, and L-azetidine-2-carboxylic acid (Table 2) [23,24,26,27,46,107–115]. In addition, *system PAT* also transported D-cysteine [23], L-cycloserine [26], and a number of amino and imino acid analogues including 3-amino-1-propanesulfonic acid (APSA or homotaurine) [114], 1-aminocyclopropanecarboxylic acid (ACPC) [114], 4-amino-5-hexynoic acid (AHA or γ-acetylenic GABA) [116], trans-4-aminocrotonic acid (TACA) [116], vigabatrin (or 4-amino-5-hexanoic acid or γ-vinyl GABA) [116], guvacine [116], cis-4-hydroxy-L-proline (CHLP) and 3,4-dehydroproline [47] (Table 2). Despite the extensive evidence for the role of this transport system in intestinal absorption of amino and imino acids, orally-delivered amino acid analogues used clinically to treat various neurological disorders, antibiotics, and a variety of GABA (receptor blockers and reuptake inhibitors) and proline analogues (including those which reduce collagen deposition in fibrotic diseases and inhibit cancer growth in vitro) [47,115–125], evidence for *system PAT* is not included in most reviews of amino acid transport. The lack of impact that these studies made on the scientific literature is probably due to the fact that the transport activity was considered by some to be an artefact of the Caco-2 cell line. This view was understandable because, firstly, there was apparently no evidence for a H^+-coupled amino or imino acid transporter in real intestinal tissues (this conclusion was incorrect, see Sections 6.2 and 7.1). Secondly, and unfortunately, the publication of the *system PAT* Caco-2 studies was coincident with the disappearance of discussion of the *imino acid carrier* from the literature so that there was apparently no evidence for a transporter with similar substrate selectivity to *system PAT*. The existence of this H^+-coupled amino and *imino acid carrier* was acknowledged in some reviews in the 1990s [92,126,127] and similarities with other transport systems discussed [92,127]. However, the apparent differences in ion-dependency between *system PAT* and 'classical' Na^+-dependent transport systems prevented confirmation of any functional equivalence [46,92,111,114,127].

6.2. The partial Na^+-dependence of system PAT in intact epithelia and the role played by the Na^+/H^+ exchanger NHE3

Overall, it is clear, that the similarity in substrate specificity between the human *system PAT* and rat *imino acid carrier* demonstrates that they represent functions of identical transport systems at the brush-border of human and rat small intestine. However, the major problem in attempting to unite the evidence for the *imino acid carrier* and *system PAT* has been the apparently incontrovertible difference in ion coupling with the *imino acid carrier* being considered, like most 'classical' amino acid transporters, to be Na^+-coupled whereas *system PAT*, in Caco-2 cells, is clearly H^+-coupled [26,46,56,107–114,116]. There is considerable evidence for a pH-dependent imino and amino acid transporter in the kidney (as described in Section 5) [93–103] and small intestine (see below) which appears identical in substrate selectivity to both *system PAT* and the *imino acid carrier*. Thompson et al. [43] demonstrated that the rat *imino acid carrier* was pH-dependent (although

experiments were performed only in the presence of extracellular Na^+). In 2004, Anderson et al. [26] demonstrated that, in Na^+-free conditions, MeAIB uptake across the mucosal surface of rat small intestine was pH-dependent and was inhibited by β-alanine, observations consistent with expression of a Na^+-independent, pH-dependent, H^+-coupled *system PAT*/*imino acid carrier*-like transporter. In contrast, no Na^+-independent pH-dependent MeAIB uptake was measured in rabbit or guinea pig small intestine [26]. Recently pH-dependent (Na^+-independent) uptake of L-proline and β-alanine was demonstrated in rat intestinal BBMV [128]. In addition, H^+-coupled L-proline transport has been demonstrated in eel (*Anguilla anguilla*) intestinal enterocytes (localised solely to the apical membrane) [129] and Na^+-independent, pH-dependent, transmural L-alanine transport (mucosal-to-serosal) has been demonstrated across lizard (*Gallotia galloti*) duodenal enterocytes [130].

There is in fact no evidence to suggest that the *imino acid carrier* is truly Na^+-coupled, only the Na^+-coupled dogma and the incorrect assumption that the Na^+-coupled *IMINO* transporter represents the same carrier. Munck and Munck [79] demonstrated that the *imino acid carrier* in rat jejunum was only partially Na^+-dependent and could function even in the absence of Na^+. A series of key experiments have led us to a model (Fig. 2) that identifies why the *imino acid carrier* is partially Na^+-dependent in intact epithelia whilst functioning as a H^+-coupled transport system. For optimal activity of the *system PAT*/*imino acid carrier* to occur it is essential that the transapical membrane pH gradient is maintained. Amino acid-induced intracellular acidification in Caco-2 cell monolayers (due to *system PAT*-mediated H^+/amino acid influx) leads to a selective activation of an apical Na^+-dependent H^+-efflux mechanism (Na^+/H^+ exchanger) without any activation of the housekeeping basolateral Na^+/H^+ exchanger NHE1 [26,46,56,109–111,113,114]. This apically-localised Na^+/H^+ exchanger was identified as NHE3 by use of selective Na^+/H^+ exchange inhibitors [26,56,113,131–133]. Pharmacological inhibition (e.g. by the NHE3-selective inhibitor S1611) or physiological inhibition (e.g. by vasoactive intestinal peptide) of NHE3 reduces the absorptive capacity for *system PAT*-mediated amino acid uptake across the apical membrane of Caco-2 cell monolayers [26,56]. Similarly, *system PAT* function in Caco-2 cells shows partial Na^+-dependence, following inactivation of NHE3 (due to the absence of Na^+) [26,56], as observed previously for the *imino acid carrier* [79]. This functional cooperativity has also been demonstrated for NHE3 and another H^+-coupled solute transporter, the intestinal di/tripeptide transporter hPepT1 [134–139]. Thus, during H^+-coupled solute absorption the driving force (the H^+ electrochemical gradient) is maintained by activity of the Na^+/H^+ exchanger NHE3 so that the overall effect is net transport of solute and Na^+ with H^+ recycling (Fig. 2). Final confirmation that the sum of *system PAT* and NHE3 functions account for that attributed previously to the *imino acid carrier* (as described in rat small intestine) required that the *system PAT*-related cDNA clone was isolated to allow definitive ion-coupling and substrate specificity to be elucidated.

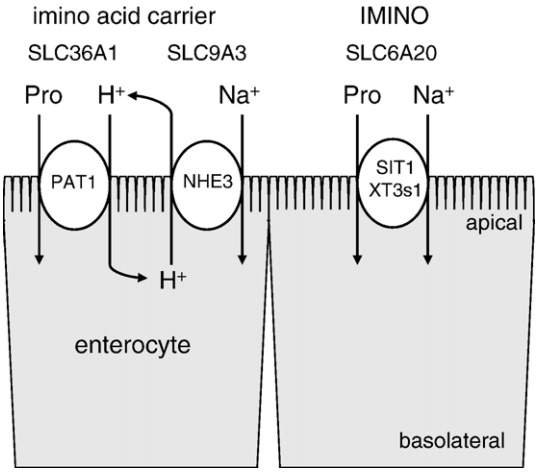

Fig. 2. Schematic representation of the ion-coupling of the *imino acid carrier* (SLC36A1 and SLC9A3) and the *IMINO* transporter (SLC6A20) at the brush-border membrane of the small intestinal epithelium.

7. The 2000s: molecular identification

7.1. The imino acid carrier or system PAT

The significant breakthrough in molecular identification of the *imino acid carrier*/*system PAT* came with the isolation of a cDNA from rat brain which was named LYAAT-1 (Lysosomal Amino Acid Transporter 1) because of its colocalisation with the lysosomal marker cathepsin D in the neurones investigated [140]. LYAAT-1 functioned in a similar manner to *system PAT* as a Na^+-independent pH-dependent transporter of GABA, L-proline and L-alanine which was inhibited by D-proline. Therefore, the primary physiological function of LYAAT-1 in neurones may be to export neutral amino acids from lysosomes into the cytosol using the outward H^+-electrochemical gradient generated by H^+-ATPase activity [140]. No reference to the extensive *imino acid carrier* or *system PAT* literature was included and the conclusion made was that LYAAT-1 was a lysosomal specific transporter [140]. LYAAT-1 has since been shown to function at the plasma-membrane of hippocampal neurones in culture [141]. Related cDNAs have been isolated from mouse [22], human [23] and rabbit [57] and named PAT1 (Table 1) due to the similarity in function between the isolated transporter when expressed in mammalian cell lines or *Xenopus laevis* oocytes and the *system PAT* transporter in Caco-2 cells. PAT1 functions as a rheogenic H^+-coupled, pH-dependent, Na^+-independent transporter of D- and L-amino and imino acids [22,23,26,45]. In PAT1-expressing oocytes, transport is dependent upon membrane potential with hyperpolarisation increasing both substrate affinity and maximal capacity [142]. It is predicted that proton binding precedes amino acid substrate binding but that translocation is simultaneous [142]. Decreasing extracellular pH decreases the K_m for substrate binding [142]. In oocytes, PAT1 can function bidirectionally with the direction of transport being governed by the relative electrical and chemical gradients for protons and amino acid

substrates [142]. Whether PAT1 can function in efflux mode under physiological conditions (e.g. in the enterocyte) remains unknown although the lower substrate affinity on the cytosolic face of the transporter favours influx [142]. The substrate selectivity of PAT1 is identical to *system PAT* at the apical membrane of Caco-2 cell monolayers (Table 2) [22,23,26,45]. In addition, PAT1 transports short-chain fatty acids (e.g. acetate and butyrate) in an electroneutral manner [143] and serotonin and L-tryptophan appear to be natural inhibitors of the carrier as they inhibit PAT1 function but are not transported [144]. As observed with the *imino acid carrier* in rat small intestine [39,61,62], and *system PAT* in human Caco-2 cell monolayers [26], PAT1 has a clear preference for aminobutyric acid when the amino group is in the β-(β-ABA) or γ-(GABA) position and has little affinity when the amino group is in the α-(α-ABA) position (Fig. 1). Similarly, PAT1 (like *system PAT* and the *imino acid carrier*) only poorly discriminates between the heterocyclic piperidine carboxylates when the amino group is in the γ-(isonipecotic acid) or β-(nipecotic acid) positions compared to the α-(pipecolic acid) position (Fig. 1) [26].

At the mRNA level, PAT1 has a ubiquitous tissue distribution (Table 2) [22,23,26]. At the protein level, immunocytochemistry using a PAT1-specific antibody localised PAT1 exclusively to the brush-border membrane of Caco-2 cell monolayers and both human and rat small intestine (Table 2) [23,26]. The identical expression pattern in Caco-2 cell monolayers and real human small intestine confirm that the Caco-2 cell line is an appropriate model for investigation of imino and amino acid absorption [26]. The brush-border localisation of PAT1-immunoreactivity in rat small intestine [26] support the role for PAT1 in pH-dependent, Na^+-independent amino and imino acid uptake in rat small intestine [26,128] and as a molecular candidate for the *imino acid carrier*.

The NHE3 selective inhibitor S1611 inhibits the Na^+-dependent component of *system PAT* function in Caco-2 cells but has no effect on amino acid uptake under conditions when NHE3 is inactive (e.g. in the absence of extracellular Na^+, at apical pH 5.5, or under conditions in which NHE3 is inactivated following an increase in $[cAMP]_i$). This demonstrates that the effect of S1611 on *system PAT* function is indirect following inhibition of maintenance of the driving force (the H^+-electrochemical gradient) for further H^+-coupled transport [26,56]. S1611 has no effect on amino acid uptake into PAT1-expressing oocytes demonstrating that selective NHE3 inhibitors have no direct effect on PAT1 function [26]. These observations confirm that in intact intestinal epithelial tissues the transport system identified as the *imino acid carrier* is the result of functional cooperativity [26] between the H^+/amino and imino acid symporter PAT1 and the Na^+/H^+ exchanger NHE3 (Fig. 2). Apart from the studies in Caco-2 cells, the only other series of studies to describe a H^+-coupled *system PAT*/*imino acid carrier*-like transporter were those that reported a pH-dependent imino and amino acid transporter in rabbit renal BBMV [93–103]. Rabbit PAT1, when expressed heterologously in human retinal pigment epithelial (HRPE) cells functions in an identical mode and with identical substrate specificity to the endogenous transporter characterised in renal BBMV [57].

Interestingly, PAT1 is the first of 4 related sequences to be identified and PAT1, therefore, is also known as SLC36A1, the first member of solute carrier family 36 [24–26,145,146]. The human PAT1 gene *SLC36A1* is localised to chromosome 5q33.1 and is clustered with *SLC36A2* and *SLC36A3* whereas *SLC36A4* is found on 11q14.3; similar clusters are observed in all mammalian genomes identified so far [23–25,145,146]. PAT2 (SLC36A2) is also a H^+-coupled, pH-dependent, Na^+-independent amino and imino acid transporter but has a more restrictive substrate specificity than PAT1 and has a higher affinity for its substrates [22,145,147,148]. PAT2 has a more restricted tissue distribution than PAT1 and is not expressed in the small intestine [22,25,145]. The physiological role of PAT2 remains unknown. However, in neurones, PAT2 is expressed in the endoplasmic reticulum, recycling endosomes and the plasma-membrane [149] and PAT2 has been suggested as a candidate [149] for the low affinity Na^+-independent glycine transporter identified functionally in rat cerebral cortex slices and homogenates [150]. An interesting and additional physiological role for PAT2 has recently been suggested by Bröer [151] which reflects the ability of PAT2 (like PAT1) to transport the three amino/imino acids (glycine, proline, hydroxyproline) lost in urine in iminoglycinuria [151]. PAT2 mRNA has been detected in kidney [22,25, 145,146]. Although there does not appear to be any functional evidence for PAT2 in renal amino acid transport, PAT2 function may have been overlooked in BBMV studies (Section 5 above) since transport is Na^+-independent and PAT2 has a pH-dependency (half-maximal transport activity at e.g. pH 8.3 [149]) that might preclude detection of function over the range of extravesicular pH values used in the studies. Bröer suggests, therefore, that iminoglycinuria is likely to be a multigene disorder which involves several transporters including PAT1, PAT2 and the *IMINO* transporter [151]. No function has yet been identified for PAT3 (SLC36A3) and PAT4 (SLC36A4).

7.2. The IMINO transporter

The *IMINO* transporter or *betaine carrier* has been identified at the molecular level and corresponds to the 20th member of solute carrier family 6 or SLC6A20 [152,153]. The transporter has been isolated from rat, human and mouse and named either SIT1 (for Sodium/Imino-acid Transporter 1) or XT3s1 [152,153]. The human *SLC6A20* gene has a distinct chromosomal localisation to the *SLC36A1* gene being found at 3p21. SIT1/XT3s1 functions as a Na^+-dependent and Cl^--dependent amino and imino acid transporter with substrate specificity and affinity identical to the earlier observations of the *IMINO* transporter and *betaine carrier* using rabbit, guinea pig and hamster small intestine [36,49,64–69,76]. The ion-coupling and substrate selectivity of SIT1/XT3s1 are distinct to those observed with PAT1 (Fig. 2).

7.3. A third transporter: system B^0

A third transporter may also play a role in amino and imino acid absorption in the small intestine and kidney. When Newey and Smyth [37] first described the *imino acid carrier* in rat small intestine they also detected a separate transporter that had a high affinity for methionine and a much lower affinity for glycine and proline. This *methionine carrier* most probably corresponds to the Na^+/L-alanine symporter characterised in the 1960s–1980s in rabbit and rat small intestine [12–14,63–65]. This transporter has been named variously as the *neutral amino acid transport system* [36,49], *neutral amino acid carrier* [49], *methionine system* [38], *methionine carrier* [40], *system 1* (using Wiseman's five-system classification for intestinal amino acid transporters) [1], *Common Neutral Amino Acid Pathway*, [64], *system NBB* (for Neutral Brush Border) [65], *system B* [90], and is now known as *system* B^0 [154]. *System* B^0 has been identified at the molecular level and corresponds to the 19th member of solute carrier family 6 or SLC6A19 [155]. The transporter has been isolated from mouse and named B^0AT1 [155]. The *system* B^0 transporter B^0AT1 (SLC6A19), like the *IMINO* transporter SIT1/XT3s1 (SLC6A20), has been immunolocalised to the brush-border membrane of the mouse small intestinal epithelium [156]. B^0AT1 prefers large aliphatic amino acids such as leucine and methionine with methionine inducing the largest inward current in B^0AT1-expressing oocytes [155,157]. Glycine, L-alanine and proline are accepted but with much lower affinity [155,157] suggesting that the *system* B^0 contribution to intestinal absorption of these substrates will be relatively small. *System* B^0 (SLC6A19) activity is reduced as extracellular pH is acidified as observed in rabbit ileum and B^0AT1-expressing oocytes [155,157,158].

7.4. A potential fourth transporter of imino acids: TauT

A fourth potential transporter of imino acids in the small intestine is the taurine transporter *TauT*. A high affinity transporter for taurine was identified in the small intestine by measurements of uptake into jejunal BBMV prepared from both rat and rabbit [159,160]. This high affinity, low capacity, Na^+ and Cl^--dependent transporter has a preference for taurine and β-alanine with a K_m for taurine of 5–40 μM. Related cDNAs have been isolated from MDCK cells [161], rat brain [162], mouse brain [163], and human thyroid and placenta [164,165] and a full-length transcript has been detected in canine ileum [161]. *TauT* corresponds to the 6th member of solute carrier family 6 or SLC6A6. Functional *TauT* activity has been demonstrated in human intestinal Caco-2 cell monolayers [166,167]. The physiological role of *TauT* is probably to mediate high affinity uptake of taurine during the fasting state (such as that released from sloughed villus cells) whereas the high capacity absorption of taurine from diet will be mediated by the *imino acid carrier* PAT1. We are unaware of any demonstration of imino acid (e.g. proline) transport via a *TauT* clone although proline can inhibit *TauT*-mediated taurine uptake but with relative (to taurine) low affinity [165]. A combination of the low capacity of the *TauT* transporter and the relative low affinity for proline mean that *TauT* is unlikely to play a significant role in imino acid absorption across the small intestinal epithelium.

8. Deciphering the mechanisms of imino (and amino) acid transport in the human small intestine

In this review we have assessed evidence primarily for two distinct imino and amino acid transporters: the H^+-coupled, Cl^--independent, *imino acid carrier* (PAT1/SLC36A1, which in intact epithelia functions in tandem with NHE3/SLC9A3) and the Na^+ and Cl^--dependent *IMINO* transporter (SIT1/XT3s1/SLC6A20) [22,23,26,140,152,153]. Evidence for the function of the *imino acid carrier* in the rat small intestine and the *IMINO* transporter in the hamster, rabbit and guinea pig small intestine has been available from the early 1960s onwards [36,37,42,49,64,69,75,76]. Surprisingly we are no nearer to understanding which of these transport systems, either or both, functions in the human small intestine. A body of evidence produced by a number of groups has identified functional expression of the *imino acid carrier* PAT1 (SLC36A1) at the brush-border of the human intestinal cell line Caco-2 [23,26,45,47,56,107–114,115,116,168]. The original demonstration of pH-dependent, Na^+-independent, proline uptake across the apical membrane of Caco-2 cells was made by Nicklin et al. [168] but unfortunately the significance of the observation was overlooked, probably due to the dominant Na^+-coupled dogma in the literature, and, therefore, the study focused on the sub-maximal uptake at pH 7.4 which was incorrectly attributed to *system A* (which is localised solely to the basolateral membrane) [64,65,78]. H^+-coupled, pH-dependent, amino and imino acid uptake across the apical membrane of Caco-2 cell monolayers has been demonstrated for a number of *imino acid carrier* substrates [23,26,45,47,56,107–116]. Similar functional characteristics (ion coupling and substrate specificity) have been demonstrated at the mucosal surface of the rat small intestine [26,37–44,61,62,79,81,128]. Immunolocalisation of PAT1 (SLC36A1) to the brush-border membrane of human Caco-2 cell monolayers, and human and rat small intestine support a role for the *imino acid carrier* in the human small intestine [26].

There are no studies reporting an *imino acid carrier*-like transport system using human intestinal BBMV but there are relatively few reports of studies using human intestinal BBMV in total and, as described above, it is highly unlikely that anyone will have performed experiments aimed at identifying a pH-dependent, Na^+-independent, transport system since such a transport system is hardly mentioned in the literature over recent years (Section 4.4) [65,84–90]. In a single study, Munck [169] measured amino and imino acid uptake into human duodenal mucosal biopsies. Proline uptake was only partially Na^+-dependent and was Cl^--independent, characteristics consistent with *imino acid carrier*-like function in the rat small intestine [79,81]. In addition uptake of the other *imino acid carrier* substrates MeAIB, β-alanine, taurine and glycine was much less sensitive to removal of extracellular Cl^- than Na^+ [169]. Munck made several conclusions: that the apparent lack of

sensitivity to extracellular Cl^- was due to residual Cl^- trapped within the tissues; that the observations provided evidence for a number of Cl^--dependent transporters in human small intestine; that the similarity in expression of Cl^--dependent carriers with observations made in rabbit and guinea pig small intestine suggested that the rat was not a good model for studies of the human small intestine. However, we feel that the observations are inconclusive and equally valid conclusions could be that: the apparent lack of sensitivity to extracellular Cl^- could be due to uptake being a function of a mixture of both Cl^--dependent transporters (e.g. *IMINO, TauT*) as well as a Cl^--independent *imino acid carrier*; the similarity in *imino acid carrier*-like function in human duodenum and rat small intestine suggest that the rat is a good model for studies of the human small intestine. Thus the results from this study using human duodenal tissue [169] could be interpreted to support expression of an *imino acid carrier* (PAT1/SLC36A1), *IMINO* transporter (SLC6A20) or both.

In contrast to the comprehensive Caco-2 studies described above [23,26,45,47,56,107–116], a single study [170] failed to demonstrate pH-dependent imino acid uptake across the apical membrane of Caco-2 cells and attributed proline uptake to the *IMINO* transporter. There may be many reasons why pH-dependent transport was not observed in this study [170] since the Caco-2 cell monolayers were prepared and experiments performed under different conditions to those in the other studies [23,26,45,47,56,107–116,170]. For example, proline accumulation within the Caco-2 cells was measured over 3 h which would represent movement through a combination of influx and efflux pathways across both apical and basolateral cell membranes rather than the function of a single transport system [170]. In addition the Na^+-dependence of proline uptake at apical pH 7.4 could be due to NHE3 inhibition, and competition experiments were performed using concentrations below the K_m for most *imino acid carrier*/*system PAT* (PAT1/SLC36A1) substrates.

Studies measuring proline uptake into human intestinal BBMV [171,172] are often quoted as providing evidence for the *IMINO* transporter although the evidence is not convincing. In both studies there was only a small increase in proline uptake in the presence of Na^+, there was no Na^+-dependent overshoot and the observations were not confirmed by competition experiments [171,172]. Indeed in human foetal small intestinal BBMV both Na^+-dependent and Na^+-independent proline uptake was observed suggesting that a Na^+-independent carrier was also involved in uptake [172]. More convincing evidence for the presence of a Na^+-dependent transport system for imino acids in the human small intestine was provided in a study that described a Na^+-dependent overshoot of MeAIB in human small intestinal BBMV, although the observations were incorrectly attributed to *system A* [173]. Thus evidence exists for expression of both *imino acid carrier* and *IMINO* transporter-like systems at the brush-border of the human small intestine. The relative contribution of these two transport systems to intestinal amino and imino acid absorption is likely to vary depending upon local conditions including substrate concentration and the ionic composition (Na^+, Cl^- and H^+) of the solution bathing the mucosal surface of the enterocyte.

9. Conclusions

It must be emphasised that any conclusions made in a review of this nature are made with the benefit of hindsight. It is clear that many of the doctrines relating to intestinal amino and imino acid absorption were based upon the observations made in a large number of well-controlled studies. Unfortunately there has often been as much opposing information available to render each doctrine redundant. For one reason or another much of the opposing information has often been omitted from key publications so that, for example, the dogma that only L-amino acids are absorbed has existed well beyond the date (1959) after which there was enough evidence available to demonstrate that it was mistaken. Many of the studies have used tissues from different species and made measurements of amino/imino acid transport using different techniques. It is important that we are fully aware of the limitations of observations made using one single technique or those using one tissue or model system. Many of the individual studies include inconsistencies or apparent controversial observations or artefacts or errors but when a group of studies in any species is reviewed an inclusive pattern of function becomes apparent. No single study is conclusive but rather each body of evidence produced identifies the existence of one transporter or another. There can be no doubt that there are species differences in the levels of expression of individual amino and imino acid transport systems in the small intestine. It is not the intention of this review to exclude any particular transport system for a role in absorption in the small intestine of any particular species but rather to highlight distinct characteristics of different transporters and species differences in functional measurements. It may be that both *imino acid carrier* (SLC36A1) and *IMINO* (SLC6A20) like transporters are expressed in the intestines of all species but that the absolute levels of expression relative to each other differ between species so that only a dominant, or highly expressed, transport system is detected. In the human small intestine the bulk of the information favours expression of an *imino acid carrier* (SLC36A1) but an *IMINO* (SLC6A20) transporter may also be expressed and the relative expression levels may vary spatially (along the length of the small intestine and crypt-villus axis), developmentally and in response to changes in diet, hormonal levels or pharmacological regimes. A role for an *imino acid carrier* such as SLC36A1 in absorption across the human small intestine is supported by the large number of SLC36A1 substrates that are orally-delivered drugs with high levels of oral bioavailability (e.g. vigabatrin) [116,122]. It is essential that any future study of neutral imino/amino acid and related drug absorption in the mammalian small intestine is designed to discriminate clearly between transport systems with overlapping function. Ultimately, understanding the mechanisms involved in absorption across the human small intestine will be of greatest value. However, an appreciation of earlier work performed using various species, and in particular

species differences, will be vital to inform future studies including those using transgenic animal models.

10. Epilogue: What's in a name? You say IMINO, I say imino

Finally, it is clear that part of the reason that the SLC36A1 (*PAT1/system PAT/imino acid carrier*) transporter is not well known, despite being identified functionally in 1964, is due to the confusion over names. Most reports have simply ignored all evidence for the *imino acid carrier* and used the *IMINO* term only. As this became more prevalent, Munck and Munck started to use both terms whilst identifying clearly which transporter was being described by indication of the species under investigation [80,92] (Table 1). When the human SLC36A1 transporter hPAT1 was isolated, this convention was followed and highlighted the similarities between hPAT1/SLC36A1 and the *rat IMINO* transporter (the term used to describe *imino acid carrier*-like function) while identifying that the *rabbit IMINO* transporter (the term used to describe *IMINO*-like activity) was distinct [23]. In the following paper, the clear distinction between the *imino acid carrier* and *IMINO* transporters was discussed in detail [26]. It has become apparent that such discriminations [23,26,80,92] are probably too subtle for some and can lead to further confusion [152]. Thus the confusion over transporter names can often reinforce dogma. Confusion can occur not only within research fields but also between them. For example, the anion exchanger SLC26A6 or CFEX (for chloride–formate exchanger) [174] is also known as the putative anion transporter (and thus PAT1) [175]. Overall the confusion over the last 40 years informs us that complete agreement on transporter names is unlikely to occur. However, in the future, as long as each transporter is also identified (in publication titles and/or abstracts) by its solute carrier family name (using the nomenclature of the Human Genome Organisation (HUGO) Nomenclature Committee Database; see http://www.gene.ucl.ac.uk/nomenclature/) [176] then further confusion and room for misinterpretation will be diminished.

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