## Rapid Report

# $Na^+$- and $Cl^-$-coupled active transport of carnitine by the amino acid transporter $ATB^{0,+}$ from mouse colon expressed in HRPE cells and *Xenopus* oocytes

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1. $ATB^{0,+}$ is an amino acid transporter energized by transmembrane gradients of $Na^+$ and $Cl^-$ and membrane potential. We cloned this transporter from mouse colon and expressed the clone functionally in mammalian (human retinal pigment epithelial, HRPE) cells and *Xenopus laevis* oocytes to investigate the interaction of carnitine and its acyl esters with the transporter.

2. When expressed in mammalian cells, the cloned $ATB^{0,+}$ was able to transport carnitine, propionylcarnitine and acetylcarnitine. The transport process was $Na^+$ and $Cl^-$ dependent and inhibitable by the amino acid substrates of the transporter. The Michaelis constant for carnitine was $0.83 \pm 0.08$ mM and the Hill coefficient for $Na^+$ activation was $1.6 \pm 0.1$.

3. When expressed in *Xenopus laevis* oocytes, the cloned $ATB^{0,+}$ was able to induce inward currents in the presence of carnitine and propionylcarnitine under voltage-clamped conditions. There was no detectable current in the presence of acetylcarnitine. Carnitine-induced currents were obligatorily dependent on the presence of $Na^+$ and $Cl^-$. The currents were saturable with carnitine and the Michaelis constant was $1.8 \pm 0.4$ mM. The analysis of $Na^+$- and $Cl^-$-activation kinetics revealed that 2 $Na^+$ and 1 $Cl^-$ were involved in the transport of carnitine via the transporter.

4. These studies describe the identification of a novel function for the amino acid transporter $ATB^{0,+}$. Since this transporter is expressed in the intestinal tract, lung and mammary gland, it is likely to play a significant role in the handling of carnitine in these tissues.

5. A $Na^+$-dependent transport system for carnitine has already been described. This transporter, known as OCTN2 (novel organic cation transporter 2), is expressed in most tissues and transports carnitine with high affinity. It is energized, however, only by a $Na^+$ gradient and membrane potential. In contrast, $ATB^{0,+}$ is a low-affinity transporter for carnitine, but exhibits much higher concentrative capacity than OCTN2 because of its energization by transmembrane gradients of $Na^+$ and $Cl^-$ as well as by membrane potential.

Carnitine ($\beta$-hydroxy-$\gamma$-trimethylaminobutyrate) is an obligate requirement for $\beta$-oxidation of long-chain fatty acids. It is synthesized endogenously in humans in the liver and kidney (Carter *et al.* 1995). It is also absorbed in the intestinal tract from dietary sources (Rebouche, 1992). The biological importance of this molecule is evident from the clinical consequences of carnitine deficiency encountered in a variety of genetic and acquired diseases (Kerner & Hoppel, 1998). The symptoms of carnitine deficiency include skeletal myopathy, cardiomyopathy, encephalopathy and failure to thrive (Treem *et al.* 1988; Kerner & Hoppel, 1998). Most tissues, including the cardiac and skeletal muscle, contain intracellular carnitine levels severalfold higher than plasma levels due to the presence of a $Na^+$-dependent high-affinity carnitine transport system (Bremer, 1983). This transport system also exists in the brush border membrane of renal tubular epithelial cells where it plays a role in the reabsorption of carnitine (Rebouche & Mack, 1984; Huang *et al.* 1999). A genetic defect in this transport system results in excessive urinary loss of carnitine, causing systemic carnitine deficiency. Since the same transport system is also responsible for active accumulation of carnitine in tissues such as the heart and skeletal muscle, the genetic defect is associated with drastically reduced intracellular levels of carnitine in

these tissues. The major clinical symptoms of this defect, known as primary carnitine deficiency, are skeletal and cardiac myopathy, resulting from impaired energy production from fatty acid oxidation as a consequence of reduced intracellular levels of carnitine. Recently, this transporter has been cloned (Wu *et al.* 1998; Tamai *et al.* 1998). Interestingly, this transporter also transports several organic cations and $\beta$-lactam antibiotics (Wu *et al.* 1998, 1999; Ohashi *et al.* 1999; Ganapathy *et al.* 2000). Furthermore, it belongs to the organic cation transporter gene family on the basis of its primary structure (Wu *et al.* 1998). Therefore, the transporter is named OCTN2 (novel organic cation transporter 2).

The present studies describe the identification of a second energy-coupled carnitine transporter. This transporter, known as $ATB^{0,+}$, is an amino acid transporter expressed in the intestine, lung and mammary gland. Functionally, $ATB^{0,+}$ is a $Na^+$- and $Cl^-$-coupled transport system for neutral and cationic amino acids. It plays an important role in the absorption of amino acids in the intestinal tract (Ganapathy *et al.* 2001). The cloning of human $ATB^{0,+}$ has been recently reported (Sloan & Mager, 1999). To date, the transport function of $ATB^{0,+}$ has been studied only with amino acids as substrates. Its transport function is highly concentrative, energized by transmembrane gradients of $Na^+$ and $Cl^-$ and membrane potential. $ATB^{0,+}$ belongs to the gene family of $Na^+$- and $Cl^-$-coupled transporters for a variety of compounds such as amino acids (e.g. glycine and proline), neurotransmitters (e.g. monoamines and $\gamma$-aminobutyrate) and osmolytes (e.g. taurine and betaine). Structurally, $ATB^{0,+}$ is very closely related to $\gamma$-aminobutyrate transporters and betaine transporter. Therefore, we tested whether $ATB^{0,+}$ is able to recognize $\gamma$-aminobutyrate and other structurally related compounds as substrates. These studies have led to an interesting finding that $ATB^{0,+}$ can transport carnitine coupled to the transmembrane gradients of $Na^+$ and $Cl^-$.

## METHODS

### Molecular cloning of $ATB^{0,+}$ cDNA from mouse colon

A cDNA library was constructed using poly(A)$^+$ RNA isolated from mouse colon. The SuperScript plasmid system (Life Technologies, Inc., Gaithersburg, MD, USA) was employed for this purpose. The cDNA probe for screening the library was prepared by RT-PCR using primers specific for the mouse $ATB^{0,+}$ cDNA reported in GenBank (accession no. AF161714). The RT-PCR product (~1.2 kbp in size) was sequenced for confirmation of its identity. Screening was done under high stringency conditions as described previously (Kekuda *et al.* 1996, 1998). The longest positive clone (~3 kbp) was used for sequencing and functional analysis.

### Carnitine transport via mouse $ATB^{0,+}$ in a mammalian cell expression system

The cloned mouse $ATB^{0,+}$ cDNA was functionally expressed in the human retinal pigment epithelial (HRPE) cell line using the vaccinia virus expression technique (Wu *et al.* 1998, 1999). These cells were originally provided by M. A. Del Monte (Kellog Eye Center, Ann Arbor, MI, USA) and have been used in our laboratory for functional expression of a variety of cloned transporters. Any mammalian cell can be used in the method involving the vaccinia virus-mediated heterologous expression of cloned transporter cDNAs. However, in our experience we found HRPE cells to be most suitable for the purpose because these cells withstand the infection with the vaccinia virus much better than other cell types (e.g. HeLa, COS-1, COS-7 and HEK293 cells). Initial studies of the interaction of carnitine with mouse $ATB^{0,+}$ in HRPE cells were done by assessing the ability of carnitine to compete with glycine for transport via $ATB^{0,+}$. Subsequent studies were carried out using [$^3$H]carnitine to assess directly the transport of carnitine via $ATB^{0,+}$. Transport measurements were made in vector-transfected cells and in mouse $ATB^{0,+}$ cDNA-transfected cells in parallel using 24-well culture plates. Incubations of the cells with radiolabelled substrates were carried out at 37 °C for 15 min. The composition of the transport buffer was 25 mM Hepes–Tris (pH 7.5), containing 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$ and 5 mM glucose. cDNA-specific transport was calculated by adjusting for the transport in vector-transfected cells. [$^3$H]Glycine, [$^3$H]carnitine, acetyl[$^3$H]carnitine and propionyl[$^3$H]carnitine were purchased from Moravek Biochemicals (Brea, CA, USA). Other radiolabelled amino acids were obtained from either American Radiolabeled Chemicals (St Louis, MO, USA) or DuPont-New England Nuclear (Boston, MA, USA).

### Carnitine transport via mouse $ATB^{0,+}$ in the *Xenopus laevis* oocyte expression system

Care and use of *Xenopus laevis* adhered to the institutional guidelines set forth by the Committee on Animal Use for Research and Education at the Medical College of Georgia. *Xenopus* oocytes were collected under anaesthesia from frogs that were humanely killed after the final collection. Mature oocytes from *X. laevis* were isolated by treatment with collagenase A (1.6 mg ml$^{-1}$). Oocytes were manually defolliculated and then used for injection with mouse $ATB^{0,+}$ cRNA or water (Fei *et al.* 2000). cRNA was synthesized using the mMESSAGE mMACHINE kit (Ambion, Austin, TX, USA). The transport of carnitine via mouse $ATB^{0,+}$ in oocytes was monitored electrophysiologically using the two-microelectrode voltage-clamp technique (Fei *et al.* 2000). The membrane potential was held steady at −50 mV. Oocytes were perifused with carnitine and the induced current was monitored. The induced current was taken as the measure of transport rate. The composition of the perifusion buffer was 10 mM Hepes–Tris (pH 7.5), containing 100 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$ and 1 mM $CaCl_2$.

### Data analysis

Experiments were repeated at least three times. Results are given as means ± S.E.M. The kinetic parameters, Michaelis constant ($K_t$) and maximal velocity ($V_{max}$), were calculated by fitting the $ATB^{0,+}$-specific transport data to the Michaelis-Menten equation describing a single saturable transport system. $Na^+$- and $Cl^-$-activation kinetics were analysed by fitting the $ATB^{0,+}$-specific transport data to the Hill equation for the determination of $K_{0.5}$ values for $Na^+$ and $Cl^-$ (concentration of $Na^+$ or $Cl^-$ necessary for half-maximal activation) and the Hill coefficient ($n_H$; the number of $Na^+$ or $Cl^-$ ions involved in the activation process). The kinetic parameters were first determined by non-linear regression methods and subsequently confirmed by linear regression methods using the commercially available computer program SigmaPlot, version 6.0 (SPSS Inc., Chicago, IL, USA).

## RESULTS

### Structural features of mouse $ATB^{0,+}$

The mouse $ATB^{0,+}$ cDNA isolated from the colon cDNA library is 3007 bp long (GenBank accession no. AF320226). The open reading frame is flanked by a 192 bp long

5′-untranslated region and an 898 bp long 3′-untranslated region. The coding region is exactly the same as the sequence of mouse $ATB^{0,+}$ previously reported in GenBank (accession no. AF161714) except for one amino acid substitution at position 192 (Ser instead of Asn). However, the two clones differ in their 3′-untranslated region. The mouse $ATB^{0,+}$ consists of 638 amino acids and the sequence is highly homologous to the human $ATB^{0,+}$ (88% identity) (Fig. 1). The protein sequence contains 12 putative transmembrane domains. Based on the amino acid sequence, mouse $ATB^{0,+}$ belongs to the gene family of $Na^+$- and $Cl^-$-coupled neurotransmitter transporters.

### Analysis of carnitine transport via mouse $ATB^{0,+}$ in the mammalian cell expression system

The recently cloned human $ATB^{0,+}$ is a $Na^+$- and $Cl^-$-coupled transporter for zwitterionic and cationic amino acids. Therefore, we first assessed the transport function of the $ATB^{0,+}$ cDNA isolated from mouse colon by comparing the transport of 12 different zwitterionic and cationic amino acids in cDNA-transfected cells and in vector (pSPORT1)-transfected cells. The cDNA-induced increase in transport was demonstrable for all amino acids examined (Gly, Ala, Ser, Thr, His, Gln, Asn, Leu, Ile, Phe, Trp and Arg). The increase was, however, the highest for glycine, the transport in cDNA-transfected cells being ~30-fold higher than in vector-transfected cells.

There is a significant structural similarity between carnitine and $\gamma$-aminobutyrate as well as between carnitine and betaine. Carnitine is a derivative of $\gamma$-aminobutyrate with an addition of a hydroxyl group at the $\beta$ carbon and the replacement of the amino group with the trimethylamino group. Because of the presence of the trimethylamino group at the terminal carbon atom, carnitine is also structurally similar to betaine. Since the primary structure of $ATB^{0,+}$ is closely related to

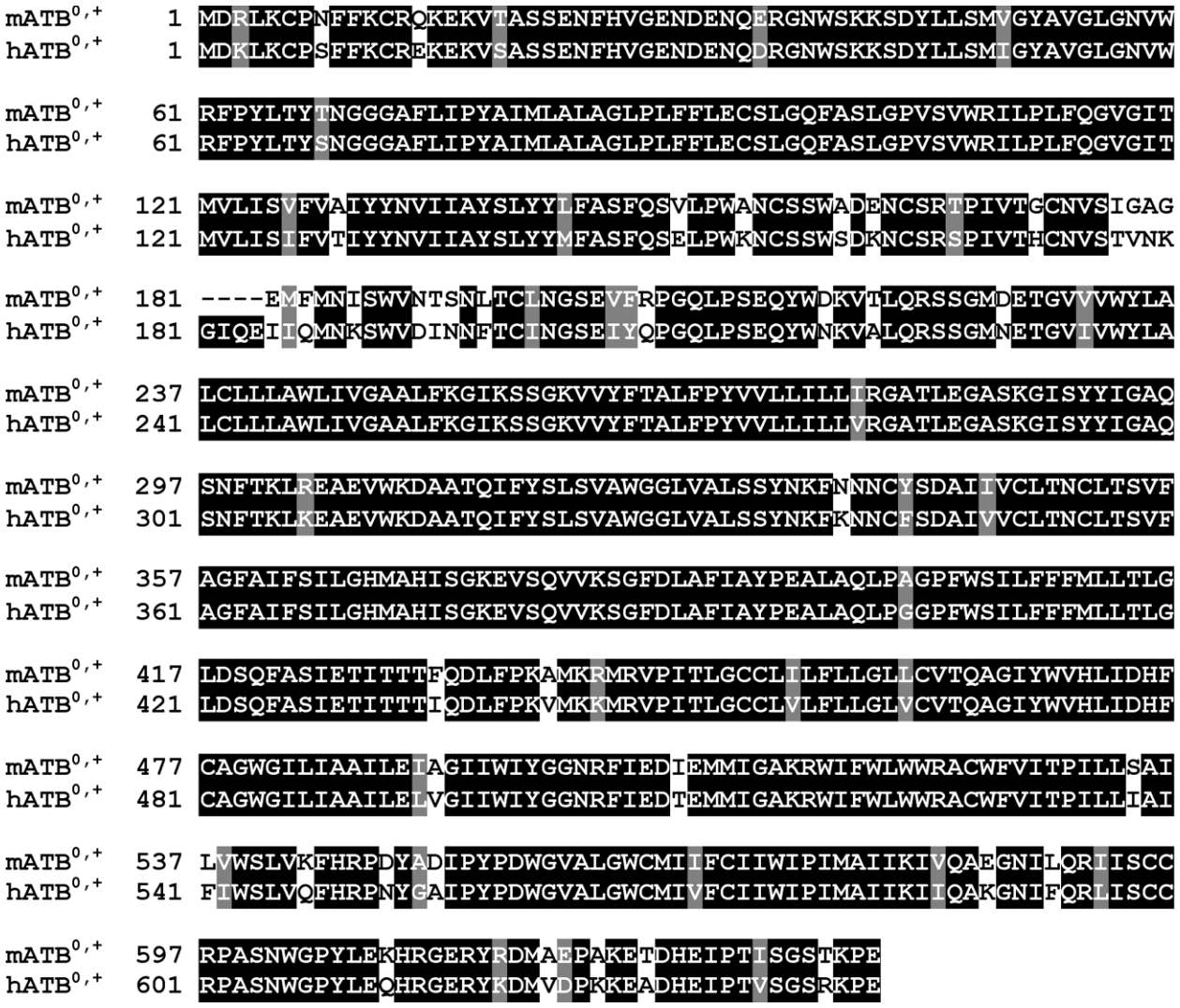


**Figure 1. Comparison of the amino acid sequence between mouse $ATB^{0,+}$ and human $ATB^{0,+}$**

Regions of sequence identity between the two species homologues are shaded.

that of $\gamma$-aminobutyrate transporters and betaine transporter in the $Na^+$- and $Cl^-$-coupled neurotransmitter transporter gene family, we investigated whether $ATB^{0,+}$ is capable of interacting with $\gamma$-aminobutyrate and betaine. In the same experiment, we also tested the ability of $ATB^{0,+}$ to interact with carnitine and its acetyl and propionyl esters. In these studies, the transport function of $ATB^{0,+}$ was monitored by measuring the transport of glycine in HRPE cells expressing the cloned mouse $ATB^{0,+}$. The expression of $ATB^{0,+}$ in these cells increased the transport of glycine by 30-fold (Fig. 2*A*). The interaction of the transporter with the test compounds was investigated by assessing their ability to inhibit $ATB^{0,+}$-mediated glycine transport (Fig. 2*B*). These studies produced interesting, but quite unexpected, results. $\gamma$-Aminobutyrate and betaine showed little or no effect on $ATB^{0,+}$-mediated glycine transport. In contrast, carnitine and propionylcarnitine inhibited $ATB^{0,+}$-mediated glycine transport markedly. The $IC_{50}$ values (concentration of the compound at which the inhibition was 50 %) for carnitine and propionylcarnitine were $0.6 \pm 0.1$ and $0.9 \pm 0.1$ mM, respectively. Acetylcarnitine was also able to inhibit $ATB^{0,+}$-mediated glycine transport, but surprisingly the inhibitory potency of this ester was much less than that of carnitine and its propionyl ester ($IC_{50}$ for acetylcarnitine was $15 \pm 3$ mM).

To determine whether or not carnitine and its esters are transportable substrates for $ATB^{0,+}$, we compared the transport of [$^3$H]carnitine and its esters between vector-transfected cells and $ATB^{0,+}$ cDNA-transfected cells (Fig. 2*C*). Expression of $ATB^{0,+}$ in HRPE cells induced the transport of carnitine (16-fold) and propionylcarnitine (6-fold) compared to transport in vector-transfected cells. The transport of acetylcarnitine was also increased by $ATB^{0,+}$ expression but to a much smaller extent (2-fold). These data show that $ATB^{0,+}$ recognizes carnitine, propionylcarnitine and acetylcarnitine as transportable substrates. Since $ATB^{0,+}$ is a member of the $Na^+$- and $Cl^-$-coupled transporter gene family, we investigated the influence of these two ions on the transport of carnitine mediated by $ATB^{0,+}$ (Fig. 2*D*). The transport was

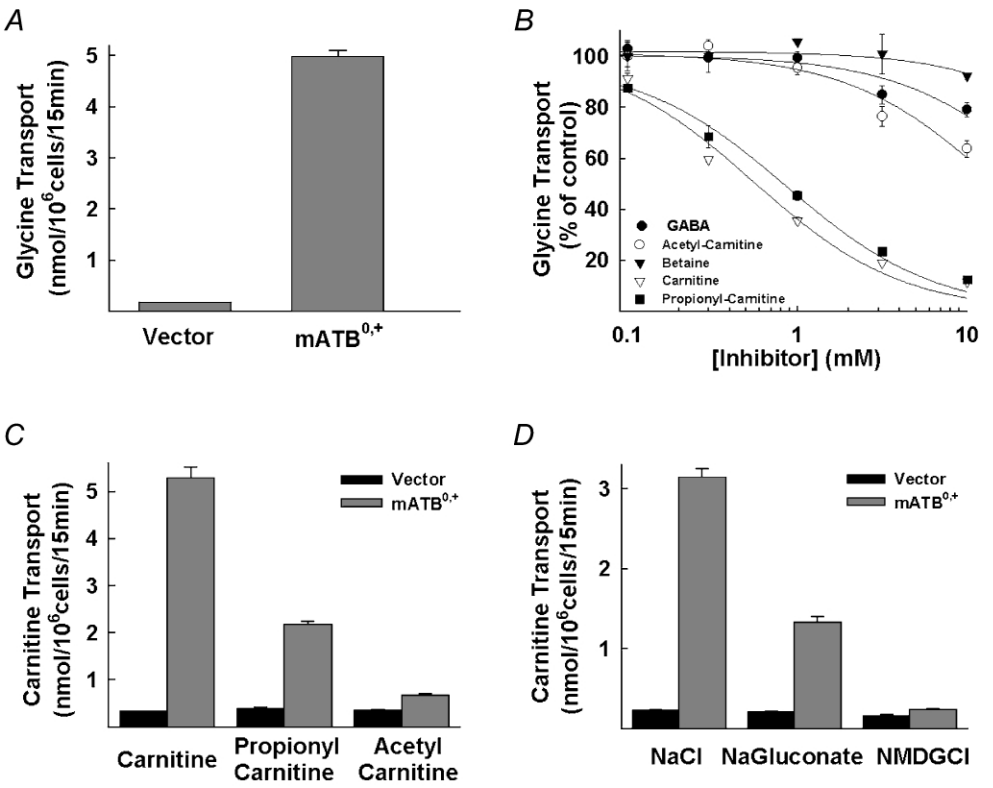


**Figure 2. Inhibition of mouse $ATB^{0,+}$-mediated glycine transport by carnitine and its acyl esters (*A* and *B*) and transport of carnitine and its acyl esters by mouse $ATB^{0,+}$ and the ion dependence of the process (*C* and *D*) in HRPE cells**

*A*, transport of glycine (10 $\mu$M) in vector-transfected cells and in cells transfected with mouse $ATB^{0,+}$ cDNA ($mATB^{0,+}$). *B*, inhibition of $ATB^{0,+}$-mediated glycine (10 $\mu$M) transport by $\gamma$-aminobutyrate (GABA), betaine, carnitine, acetylcarnitine and propionylcarnitine. Transport in the absence of inhibitors was taken as 100 %. *C*, transport of carnitine (25 $\mu$M), propionylcarnitine (25 $\mu$M) and acetylcarnitine (25 $\mu$M) in vector-transfected cells and in cells transfected with mouse $ATB^{0,+}$ cDNA. *D*, transport of carnitine (15 $\mu$M) in vector-transfected cells and in cells transfected with mouse $ATB^{0,+}$ cDNA in the presence of NaCl, in the presence of $Na^+$ but in the absence of $Cl^-$ (sodium gluconate), and in the presence of $Cl^-$ but in the absence of $Na^+$ (NMDG-Cl).

completely abolished when $Na^+$ in the uptake buffer was replaced with *N*-methyl-D-glucamine. Removal of $Cl^-$ from the uptake buffer by replacement with gluconate reduced the transport by ~60 %. These results show that $ATB^{0,+}$-mediated carnitine transport is coupled to both $Na^+$ and $Cl^-$. The removal of $Cl^-$ did not abolish the transport completely because of the possible release of $Cl^-$ from the cells during transport measurements. The transporter interacts with $Cl^-$ with high affinity and therefore the transporter is significantly active even at low concentrations of $Cl^-$ (see below).

Since $ATB^{0,+}$ transports several zwitterionic and cationic amino acids, we tested whether the $ATB^{0,+}$-mediated carnitine transport is inhibitable by the amino acid substrates of the transporter (Fig. 3*A*). All zwitterionic and cationic amino acids examined inhibited carnitine transport mediated by $ATB^{0,+}$. The anionic amino acid aspartate and the *N*-methylated amino acid α-(methylamino)isobutyric acid (MeAIB), which are not substrates for $ATB^{0,+}$, did not inhibit $ATB^{0,+}$-mediated carnitine transport.

The transport of carnitine mediated by $ATB^{0,+}$ was saturable (Fig. 3*B*). The values for the kinetic parameters $K_t$ and $V_{max}$ were $0.83 \pm 0.08$ mM and $72 \pm 2$ nmol $(10^6 \text{ cells})^{-1}$ $(15 \text{ min})^{-1}$. The relationship between $ATB^{0,+}$-mediated carnitine transport and $Na^+$ concentration was sigmoidal (Fig. 3*C*). The $K_{0.5}$ for $Na^+$ was $54 \pm 4$ mM and the Hill coefficient ($n_H$) was $1.6 \pm 0.1$. The $Cl^-$-activation kinetics were not investigated in this expression system because of the efflux of significant amounts of $Cl^-$ from the cells during the experiment.

To determine whether the ability to transport carnitine is also a characteristic of human $ATB^{0,+}$, we isolated a functional $ATB^{0,+}$ cDNA from a MCF-7 (a human mammary tumour cell line) cDNA library and examined its ability to transport carnitine in HRPE cells following heterologous expression. The amino acid sequence of $ATB^{0,+}$ cloned from the MCF-7 cell line was exactly the same as the recently published sequence of human $ATB^{0,+}$ (Sloan & Mager, 1999). The transport of carnitine was increased 4.5-fold in cells transfected with human $ATB^{0,+}$ cDNA compared to transport in cells transfected with vector alone. These data show that human $ATB^{0,+}$ is also capable of carnitine transport.

### Analysis of carnitine transport via mouse $ATB^{0,+}$ in the *X. laevis* oocyte expression system

Transport of amino acid substrates via mouse $ATB^{0,+}$ is electrogenic (Sloan & Mager, 1999). To investigate the electrogenic nature of $ATB^{0,+}$-mediated carnitine transport, we employed the *X. laevis* oocyte expression system. When oocytes expressing the mouse $ATB^{0,+}$ were perifused with carnitine, marked inward currents were detectable by the two-microelectrode voltage-clamp technique (~300 nA at 10 mM carnitine). Under similar conditions, propionylcarnitine induced ~200 nA currents. In contrast, acetylcarnitine was unable to induce any detectable currents. With carnitine and propionylcarnitine, the induced currents were absolutely dependent on the presence of $Na^+$ as well as $Cl^-$. Removal of either of the two ions abolished the currents completely.

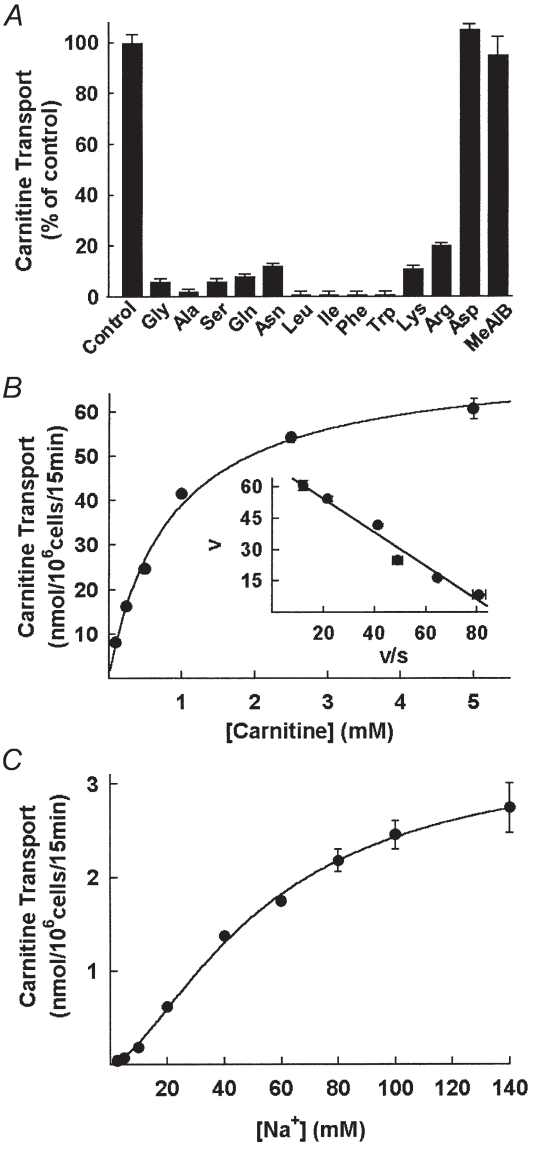


**Figure 3. Characteristics of mouse $ATB^{0,+}$-mediated carnitine transport in HRPE cells**

*A*, inhibition of $ATB^{0,+}$-mediated carnitine (10 μM) transport by zwitterionic and cationic amino acids (2.5 mM). Transport in the absence of inhibitors was taken as 100 %. *B*, transport of carnitine via mouse $ATB^{0,+}$ over a carnitine concentration range of 0.1–5 mM (inset, Eadie-Hofstee plot; *V*, carnitine transport in nmol $(10^6 \text{ cells})^{-1}$ $(15 \text{ min})^{-1}$; *S*, carnitine concentration in mM). *C*, transport of carnitine (15 μM) via mouse $ATB^{0,+}$ over a $Na^+$ concentration range of 2.5–140 mM. Concentration of $Cl^-$ was kept constant (140 mM) and concentration of $Na^+$ was varied by appropriately replacing NaCl with *N*-methyl-D-glucamine chloride.

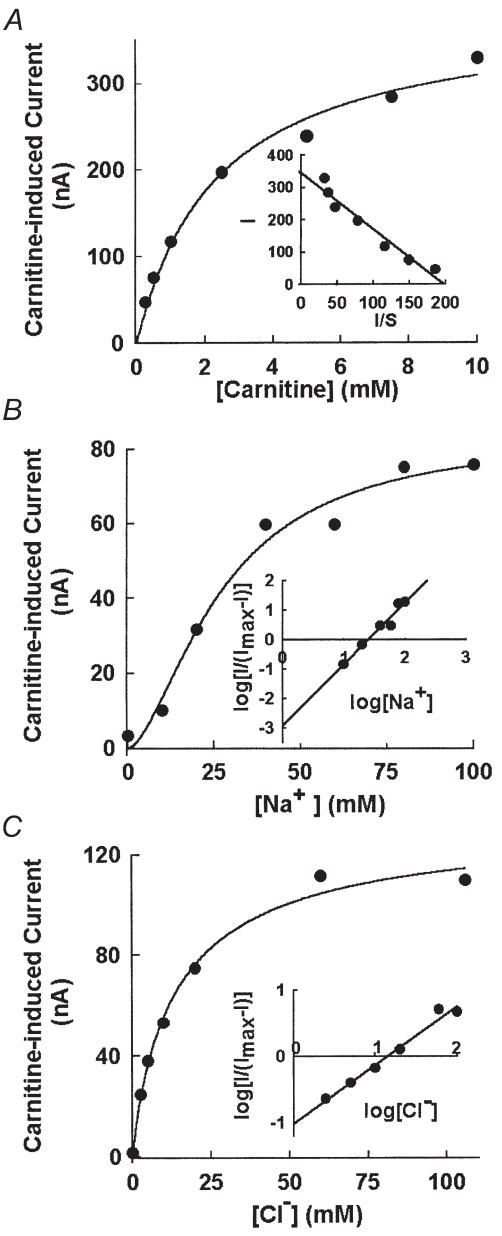


**Figure 4. Characteristics of carnitine transport mediated by mouse ATB$^{0,+}$ in *X. laevis* oocytes**

*A*, saturation kinetics for carnitine-induced current in oocytes expressing mouse ATB$^{0,+}$ (inset, Eadie-Hofstee plot; *I*, carnitine-induced current in nA; *S*, carnitine concentration in mM). *B*, $Na^+$ dependence of carnitine (1 mM)-induced current in oocytes expressing mouse ATB$^{0,+}$ (inset, Hill plot; *I*, carnitine-induced current; $I_{max}$, maximal current induced by 1 mM carnitine). Concentration of $Cl^-$ was kept constant (100 mM) and concentration of $Na^+$ was varied by appropriately replacing NaCl with *N*-methyl-D-glucamine chloride. *C*, $Cl^-$ dependence of carnitine (1 mM)-induced current in oocytes expressing mouse ATB$^{0,+}$ (inset, Hill plot; *I*, carnitine-induced current; $I_{max}$, maximal current induced by 1 mM carnitine). Concentration of $Na^+$ was kept constant (100 mM) and concentration of $Cl^-$ was varied by appropriately replacing NaCl with sodium gluconate.

We analysed the kinetic parameters of ATB$^{0,+}$-mediated carnitine transport using the carnitine-induced inward currents as a measure of the transporter function. The currents were saturable with increasing concentrations of carnitine (Fig. 4*A*). The $K_{0.5}$ for carnitine was $1.8 \pm 0.4$ mM. The $Na^+$-activation kinetics of carnitine-induced currents showed a sigmoidal relationship (Fig. 4*B*). The $K_{0.5}$ for $Na^+$ was $25 \pm 4$ mM and the Hill coefficient ($n_H$) was $1.9 \pm 0.5$. Since the removal of $Cl^-$ abolished completely the carnitine-induced currents, there was apparently no efflux of $Cl^-$ from the oocytes under the experimental conditions. Therefore, we used this expression system to analyse the $Cl^-$-activation kinetics. The relationship between carnitine-induced currents and $Cl^-$ concentration was hyperbolic (Fig. 4*C*). The $K_{0.5}$ for $Cl^-$ was $15 \pm 5$ mM and the Hill coefficient ($n_H$) was $0.9 \pm 0.2$. These data show that the $Na^+$:$Cl^-$:carnitine stoichiometry for the ATB$^{0,+}$-mediated transport process was 2:1:1.

## DISCUSSION

We have demonstrated in the present study that the amino acid transporter ATB$^{0,+}$ is able to mediate the transport of carnitine in a $Na^+$- and $Cl^-$-coupled manner. This property is demonstrable with mouse ATB$^{0,+}$ cloned from colon and human ATB$^{0,+}$ cloned from a mammary tumour cell line. The characteristics of carnitine transport via mouse ATB$^{0,+}$ were investigated using two different expression systems: a mammalian cell expression system and the *X. laevis* oocyte expression system. In the mammalian cell expression system, carnitine transport via ATB$^{0,+}$ was obligatorily dependent on $Na^+$. The transport process was also $Cl^-$ dependent even though removal of $Cl^-$ from the uptake buffer did not abolish carnitine transport completely. The persistence of significant carnitine transport activity in a $Cl^-$-free uptake medium is most likely to be due to $Cl^-$ ions that might be released from the cells into the extracellular medium during the 15 min period used in uptake measurements. This is supported by the findings that in the *X. laevis* oocyte expression system in which the transport-associated currents were measured over a very short time period, removal of $Cl^-$ from the perifusion buffer abolished the carnitine-induced currents almost completely. There are additional differences in the kinetic parameters of ATB$^{0,+}$-mediated carnitine transport between the two expression systems. Possible reasons for these differences might include the different time periods employed in the two expression systems to measure carnitine transport and also the fact that the transport was measured in HRPE cells without clamping the membrane potential whereas transport in *X. laevis* oocytes was measured under voltage-clamped conditions.

To date, OCTN2 is the only transporter that has been shown to transport carnitine in an ion gradient-coupled manner (Wu *et al.* 1998, 1999; Tamai *et al.* 1998). The

transport function of OCTN2 is dependent on the presence of $Na^+$. $Cl^-$ does not have any role in the function of this transporter. The transporter is, however, likely to be electrogenic due to the zwitterionic nature of carnitine and the coupling of the transport process with $Na^+$ cotransport. OCTN2-mediated transport of carnitine is therefore energized by the transmembrane $Na^+$ gradient and membrane potential. The present studies describe the identification of a second ion gradient-coupled transporter for carnitine. $ATB^{0,+}$ transports carnitine in a $Na^+$- and $Cl^-$-coupled manner. The transport process is electrogenic. Thus, the transport of carnitine via $ATB^{0,+}$ is energized by transmembrane gradients of $Na^+$ and $Cl^-$ as well as membrane potential. The concentrative capacity of $ATB^{0,+}$ for carnitine is much greater than that of OCTN2. However, $ATB^{0,+}$ is a low-affinity transporter for carnitine ($K_t = 1–2$ mM). In contrast, OCTN2 is a high-affinity transporter for carnitine ($K_t = 5–15$ $\mu$M) (Tamai *et al.* 1998; Wu *et al.* 1999). The concentrations of carnitine in blood are in the range of 30–50 $\mu$M and therefore OCTN2 is more important that $ATB^{0,+}$ for cellular uptake of carnitine in most tissues under physiological conditions. Interestingly, the tissue distribution of the two transporters is quite different. OCTN2 is expressed in most tissues whereas $ATB^{0,+}$ is expressed primarily in the mammary gland, lung and intestinal tract (Sloan & Mager, 1999). $ATB^{0,+}$ is likely to play a significant role in tissues in which it is expressed. Recent studies with JVS mice, which have a genetic defect in OCTN2 transport function, have shown that the intestinal absorption of carnitine is reduced by only about 50 % due to the defect (Yokogawa *et al.* 1999). The finding that defects in OCTN2 function do not eliminate intestinal absorption completely suggests that some additional, hitherto unidentified, transporters participate in the intestinal absorption of carnitine. Since functional studies have shown that system $B^{0,+}$ is expressed in the brush border membrane of the absorptive cells of the intestinal tract, it is possible that $ATB^{0,+}$, along with OCTN2, participates in the intestinal absorption of carnitine. Furthermore, $ATB^{0,+}$ is expressed not only in the small intestine but also in the colon. The present studies were done with $ATB^{0,+}$ cloned from mouse colon. Microbial flora utilize carnitine as a carbon source (Rebouche & Seim, 1998). Therefore, we speculate that $ATB^{0,+}$ in the colon may play a role in the absorption of carnitine and thus compete with colonic bacteria for carnitine in the lumen. In addition, the intestinal transport of carnitine via $ATB^{0,+}$ is likely to be very relevant to carnitine homeostasis in patients with genetic defects in OCTN2.

OCTN2 and $ATB^{0,+}$ differ not only in their affinity and driving forces but also in their substrate specificity. OCTN2 transports carnitine, acetylcarnitine and propionylcarnitine with comparable affinity (Wu *et al.* 1999). In contrast, $ATB^{0,+}$ transports only carnitine and propionylcarnitine. The transporter shows very low affinity for acetylcarnitine. Acetylcarnitine is the predominant acylcarnitine ester inside the cell as well as in the circulation. It is a key intermediate in anabolic and catabolic pathways of metabolism. The differential affinity of OCTN2 and $ATB^{0,+}$ for acetylcarnitine may have physiological implications. OCTN2 belongs to the organic cation transporter gene family, the members of which mediate the transport of structurally diverse xenobiotics (Burckhardt & Wolff, 2000). In contrast, $ATB^{0,+}$ belongs to the neurotransmitter transporter gene family, the members of which mediate $Na^+$- and $Cl^-$-coupled transport of several cationic and zwitterionic organic solutes including monoamines, amino acids and osmolytes. The structural differences between OCTN2 and $ATB^{0,+}$ must underlie the differential substrate specificities of these two transporters.


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