

# Human solute carrier SLC6A14 is the $\beta$ -alanine carrier

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The  $\beta$ -alanine carrier was characterized functionally in the 1960s to 1980s at the luminal surface of the ileal mucosal wall and is a Na<sup>+</sup>- and Cl<sup>-</sup>-dependent transporter of a number of essential and non-essential cationic and dipolar amino acids including lysine, arginine and leucine.  $\beta$ -Alanine carrier-like function has not been demonstrated by any solute carrier transport system identified at the molecular level. A series of experiments were designed to determine whether solute carrier SLC6A14 is the molecular correlate of the intestinal  $\beta$ -alanine carrier, perhaps the last of the classical intestinal amino acid transport systems to be identified at the molecular level. Following expression of the human SLC6A14 transporter in *Xenopus laevis* oocytes, the key functional characteristics of the  $\beta$ -alanine carrier, identified previously *in situ* in ileum, were demonstrated for the first time. The transport system is both Na<sup>+</sup> and Cl<sup>-</sup> dependent, can transport non- $\alpha$ -amino acids such as  $\beta$ -alanine with low affinity, and has a higher affinity for dipolar and cationic amino acids such as leucine and lysine. *N*-methylation of its substrates reduces the affinity for transport. These observations confirm the hypothesis that the *SLC6A14* gene encodes the transport protein known as the  $\beta$ -alanine carrier which, due to its broad substrate specificity, is likely to play an important role in absorption of essential nutrients and drugs in the distal regions of the human gastrointestinal tract.

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Amino acids are required for many fundamental biological functions such as protein synthesis, neurotransmission, nitrogen metabolism, and cell growth. In humans and mammals the requirements for most amino acids are met by assimilation from diet. Transepithelial amino acid absorption across the intestinal wall is mediated by a number of amino acid transporters arranged in series and parallel at the luminal and serosal membranes of the intestinal epithelium (Ganapathy *et al.* 2006). Although amino acid transporters vary in substrate selectivity, ion dependency and substrate affinity, the attribution of function to any particular transport system in intact tissues is often hampered by overlapping substrate specificity. One such amino acid transport system was described at the mucosal surface of rabbit ileum and named the  $\beta$ -alanine carrier (Munck & Schultz, 1969; Paterson *et al.* 1981; Munck, 1985; Anderson & Munck, 1987). This carrier system transports a range of both essential and non-essential amino acids and accepts non- $\alpha$ -amino acids such as  $\beta$ -alanine but has a higher affinity for dipolar (e.g. leucine) and cationic (e.g. lysine) amino acids; is Na<sup>+</sup> and Cl<sup>-</sup> dependent; is only moderately stereospecific; and has

a much lower affinity for the *N*-methylated derivatives of its dipolar amino acid substrates (Munck, 1985; Munck & Munck, 1990, 1992*a,b*, 1995). The  $\beta$ -alanine carrier is unusual in that, under normal circumstances, its small intestinal expression is limited to the ileum (Munck & Munck, 1992*a,b*).

The cloning of transporter related genes over recent years has allowed molecular identification of most of the 'classical' amino acid transport systems characterized functionally in specific cells and tissues during the 1960s to 1980s. The purpose of this investigation was to establish the molecular identity of the  $\beta$ -alanine carrier. The solute carrier SLC6A14 has been cloned from human mammary gland, mouse colon and rat lung (Sloan & Mager, 1999; Hatanaka *et al.* 2001; Nakanishi *et al.* 2001; Ugawa *et al.* 2001; Umapathy *et al.* 2004). SLC6A14 is the 14th member of solute carrier family 6, a family of Na<sup>+</sup>- and Cl<sup>-</sup>-dependent solute transport systems many of which are involved in transmembrane movement of neurotransmitters (Chen *et al.* 2004). SLC6A14 (also named ATB<sup>0,+</sup>) functions as a dipolar and cationic amino acid transporter with characteristics similar to

system B<sup>0+</sup> (identified originally in mouse blastocysts; Van Winkle *et al.* 1985). Thus far,  $\beta$ -alanine carrier-like function has not been demonstrated by any solute carrier transport system identified at the molecular level. In this investigation, a series of experiments were designed to determine whether SLC6A14 is the molecular correlate of the intestinal  $\beta$ -alanine carrier, perhaps the last of the classical intestinal amino acid transport systems to be identified at the molecular level.

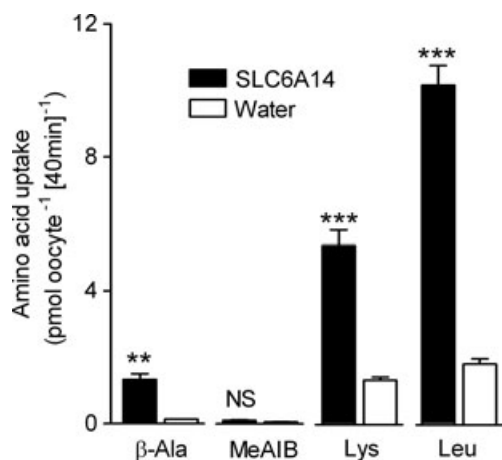
## Methods

### Materials

[<sup>3</sup>H] $\beta$ -Alanine (50 Ci mmol<sup>-1</sup>) was from American Radiolabelled Chemicals. [<sup>3</sup>H]Lysine (99 Ci mmol<sup>-1</sup>), [<sup>3</sup>H]leucine (115 Ci mmol<sup>-1</sup>) and [<sup>14</sup>C]MeAIB ( $\alpha$ -(methylamino)isobutyric acid) (51 mCi mmol<sup>-1</sup>) were from PerkinElmer.

### Functional expression in *Xenopus laevis* oocytes

Human SLC6A14 cRNA was produced by *in vitro* transcription (using mMessage mMachine T7 Ultra kit (Ambion)) of pSPORT1 plasmid containing the SLC6A14 sequence isolated originally from MCF-7 cells (Nakanishi *et al.* 2001). Female *Xenopus laevis* were killed humanely by cervical dislocation following Schedule 1 procedures. Oocytes were prepared and injected with 50 nl cRNA (1 mg ml<sup>-1</sup>) or water, as previously described (Kennedy *et al.* 2002, 2005), and incubated at 18°C in Barth's solution until required.



**Figure 1. Amino acid uptake in SLC6A14-injected and water-injected oocytes**

Uptake of various radiolabelled amino acids (2–5  $\mu$ Ci ml<sup>-1</sup>; all 2  $\mu$ M except [<sup>14</sup>C]MeAIB which was 20  $\mu$ M) was measured over 40 min in a NaCl-containing pH 7.4 solution into oocytes injected with either SLC6A14 cRNA or water ( $n = 18$ –20). NS,  $P > 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$  all *versus* water-injected oocytes.

### Uptake of radiolabelled amino acids

Uptake of radiolabelled amino acids (2–5  $\mu$ Ci ml<sup>-1</sup>) was measured in oocytes 2–5 days after injection, as previously described (Kennedy *et al.* 2002). Oocytes were washed in a NaCl-containing pH 7.4 solution (100 mM NaCl, 2 mM KCl, 1 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 10 mM Hepes adjusted to pH 7.4 with Tris base) and uptake measured at 22°C for 40 min. Uptake measurements were performed in this NaCl-containing pH 7.4 solution or using a solution adjusted as follows: for the Na<sup>+</sup>-free solution, NaCl was replaced with choline chloride; for the Cl<sup>-</sup>-free solution, NaCl, KCl, CaCl<sub>2</sub> and MgCl<sub>2</sub> were replaced with sodium gluconate, potassium gluconate, calcium gluconate and MgSO<sub>4</sub>, respectively; for the pH 5.5 solution, Hepes was replaced by Mes. After uptake, oocytes were washed three times in ice-cold buffer and lysed in 10% SDS. Radioactivity was measured by scintillation counting.

### Two-electrode voltage clamp

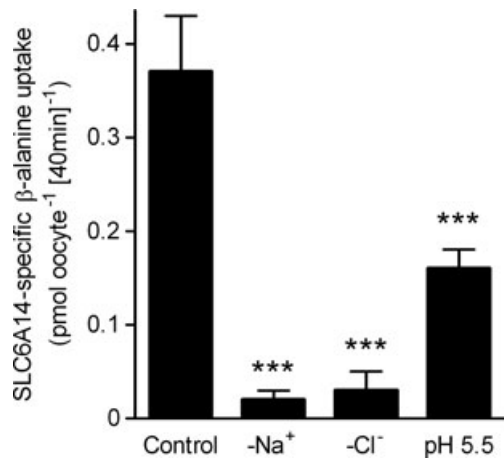
Oocytes (2–8 days post-injection) were superfused in an open chamber with a NaCl-containing pH 7.4 solution (see above). Oocytes were clamped at –60 mV and exposed to various concentrations of  $\beta$ -alanine (0.2–20 mM, 2 min) or different amino acids (all at 20 mM, 2 min) to allow amino acid-induced currents to be measured using a Geneclamp 500 amplifier, Digidata 1200 (Axon Instruments) and Clampex software (Kennedy *et al.* 2005). Currents were analysed using Clampfit 8.2. To determine the current evoked by a 2 min exposure to an amino acid, the current measured over the last 15 s of the 2 min exposure was averaged. The baseline current (taken as the average current over the 15 s before exposure to the amino acid) was then subtracted to determine SLC6A14-specific current.

### Statistics

Data are means  $\pm$  S.E.M. Statistical comparisons were made using ANOVA and Tukey's *post hoc* test using GraphPad Prism 4 (GraphPad Software Inc., San Diego, CA, USA). Curves were fitted with GraphPad Prism 4.

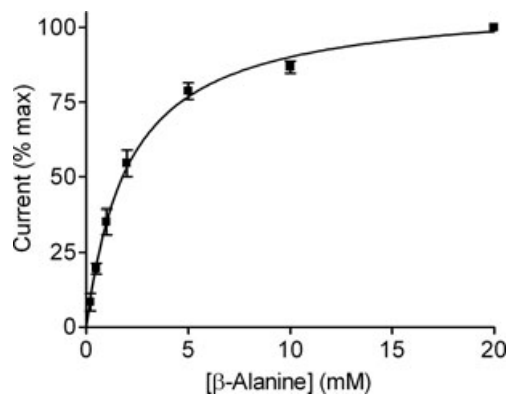
### Results

At a tracer concentration of 2  $\mu$ M there was a 9.5-fold increase in [<sup>3</sup>H] $\beta$ -alanine uptake in SLC6A14 cRNA-injected oocytes compared to water-injected oocytes ( $P < 0.01$ ) (Fig. 1). Similarly, other amino acids identified as substrates for the  $\beta$ -alanine carrier in rabbit ileum (Munck, 1985; Munck & Munck, 1992b) also showed significant uptake into SLC6A14-injected oocytes with 5.7-fold and 4.1-fold increases in uptake, compared



**Figure 2. Na<sup>+</sup>- and Cl<sup>-</sup>-dependent  $\beta$ -alanine uptake by SLC6A14**  
 $[^3\text{H}]\beta$ -Alanine uptake (2  $\mu\text{M}$ ) was measured under control conditions (NaCl-containing solution, pH 7.4), in Na<sup>+</sup>-free pH 7.4 (-Na<sup>+</sup>) solution, in Cl<sup>-</sup>-free pH 7.4 (-Cl<sup>-</sup>) solution, or in a NaCl pH 5.5 solution. Under each condition, uptake in water-injected oocytes was subtracted from that in cRNA-injected oocytes to give SLC6A14-specific uptake ( $n = 20$ ). \*\*\* $P < 0.001$  versus control.

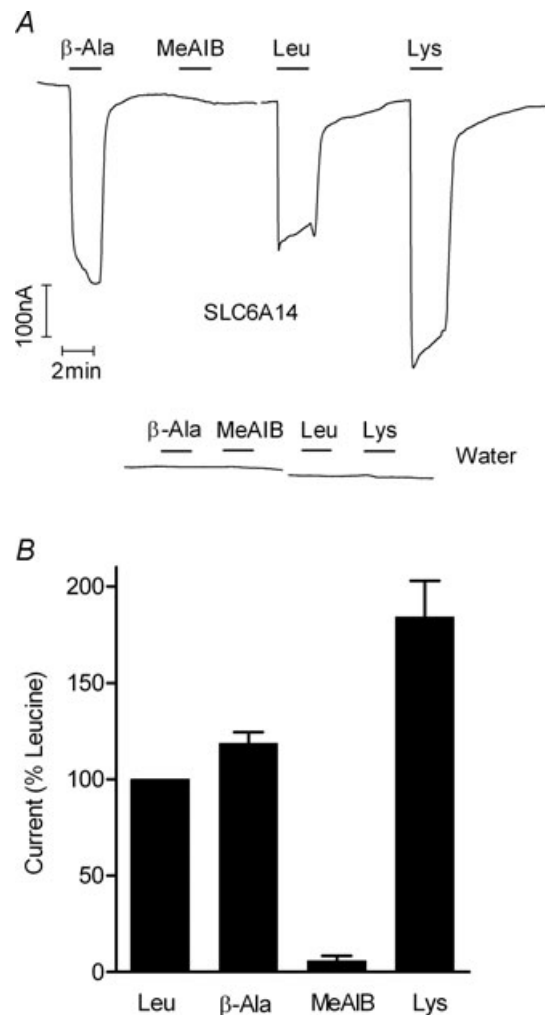
to water-injected oocytes, being observed for the dipolar amino acid  $[^3\text{H}]$ leucine and the cationic amino acid  $[^3\text{H}]$ lysine (both  $P < 0.001$  versus water) (Fig. 1). The greater uptake of  $[^3\text{H}]$ leucine and  $[^3\text{H}]$ lysine compared to  $[^3\text{H}]\beta$ -alanine at tracer concentrations (Fig. 1) reflects the earlier observation in rabbit ileum that leucine and lysine are higher affinity substrates for the  $\beta$ -alanine carrier (Munck, 1985). There was no significant uptake ( $P > 0.05$  versus water) of  $[^{14}\text{C}]\text{MeAIB}$  into SLC6A14-injected oocytes (Fig. 1). MeAIB interacts very poorly with the  $\beta$ -alanine carrier in rabbit ileum (Munck, 1985). SLC6A14-mediated  $[^3\text{H}]\beta$ -alanine uptake was abolished in the absence of either extracellular Na<sup>+</sup> or Cl<sup>-</sup> (Fig. 2)



**Figure 3. Concentration-dependent  $\beta$ -alanine-induced current in SLC6A14-expressing oocytes**  
 $\beta$ -Alanine-induced current in two-electrode voltage-clamped oocytes was measured at -60 mV following exposure to  $\beta$ -alanine (0.2–20 mM, 2 min) in a NaCl pH 7.4 solution. Data are expressed as percentage current produced by 20 mM  $\beta$ -alanine ( $n = 4$ ).

demonstrating that  $[^3\text{H}]\beta$ -alanine uptake via SLC6A14 is a Na<sup>+</sup>- and Cl<sup>-</sup>-dependent process as observed with the  $\beta$ -alanine carrier in rabbit ileum (Munck & Munck, 1990, 1992a). Na<sup>+</sup>- and Cl<sup>-</sup>-dependent  $[^3\text{H}]\beta$ -alanine uptake is reduced as extracellular pH becomes acidic (pH 5.5) (Fig. 2).

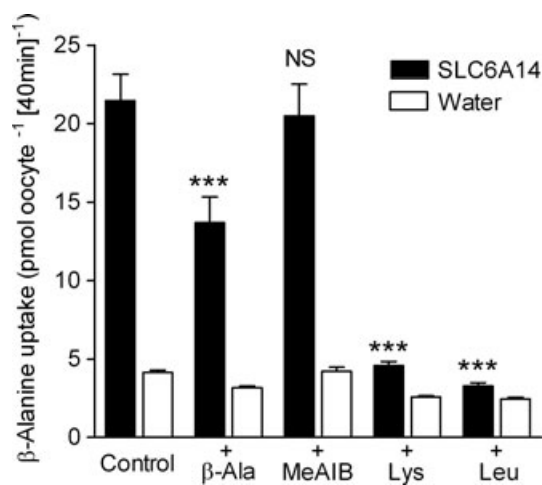
SLC6A14-mediated  $\beta$ -alanine/Na<sup>+</sup>/Cl<sup>-</sup> cotransport is a rheogenic process which generates inward current under two-electrode voltage-clamp conditions (Figs 3 and 4) consistent with a stoichiometry of 2–3 Na<sup>+</sup> : 1 Cl<sup>-</sup> : 1 amino acid, as suggested previously with other substrates for SLC6A14 (Sloan & Mager, 1999). The relative low affinity of SLC6A14 for  $\beta$ -alanine ( $K_m = 2.1 \pm 0.9$  mM, Fig. 3), compared to leucine and lysine, is consistent with



**Figure 4. Amino acid induced inward current in SLC6A14-expressing oocytes**  
A, example traces of amino acid-induced currents in SLC6A14-expressing and water-injected oocytes following exposure (2 min) to saturating concentrations (20 mM) of different amino acids in NaCl pH 7.4 solution. B, mean results for experiments described in A using SLC6A14-expressing oocytes where data are expressed as the percentage current induced by 20 mM leucine ( $n = 7$ ).

earlier observations of the  $\beta$ -alanine carrier in rabbit ileum ( $K_m = 2$  mM) (Munck, 1985; Munck & Munck, 1992a). At saturating substrate concentrations (20 mM),  $\beta$ -alanine transport via SLC6A14 is associated with a large inward current of similar magnitude ( $P > 0.05$ ) to that observed with the other dipolar substrate leucine (Fig. 4). The cationic amino acid lysine was associated with a larger current ( $P < 0.01$ , lysine *versus*  $\beta$ -alanine) which raises the possibility that a component of the current is carried by the positively charged amino acid (Fig. 4). MeAIB, which is excluded from the  $\beta$ -alanine carrier, failed to induce significant current ( $P > 0.05$ ) in the SLC6A14-injected oocytes (Fig. 4). None of the three  $\beta$ -alanine carrier substrates induced inward current in water-injected oocytes (Fig. 4A). The higher affinity of SLC6A14 for lysine and leucine compared to  $\beta$ -alanine is confirmed by the greater inhibition of [ $^3$ H] $\beta$ -alanine uptake observed (Fig. 5) when all competing cold substrates were present at a concentration (2 mM) around the  $K_m$  for  $\beta$ -alanine (leucine and lysine both  $P < 0.001$  *versus* cold  $\beta$ -alanine;  $\beta$ -alanine  $P < 0.001$  *versus* control).

A key functional characteristic of the  $\beta$ -alanine carrier in rabbit ileum (Munck, 1985) is the reduction in affinity of the carrier for dipolar substrates following *N*-methylation. As measured in rabbit ileum (Munck, 1985), the effects of *N*-methylation on the ability of leucine, glycine, alanine and aminoisobutyric acid (AIB) to inhibit [ $^3$ H] $\beta$ -alanine transport via the  $\beta$ -alanine carrier were determined here in SLC6A14-injected oocytes (Fig. 6). An identical pattern of results was obtained in SLC6A14-injected oocytes (Fig. 6) to those measured



**Figure 5.** Inhibition of SLC6A14-mediated  $\beta$ -alanine uptake by unlabelled amino acids

Inhibition of [ $^3$ H] $\beta$ -alanine (100  $\mu$ M) transport into SLC6A14-expressing oocytes by various amino acids (all 2 mM) in NaCl pH 7.4 solution ( $n = 18$ –21). NS,  $P > 0.05$ ; \*\*\* $P < 0.001$ ; all *versus* SLC6A14 control.

earlier for the  $\beta$ -alanine carrier in rabbit ileum (Munck, 1985). The ability of each amino acid to inhibit [ $^3$ H] $\beta$ -alanine transport was reduced by *N*-methylation. For example, greater inhibition of [ $^3$ H] $\beta$ -alanine uptake into SLC6A14-injected oocytes was observed at 10 mM for leucine (Leu) *versus* *N*-methyl-leucine (*N*-Me-Leu) ( $P < 0.001$ ) (Fig. 6A), glycine (Gly) *versus* *N*-methyl-glycine or sarcosine (Sar) ( $P < 0.001$ ) (Fig. 6B), alanine (Ala) *versus* *N*-methyl-alanine (*N*-Me-Ala) ( $P < 0.001$ ) (Fig. 6C), and AIB *versus* MeAIB ( $P < 0.001$ ) (Fig. 6D).

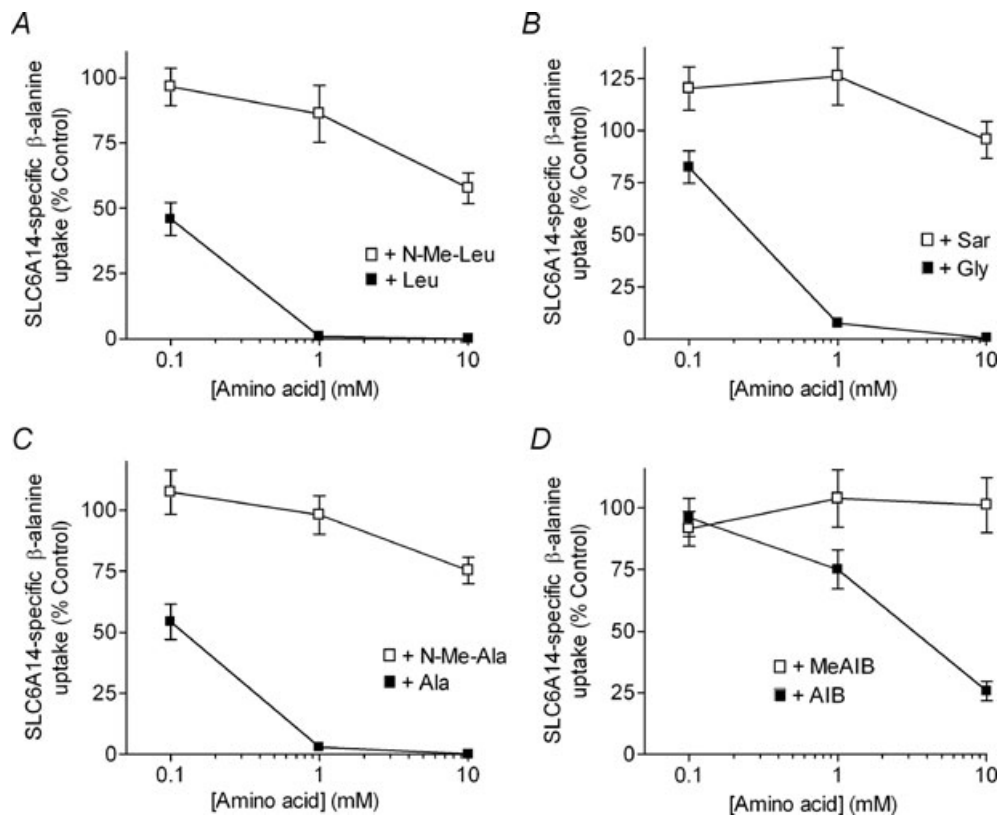
## Discussion

The key functional characteristics of the  $\beta$ -alanine carrier, as previously described only *in situ* in rabbit ileum (Munck, 1985), are demonstrated following expression of the SLC6A14 transporter in *Xenopus laevis* oocytes (Figs 1–6). The transport system is both Na<sup>+</sup> and Cl<sup>-</sup> dependent, can transport non- $\alpha$ -amino acids such as  $\beta$ -alanine with low affinity, and has a higher affinity for dipolar and cationic amino acids such as leucine and lysine (Figs 1–5). *N*-methylation of the substrates leucine, glycine, alanine and AIB reduces the affinity for transport (Fig. 6). In addition, the characteristic of weak stereoselectivity has been demonstrated previously with the identification of D-serine transport via SLC6A14 (Hatanaka *et al.* 2002). These observations confirm the hypothesis that the SLC6A14 gene encodes the transport protein known as the  $\beta$ -alanine carrier (Munck, 1985; Anderson & Munck, 1987). The  $\beta$ -alanine carrier is considered to be the Na<sup>+</sup>-dependent transporter of leucine, lysine and alanine observed originally in rabbit ileum by Munck & Schultz (1969) and Paterson *et al.* (1981). Importantly, these observations also indicate that the studies of the  $\beta$ -alanine carrier in the 1960s to 1980s can now be considered to represent *in situ* measurements of SLC6A14 function in the intestine.

The tissue distribution of the  $\beta$ -alanine carrier is unusual as the only intestinal location where function has been described is at the luminal surface of the ileum (Munck, 1985; Anderson & Munck, 1987; Munck & Munck, 1990, 1992a,b, 1995). This predominantly distal intestinal expression pattern is supported by observations using mouse gastrointestinal tract that demonstrate greater expression of SLC6A14 mRNA in the ileum and colon compared to duodenum and jejunum (Hatanaka *et al.* 2001, 2002; Ugawa *et al.* 2001; Sloan *et al.* 2003). In the rabbit, the physiological role of the  $\beta$ -alanine carrier seems certain to lie in nutrient absorption from the diet as the ileum is the site of maximal amino acid absorption in rabbit small intestine (Munck & Munck, 1992b). The physiological role in the human intestine is not known but the broad substrate specificity of SLC6A14 means that

this distal intestinal site of absorption could be important in both nutrient and drug absorption.  $\beta$ -Alanine is a non-proteinogenic amino acid and is a component of the dipeptide carnosine ( $\beta$ -alanine-L-histidine), found at high concentrations in both vertebrate and non-vertebrate skeletal muscle. In humans, dietary supplementation with  $\beta$ -alanine (at concentrations relevant to diet) leads to an increase in skeletal muscle carnosine (Harris *et al.* 2006) which is associated with an improvement in performance during exercise, proposed to be due to the enhanced pH buffering effect of carnosine (Harris *et al.* 2006; Stout *et al.* 2007; Zoeller *et al.* 2007). In addition to cationic and dipolar amino acids, SLC6A14 transports carnitine, a range of nitric oxide synthase inhibitors, and the antiviral prodrugs valacyclovir and valganciclovir (Hatanaka *et al.* 2001, 2004; Nakanishi *et al.* 2001; Umapathy *et al.* 2004). Thus, SLC6A14 has great potential as a target for drug delivery programmes using slow release formulations or rectal suppositories. In addition, the colonic expression may have relevance for the absorption of bacterially derived D-amino acids as D-serine is

transported by SLC6A14 (Hatanaka *et al.* 2002). Interestingly, the distribution pattern of SLC6A14 parallels the regions of the gastrointestinal tract that are generally colonized by bacteria. The hypothesis that SLC6A14 expression might be up-regulated following bacterial colonization is consistent with the recent demonstration of selective up-regulation of SLC6A14 mRNA and protein expression in acute cholera patients compared to convalescence such that SLC6A14 protein has been immunolocalized to the luminal surface of the human duodenum during acute infection (Flach *et al.* 2007). After induction, e.g. following infection with cholera, SLC6A14 could provide a highly concentrating mechanism (due to its  $\text{Na}^+$  and  $\text{Cl}^-$  dependence) for absorption of essential amino acids such as lysine and leucine that could otherwise be lost during excess intestinal fluid and electrolyte secretion. The *SLC6A14* gene seems highly regulated being associated with obesity (Suviolahti *et al.* 2003; Durand *et al.* 2004), and being up-regulated in colorectal cancer (Gupta *et al.* 2005), cervical cancer (Gupta *et al.* 2006), and ulcerative colitis (Flach *et al.* 2006).



**Figure 6. The effects of substrate N-methylation on  $\beta$ -alanine carrier-like transport by SLC6A14**

SLC6A14-mediated [ $^3\text{H}$ ] $\beta$ -alanine (100  $\mu\text{M}$ ) uptake (measured in NaCl pH 7.4 solution) in the presence of various amino acids (filled squares) and their N-methylated analogues (open squares) at 0.1, 1 and 10 mM. Amino acids are: A, leucine and N-methyl-leucine (N-Me-Leu); B, glycine and sarcosine (Sar; N-methyl-glycine); C, alanine and N-methyl-alanine (N-Me-Ala); D, AIB (aminoisobutyric acid) and MeAIB ( $\alpha$ -(methylamino)isobutyric acid). Data are expressed as percentage of control (absence of competing amino acids) after subtraction of uptake in water-injected oocytes under each condition ( $n = 18$ –20).

In summary, this study describes the first demonstration of  $\beta$ -alanine carrier-like function by any cloned transporter. The functional characteristics described here, which are identical to those determined by measurement of amino acid uptake across the luminal surface of flat sheets of rabbit ileal mucosa (Munck, 1985; Munck & Munck, 1990, 1992a,b, 1994, 1995), can account for all  $\beta$ -alanine carrier-like function and emphasize the added value of *in situ* measurements of physiological function over theoretical predictions of transport protein function based solely upon measurements of mRNA distribution.

SLC6A14 was isolated originally from human mammary gland (Sloan & Mager, 1999) and named ATB<sup>0,+</sup> due to its similarity in function to the amino acid transport system B<sup>0,+</sup>, described in mouse blastocysts (Van Winkle *et al.* 1985). The observations reported in this study demonstrate that SLC6A14 is the  $\beta$ -alanine carrier and that the blastocyst ATB<sup>0,+</sup> and intestinal  $\beta$ -alanine carrier are one and the same transport system (Munck & Munck, 1995). The term  $\beta$ -alanine carrier is somewhat inappropriate as a means of identification of this intestinal transport system as it has a low affinity for  $\beta$ -alanine, and the intestinal tract contains another transport system for  $\beta$ -alanine namely the H<sup>+</sup>-coupled amino acid transporter SLC36A1 which is also known variously as system PAT, PAT1 or the imino acid carrier (Thwaites *et al.* 1993; Chen *et al.* 2003; Anderson *et al.* 2004; Thwaites & Anderson, 2007). To reduce confusion in the literature, the term  $\beta$ -alanine carrier should be avoided and the names SLC6A14 and ATB<sup>0,+</sup> adopted to be consistent with descriptions in other tissues (Van Winkle *et al.* 1985; Munck & Munck, 1994, 1995; Sloan & Mager, 1999; Sloan *et al.* 2003).

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