Transport of Amino Acid Esters and the Amino-Acid–Based Prodrug Valganciclovir by the Amino Acid Transporter $ATB^{0,+}$

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Purpose. The purpose of this study was to analyze the transport of amino acid esters and the amino-acid–based prodrug valganciclovir by the Na^+/Cl^--coupled amino acid transporter $ATB^{0,+}$.

Methods. The interaction of amino acid esters and valganciclovir with the cloned rat $ATB^{0,+}$ was evaluated in a mammalian cell expression system and in the *Xenopus* oocyte expression system.

Results. In mammalian cells, expression of $ATB^{0,+}$ induced glycine uptake. This uptake was inhibited by valine and its methyl, butyl, and benzyl esters. The benzyl esters of other neutral amino acids were also effective inhibitors. Valganciclovir, the valyl ester of ganciclovir, was also found to inhibit $ATB^{0,+}$-mediated glycine uptake competitively. Exposure of $ATB^{0,+}$-expressing oocytes to glycine induced inward currents. Exposure to different valyl esters (methyl, butyl, and benzyl), benzyl esters of various neutral amino acids, and valganciclovir also induced inward currents in these oocytes. The current induced by valganciclovir was saturable with a $K_{0.5}$ value of 3.1 ± 0.7 mM and was obligatorily dependent on Na^+ and Cl^-. The Na^+:Cl^-:valganciclovir stoichiometry was 2 or 3:1:1.

Conclusions. Amino acid esters and the amino-acid–based prodrug valganciclovir are transported by $ATB^{0,+}$. This shows that $ATB^{0,+}$ can serve as an effective delivery system for amino acid–based prodrugs.

KEY WORDS: amino acid esters; amino acid transporter $ATB^{0,+}$; drug delivery; valganciclovir.

INTRODUCTION

$ATB^{0,+}$ is an amino acid transporter with a broad substrate specificity (1,2). It interacts with neutral amino acids as well as cationic amino acids. In addition, it also recognizes several D-amino acids as substrates (3). A unique feature of this transporter is that it is energized by a Na^+ gradient and a Cl^- gradient (3–6). Thus, $ATB^{0,+}$ is the only transporter in mammalian cells that has the ability to accumulate the cationic amino acid arginine in a Na^+/Cl^--coupled manner. This has relevance to the cellular generation of nitric oxide under various physiological and pathological conditions. Recently, we have shown that $ATB^{0,+}$ is capable of transporting a variety of amino-acid–based inhibitors of nitric oxide synthases (6), indicating that the transporter may have potential as a drug target for suppression of nitric oxide production under pathological conditions.

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$ATB^{0,+}$ is expressed primarily in the colon, lung, and eye, the tissues easily amenable for drug delivery (7). This prompted us recently to evaluate the utility of this transporter as a potential drug delivery system (7). Because this transporter recognizes glutamine and asparagine as substrates but excludes glutamate and aspartate, we hypothesized that if the side chains of the anionic amino acids can be used to couple drugs in the form of esters or amides, such derivatives can be recognized as substrates by $ATB^{0,+}$. We tested this hypothesis by synthesizing glutamate γ-ester of acyclovir and examining its transport via $ATB^{0,+}$. These studies showed that whereas acyclovir does not interact with the transporter, glutamate γ-ester of acyclovir is recognized as a substrate (7), thus validating our hypothesis. We then tested valacyclovir, which is an α-ester of valine, as a substrate for $ATB^{0,+}$, expecting this compound not to be transported by the transporter. We thought that because $ATB^{0,+}$ is an amino acid transporter, the presence of a free α-carboxyl group and a free α-amino group may be obligatory for recognition as a substrate by the transporter. But, these studies produced surprising results (7). Valacyclovir was found to be a transportable substrate. Furthermore, the ability of $ATB^{0,+}$ to transport valacyclovir was comparable to that of the peptide transporter PEPT1. This suggested that therapeutically active drugs can be coupled to the α-carboxyl groups of the amino acid substrates of $ATB^{0,+}$ as a means of facilitating their delivery into cells. In the current study, we evaluated in a greater detail the potential of this approach as a drug delivery mechanism by examining the interaction of $ATB^{0,+}$ with a variety of α-carboxyl derivatives of neutral amino acids. In addition, we investigated the ability of $ATB^{0,+}$ to transport the prodrug valganciclovir, an α-carboxyl ester of the neutral amino acid valine with the antiviral drug ganciclovir. These studies show that $ATB^{0,+}$ is able to transport a variety of α-carboxyl esters of neutral amino acids, thus enhancing the potential utility of this transporter as a delivery system for amino-acid–based drugs and prodrugs.

MATERIALS AND METHODS

Materials

[2-^{3}H]Glycine (sp. radioactivity, 30 Ci/mmol) was purchased from NEN Life Science Products (Boston, MA, USA). All amino acids and amino acid derivatives were from Sigma Chemical Co. (St. Louis, MO, USA). Ganciclovir and valganciclovir were provided by Roche Diagnostics GmbH (Mannheim, Germany).

Functional Expression of $ATB^{0,+}$ in Human Retinal Pigment Epithelial Cells

The rat $ATB^{0,+}$ cDNA was cloned from a lung cDNA library. This clone was used for heterologous expression of the transporter in a human retinal pigment epithelial (HRPE) cell line. The vaccinia virus expression system was used for this purpose (3,5–7). This procedure involves infection of the cells with a recombinant vaccinia virus carrying the gene for T7 RNA polymerase, followed by lipofectin-mediated transfection of the cells with plasmid DNA in which the cDNA insert is under the control of T7 promoter. Glycine was used as the substrate for $ATB^{0,+}$ (5–7). Transport of 10 μM glycine

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(radiolabeled glycine, 0.05 μM; unlabeled glycine, 9.95 μM) in cDNA-transfected cells was measured at 37°C for 30 min. In some experiments, the concentration of glycine was 5 μM (radiolabeled glycine, 0.05 μM; unlabeled glycine, 4.95 μM). The transport was linear under these conditions. 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. After incubation for 30 min, transport was terminated by aspiration of the transport buffer followed by two washes with 2 ml of ice-cold transport buffer. The cells were lysed with 0.5 ml of 1% sodium dodecylsulfate in 0.2 M NaOH and the lysate was used for determination of radioactivity. Interaction of various amino acid derivatives, ganciclovir, and valganciclovir with the transporter was assessed by monitoring the ability of these compounds to inhibit $ATB^{0,+}$-mediated glycine transport. The nature of inhibition was analyzed by monitoring the saturation kinetics of $ATB^{0,+}$-mediated glycine transport in the absence and presence of inhibitors.

Functional Expression of $ATB^{0,+}$ in *Xenopus laevis* Oocytes

Capped cRNA from the cloned rat $ATB^{0,+}$ cDNA was synthesized using the mMESSAGE mMACHINE kit (Ambion Inc., Austin, TX, USA). Mature oocytes (stage IV or V) from *Xenopus laevis* were injected with 50 ng cRNA. Uninjected oocytes served as controls. The oocytes were used for electrophysiological studies 6 days after cRNA injection. Electrophysiological studies were performed by the two-microelectrode voltage-clamp method (5–7). Oocytes were perfused with a NaCl-containing buffer (100 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 3 mM Hepes, 3 mM Mes, and 3 mM Tris, pH 7.5), followed by the same buffer containing different amino acid derivatives. The membrane potential was clamped at –50 mV. The differences between the steady-state currents measured in the presence and absence of substrates were considered as the substrate-induced currents. In the analysis of the saturation kinetics of substrate-induced currents, the kinetic parameter $K_{0.5}$ (i.e., the substrate concentration necessary for the induction of half-maximal current) was calculated by fitting the values of the substrate-induced currents to Michaelis-Menten equation. The Na^+ and Cl^- activation kinetics of substrate-induced currents were analyzed by measuring the substrate-specific currents in the presence of increasing concentrations of Na^+ (the concentration of Cl^- kept constant at 100 mM) or in the presence of increasing concentrations of Cl^- (the concentration of Na^+ kept constant at 100 mM). In these experiments, the composition of the perfusion buffer was modified to contain 2 mM potassium gluconate, 1 mM $MgSO_4$, and 1 mM calcium gluconate in place of KCl, $MgCl_2$, and $CaCl_2$, respectively. The data from these experiments were analyzed by the Hill equation to determine the $K_{0.5}$ values for Na^+ and Cl^- (i.e., the concentrations of Na^+ and Cl^- necessary for half-maximal activation) and the Hill coefficient (h; the number of Na^+ and Cl^- ions involved in the activation process). The kinetic parameters were determined using the commercially available computer program Sigma Plot, version 6.0 (SPSS, Inc., Chicago, IL, USA).

Uninjected oocytes were used to determine endogenous currents induced by neutral amino acids and their α-benzyl esters as well as by ganciclovir and valganciclovir. At a concentration of 2.5 mM, the currents induced by these compounds were in the range 0–5 nA. The endogenous currents elicited by these compounds were less than 3% of the corresponding currents induced in $ATB^{0,+}$ cRNA-injected oocytes. Therefore, the endogenous currents were not considered in the analysis of kinetic parameters of $ATB^{0,+}$ transport activity.

Data Analysis

Experiments with HRPE cells were repeated three times with three independent transfections, and transport measurements were made in duplicate in each experiment. Electrophysiological measurements of substrate-induced currents were repeated at least three times with separate oocytes. The data are presented as means ± SE of these replicates. Statistical analysis was done by Student's *t* test, and a $p < 0.05$ was considered significant. Data for saturation kinetics were analyzed according to the Michaelis-Menten equation

$$y = a \times x/(b + x)$$

where y is uptake rate or substrate-induced current, a is the maximal uptake velocity (V_{max}) or maximal substrate-induced current (I_{max}), b is the Michaelis-Menten constant (K_t in uptake measurement studies or $K_{0.5}$ in current measurement studies), and x is the substrate concentration. Data for Na^+ or Cl^- activation kinetics were analyzed according to the Hill equation

$$I = I_{max} \times x^h/(K_{0.5}{}^h + x^h)$$

where I is the substrate-induced current, I_{max} is the current induced by maximal concentrations of activating ions (Na^+ or Cl^-), x is the activating ion concentration, $K_{0.5}$ is the concentration of activating ion needed for the induction of half-maximal current, and h is the Hill coefficient. The IC_{50} (i. e., the concentration of inhibitor needed to cause 50% inhibition of glycine uptake) values were calculated by fitting the data from the dose-response experiments to the equation

$$y = y^0 + a \times b/(b + x)$$

where y is the uptake in the presence of the competitor, y^0 is the uptake in the presence of maximal concentration of the competitor, a is the uptake in the absence of the competitor, b is the concentration of the competitor needed for 50% inhibition (IC_{50}), and x is the concentration of the competitor.

RESULTS

Interaction of Amino Acid Esters and Valganciclovir with $ATB^{0,+}$ in the Mammalian Cell Expression System

Valine is a neutral amino acid that is an excellent substrate for $ATB^{0,+}$. Therefore, we first investigated the interaction of a variety of α-carboxyl derivatives of this amino acid with the cloned rat $ATB^{0,+}$. This was done by assessing the influence of these derivatives on the transport of glycine mediated by $ATB^{0,+}$ in HRPE cells after heterologous expression of the cloned transporter. As expected, L-valine at a concentration of 2.5 mM inhibited $ATB^{0,+}$-mediated glycine transport almost completely (~95%) (Table I). When the

Table I. Inhibition of $ATB^{0,+}$-Mediated Glycine Uptake by L-Valine and Its Derivatives

L-Valine derivatives	$ATB^{0,+}$-mediated glycine uptake (pmol/10^6 cells per 30 min)	%
Control	2.66 ± 0.22	100
L-Valine	0.13 ± 0.02	5
L-Valinol	2.64 ± 0.37	100
L-Valinamide	2.13 ± 0.32	81
L-Val-methyl ester	1.51 ± 0.10	57
L-Val-butyl ester	1.55 ± 0.09	59
L-Val-benzyl ester	0.81 ± 0.08	19

Uptake of glycine (10 μM) was measured in HRPE cells transfected with rat $ATB^{0,+}$ cDNA. Concentration of L-valine and its derivatives was 2.5 mM.

α-carboxyl group of L-valine is replaced by a hydroxymethyl group (valinol), the resultant derivative did not interfere with $ATB^{0,+}$-mediated glycine transport. Similarly, when the α-carboxyl group of L-valine is amidated, the inhibitory potency was markedly reduced. However, three different esters of L-valine at its α-carboxyl group (L-Val-methyl ester, L-Val-butyl ester, and L-Val-benzyl ester) inhibited $ATB^{0,+}$-mediated glycine transport to a marked extent. The benzyl ester was the most effective, with 80% inhibition at a concentration of 2.5 mM. We then compared the inhibition of $ATB^{0,+}$-mediated glycine transport by six different neutral amino acids (glycine, L-alanine, L-serine, L-valine, L-leucine, and L-phenylalanine) and their respective benzyl esters at the α-carboxyl group (Table II). In each case, the benzyl ester was a very effective inhibitor. Studies of dose-response relationship for the inhibition of $ATB^{0,+}$-mediated glycine transport by L-valine and L-Val-benzyl ester showed that the IC_{50} values (i.e., concentration of the inhibitor necessary for 50% inhibition) for L-valine and L-Val-benzyl ester were 53 ± 5 μM and 1.0 ± 0.1 mM, respectively (Fig. 1A). Kinetic analysis revealed that the inhibition caused by the L-Val-benzyl ester was competitive (Fig. 1B). In the absence of the inhibitor, the Michaelis-Menten constant (K_t) and the maximal velocity (V_{max}) for $ATB^{0,+}$-mediated glycine transport were 201 ± 36 μM and 106 ± 9 nmol/10^6 cells/30 min. In the presence of 2 mM L-Val-benzyl ester, the corresponding values were 729 ± 242 μM and 95 ± 18 nmol/10^6 cells/30 min. Thus, L-Val-benzyl ester inhibited $ATB^{0,+}$-mediated glycine transport primarily by reducing the substrate affinity with no statistically significant effect on the maximal velocity.

We then examined the influence of the L-valyl ester of ganciclovir (valganciclovir) on $ATB^{0,+}$-mediated glycine transport. The chemical structure of valganciclovir is shown (Fig. 2A). Valganciclovir was found to be an effective inhibitor of glycine transport via $ATB^{0,+}$, the inhibition being ~60% at 2.5 mM (Fig. 2B). Under similar conditions, ganciclovir did not inhibit glycine transport. As in the case of L-Val-benzyl ester, the inhibition caused by valganciclovir was also competitive (Fig. 2C). In the presence of 3 mM valganciclovir, the K_t and V_{max} values for $ATB^{0,+}$-mediated glycine transport were 683 ± 26 μM and 108 ± 2 nmol/10^6 cells/30 min. The corresponding values in the absence of valganciclovir were 217 ± 27 μM and 110 ± 6 nmol/10^6 cells/30 min. Thus, valganciclovir decreased the substrate affinity for the transporter with no statistically significant effect on the maximal velocity.

Table II. Transport of Neutral Amino Acids and Their Corresponding α-Benzylesters via Rat $ATB^{0,+}$ in a Mammalian Cell Expression System and in *Xenopus* Oocyte Expression System

Amino acids and their α-benzylesters	$ATB^{0,+}$-mediated [^{3}H]glycine uptake in HRPE cells (pmol/10^6 cells per 30 min)	$ATB^{0,+}$-mediated currents in oocytes (nA)
Control	1.12 ± 0.06 (100)	
Glycine	0.10 ± 0.01 (9)	357 ± 137 (100)
Gly-benzylester	0.22 ± 0.01 (20)	203 ± 109 (57)
L-Alanine	0.06 ± 0.01 (5)	330 ± 107 (100)
L-Ala-benzylester	0.12 ± 0.01 (11)	288 ± 131 (87)
L-Serine	0.36 ± 0.02 (33)	382 ± 148 (100)
L-Ser-benzylester	0.05 ± 0.01 (4)	245 ± 72 (64)
L-valine	0.06 ± 0.01 (6)	268 ± 82 (100)
L-Val-benzylester	0.20 ± 0.01 (19)	155 ± 58 (58)
L-Leucine	0.04 ± 0.01 (3)	195 ± 83 (100)
L-Leu-benzylester	0.01 ± 0.01 (1)	249 ± 75 (128)
L-Phenylalanine	0.06 ± 0.01 (6)	211 ± 115 (100)
L-Phe-benzylester	0.08 ± 0.01 (7)	164 ± 72 (78)

In the mammalian cell expression system, rat $ATB^{0,+}$ was expressed heterologously in HRPE cells by the vaccinia virus technique and the transporter activity was measured as [^{3}H]glycine (5 μM) uptake. Interaction of neutral amino acids and their α-benzylesters (2.5 mM) with the transporter was monitored by their ability to compete with [^{3}H]glycine for the uptake process. Values in parentheses represent [^{3}H]glycine uptake in the presence of competitors as percent of control uptake measured in the absence of competitors. In the *Xenopus* oocyte expression system, rat $ATB^{0,+}$ was expressed by injection of rat $ATB^{0,+}$ cRNA. Currents induced by neutral amino acids and their α-benzylesters (2.5 mM) were measured in three different oocytes. Values in parentheses represent currents induced by benzylesters as percent of currents induced by their corresponding parent amino acids.

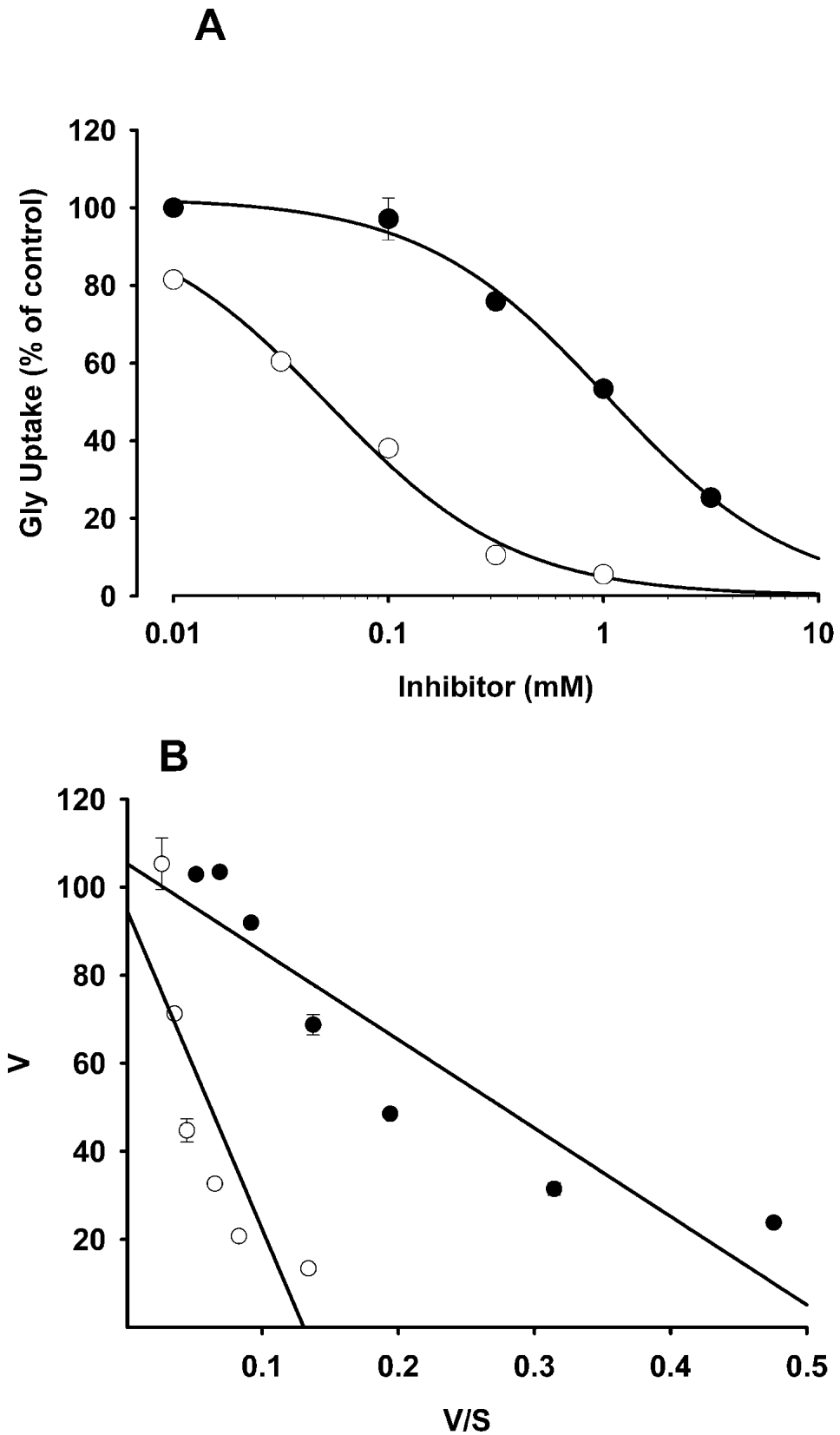

Fig. 1. Kinetic analysis of the interaction of L-valine and L-Val-benzyl ester with $ATB^{0,+}$. Rat $ATB^{0,+}$ was expressed heterologously in HRPE cells and its transport function was monitored by the uptake of glycine. (A) Dose-response relationship for the inhibition of $ATB^{0,+}$-mediated uptake of glycine (10 μM) by L-valine (○) and L-Val-benzyl ester (●). (B) Saturation kinetics for $ATB^{0,+}$-mediated glycine uptake in the absence (●) and presence (○) of 2 mM L-Val-benzyl ester. Data are given as Eadie-Hofstee plots (V, glycine uptake in nmol/10^6 cells/30 min; S, glycine concentration in mM).

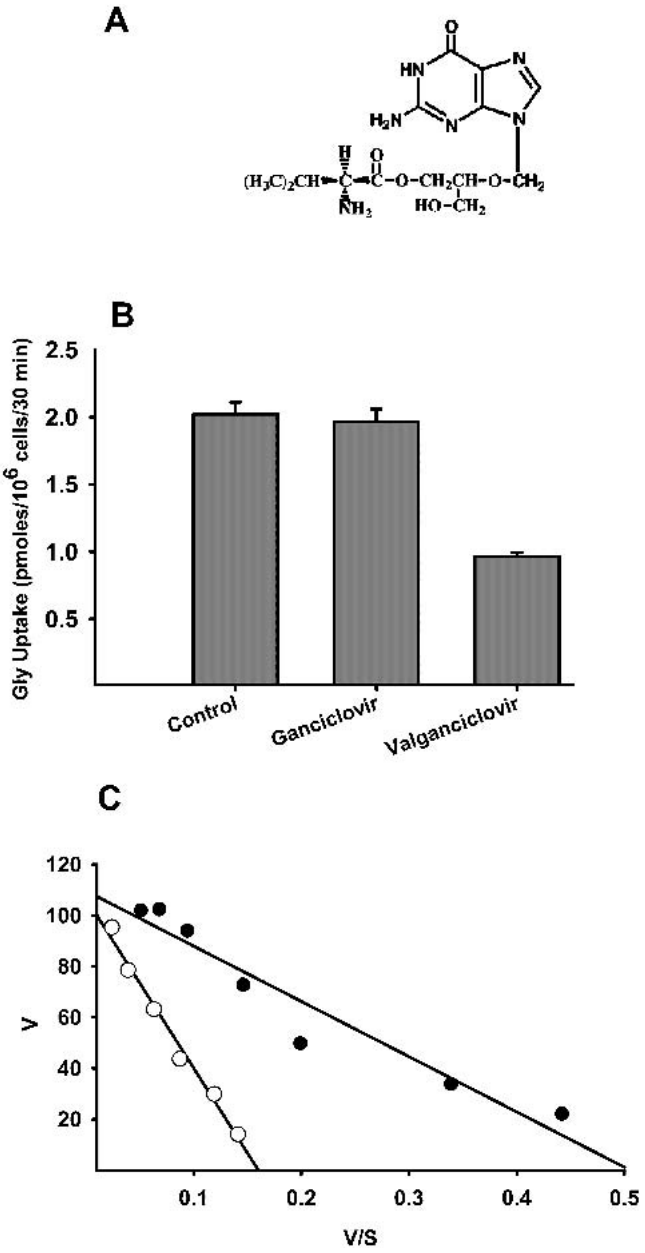

Fig. 2. Interaction of ganciclovir and valganciclovir with ATB$^{0,+}$. (A) Chemical structure of valganciclovir. (B & C) Rat ATB$^{0,+}$ was expressed heterologously in HRPE cells and its transport function was monitored by the uptake of glycine. (B) Influence of ganciclovir (2.5 mM) and valganciclovir (2.5 mM) on ATB$^{0,+}$-mediated uptake of glycine (10 μM). (C) Saturation kinetics for ATB$^{0,+}$-mediated glycine uptake in the absence (●) and presence (○) of 3 mM valganciclovir. Data are given as Eadie-Hofstee plots (V, glycine uptake in nmol/10^6 cells/30 min; S, glycine concentration in mM).

Interaction of Amino Acid Esters and Valganciclovir with ATB$^{0,+}$ in *Xenopus laevis* Oocyte Expression System

The studies described above with the mammalian cell expression system clearly show that α-carboxyl esters of neutral amino acids, including valganciclovir, compete effectively with glycine for transport via ATB$^{0,+}$. But, these studies do not prove that the amino acid derivatives are actually transported into cells via the transporter. It is possible that these amino acid derivatives compete with glycine for binding to the substrate-binding site without themselves being actually translocated across the membrane. Therefore, it was necessary to show whether or not the α-carboxyl esters of neutral amino acids are transportable substrates for ATB$^{0,+}$. For this purpose, we used the *Xenopus* oocyte expression system in which the cloned rat ATB$^{0,+}$ was expressed heterologously by injection of corresponding cRNA and translocation of substrates via the transporter was monitored by inward currents when the oocytes were exposed to putative substrates under voltage-clamp conditions. Exposure of ATB$^{0,+}$-expressing oocytes to various neutral amino acids induced marked inward currents. Such currents were not detected with uninjected oocytes (data not shown). Similarly, amino acid-induced currents in ATB$^{0,+}$-expressing oocytes were not detected when the perfusion medium lacked either Na^+ or Cl^-, indicating the obligatory requirement of these ions for ATB$^{0,+}$-mediated transport process (data not shown). We then tested various derivatives of L-valine to see if they induce inward currents in ATB$^{0,+}$-expressing oocytes. Valinol and valinamide did not induce detectable currents (Fig. 3), showing that these two derivatives are not transportable substrates for ATB$^{0,+}$. These data corroborated the findings from the mammalian cell expression studies in which valinol and valinamide failed to inhibit ATB$^{0,+}$-mediated glycine transport. In contrast, the methyl, butyl, and benzyl esters of L-valine induced appreciable inward currents, even though the magnitude of currents induced by these esters was significantly less than that induced by L-valine. We then monitored the ability of six different neutral amino acids and their corresponding α-benzyl esters to induce currents in ATB$^{0,+}$-expressing oocytes (Table II). At a concentration of 2.5 mM, L-alanine, L-serine, and glycine induced maximal currents. L-Valine, L-phenylalanine, and L-leucine also induced marked currents, though the magnitude of the currents induced was less than that induced by L-alanine, L-serine, or glycine. In the case of corresponding benzyl esters, L-Ala-benzyl ester induced the maximal current followed by L-Ser-benzyl ester. The benzyl

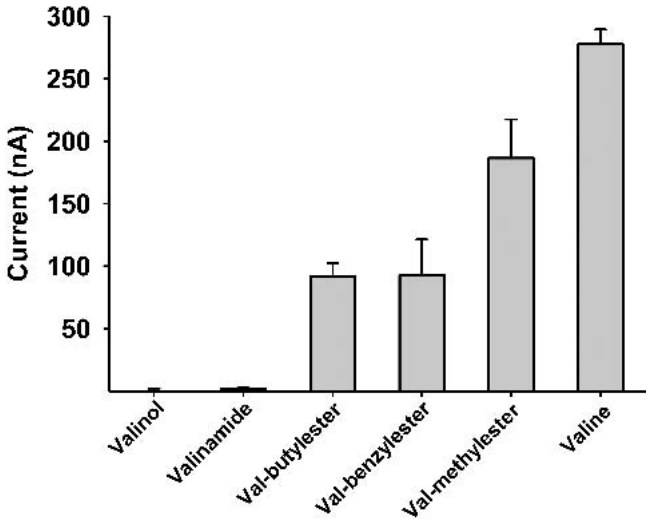

Fig. 3. Transport of L-valine derivatives via rat ATB$^{0,+}$ as assessed in *Xenopus* oocytes by substrate-induced currents. Rat ATB$^{0,+}$ was expressed in *Xenopus* oocytes heterologously and the currents induced by L-valine and its derivatives (2.5 mM) in these oocytes were measured.

esters of L-valine, L-phenylalanine, L-leucine, and glycine also induced marked currents, but the magnitude of the currents was 50–60% of the currents induced by L-Ala-benzyl ester. When the magnitudes of the currents induced by each of the amino acids and the corresponding benzyl esters were compared, an interesting pattern emerged. L-Leu-benzyl ester induced more currents than the parent amino acid. With L-alanine and L-phenylalanine, the benzyl esters induced currents that were about 80% compared to the currents induced by the parent amino acids. With L-serine, L-valine, and glycine, the benzyl esters induced currents that were about 60% compared to the currents induced by the corresponding parent amino acids.

We then compared the abilities of ganciclovir and valganciclovir to induce currents in $ATB^{0,+}$-expressing oocytes. Ganciclovir failed to induce detectable inward currents whereas valganciclovir induced marked currents under identical conditions (data not shown). There were no detectable currents with valganciclovir in uninjected oocytes. The currents induced by valganciclovir in $ATB^{0,+}$-expressing oocytes were obligatorily dependent on the presence of Na^+ and Cl^- as was expected for $ATB^{0,+}$-mediated transport (data not shown). Because these preliminary studies provided evidence for the transport of valganciclovir via $ATB^{0,+}$, we analyzed the kinetics of this transport process in a greater detail. The currents induced by valganciclovir in $ATB^{0,+}$-expressing oocytes were saturable with increasing concentrations of valganciclovir (Fig. 4). The value for $K_{0.5}$ (i.e, concentration of the substrate necessary for induction of half-maximal current) was 3.1 ± 0.6 mM. Analysis of the activation of valganciclovir (2.5 mM)-induced currents by increasing concentrations of Na^+ showed a sigmoidal relationship, indicating involvement of multiple Na^+ ions in the activation process (Fig. 5A). The Hill coefficient (h) for Na^+, which is an estimate of the number of Na^+ ions involved in the activation process, was 2.4 ± 0.2. The value for $K_{0.5}$ for Na^+ (i.e., concentration of Na^+ needed for half-maximal activation) was 17 ± 1 mM. We carried out similar studies for the activation of valganciclovir (2.5 mM)-induced currents by Cl^- (Fig. 5B). In contrast to the kinetics of Na^+ activation, the relationship between valganciclovir-induced currents and Cl^- concentration was hyperbolic, indicating involvement of a single Cl^- ion in the activation process. Accordingly, the Hill coefficient for Cl^- was 0.71 ± 0.03. The value for $K_{0.5}$ for Cl^- (i.e., concentration of Cl^- needed for half-maximal activation) was 9.9 ± 1.7 mM. Thus, the Na^+:Cl^-:valganciclovir stoichiometry for the transport of valganciclovir via $ATB^{0,+}$ appears to be 2 or 3:1:1.

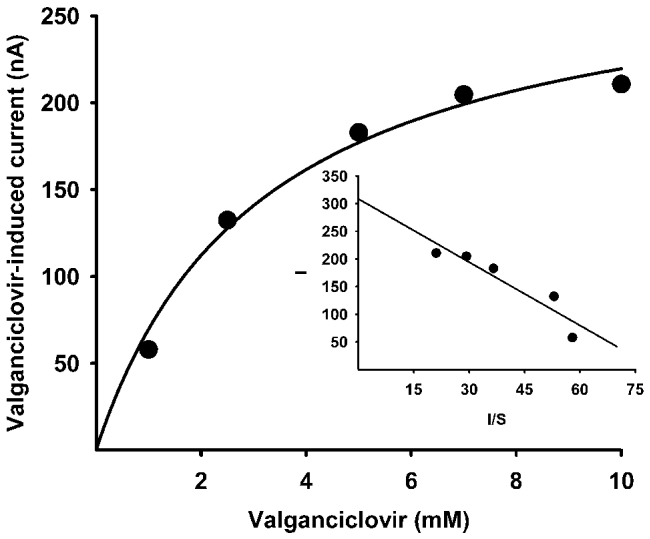

Fig. 4. Saturation kinetics of valganciclovir transport via rat $ATB^{0,+}$ in *Xenopus* oocytes. Rat $ATB^{0,+}$ was expressed in *Xenopus* oocytes heterologously and the currents induced by increasing concentrations of valganciclovir were measured. Inset: Eadie-Hofstee plot (I, current induced by valganciclovir; S, concentration of valganciclovir).

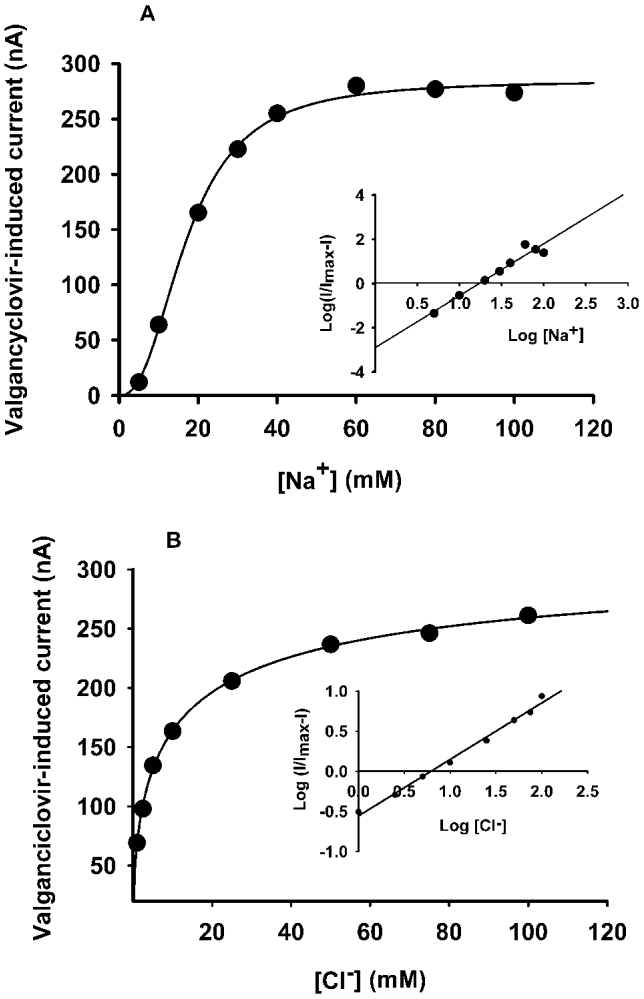

Fig. 5. Na^+- and Cl^- activation kinetics of valganciclovir-induced currents in *Xenopus* oocytes expressing rat $ATB^{0,+}$. Rat $ATB^{0,+}$ was expressed in *Xenopus* oocytes heterologously. (A) For Na^+-activation kinetics, the currents induced by 2.5 mM valganciclovir were measured in the presence of increasing concentrations of Na^+ with a fixed concentration of Cl^- (100 mM). Inset: Hill plot. (B) For Cl^--activation kinetics, the currents induced by 2.5 mM valganciclovir were measured in the presence of increasing concentrations of Cl^- with a fixed concentration of Na^+ (100 mM). Inset: Hill plot.

DISCUSSION

In this paper, we provide evidence that the amino acid transporter $ATB^{0,+}$ is capable of transporting a variety of α-carboxyl esters of neutral amino acids. These results are of biochemical interest and of pharmacological and therapeutic relevance. First, the data provide interesting clues as to the

structural features to be present in a potential substrate in order to be recognized by $ATB^{0,+}$. The α-carboxyl group of any of the neutral amino acid can be modified by esterification with methyl, butyl, or benzyl group and such derivatives are still recognized as transportable substrates by $ATB^{0,+}$. However, when the α-carboxyl group is amidated or replaced with a hydroxymethyl group, it interferes with their recognition by the transporter. The esterification of the α-carboxyl group does not have to be restricted to a small aliphatic group as in methyl ester to retain recognition by the transporter. It can be a large aliphatic group as in butyl ester or a bulky aromatic group as in benzyl ester. Similarly, the neutral amino acid used for esterification can be any of the small aliphatic amino acids such as glycine or alanine or a bulky hydrophobic branched chain amino acid such as valine, leucine and isoleucine or an aromatic amino acid such as phenylalanine.

Second, these studies provide evidence for potential utility of $ATB^{0,+}$ as a delivery system for amino-acid–based prodrugs. Ganciclovir, an antiviral drug, is not recognized as a substrate by $ATB^{0,+}$. But, if this drug is coupled to the α-carboxyl group of valine by an ester linkage, the resultant valganciclovir becomes an excellent substrate for the transporter. We have reported similar results with valacyclovir (7). These data show that therapeutically active drugs can be coupled to any of the neutral amino acid substrates of $ATB^{0,+}$ as an ester with the α-carboxyl group of the amino acid to facilitate their delivery into cells via the transporter. It is already known that the oral bioavailability of valacyclovir and valganciclovir is much higher than that of the corresponding parent drugs (8–11). Previous studies have shown that transport of these valyl esters by the intestinal peptide transporter PEPT1 is at least partly responsible for their enhanced oral bioavailability (12–17). Though acyclovir and ganciclovir are not transported by PEPT1, valacyclovir and valganciclovir are recognized as transportable substrates by this transporter (12–17).

The present findings together with our earlier data (7) on the ability of $ATB^{0,+}$ to transport the amino acid esters of the antiviral drugs acyclovir and ganciclovir are of potential therapeutic significance. PEPT1 and $ATB^{0,+}$ are expressed in a complementary manner in the mammalian intestinal tract (3,6). The former is expressed in the small intestine and the latter is expressed predominantly in the large intestine. This expression pattern is of relevance to the oral bioavailability of the amino-acid–based prodrugs valacyclovir and valganciclovir. Even though PEPT1 is known to transport these prodrugs, its role in the absorption of these prodrugs may be compromised *in vivo* because of the presence of large amounts of diet-derived di- and tripeptides in the small intestine which are likely to compete with the prodrugs for transport via PEPT1. Since most of the protein digestion end products are absorbed in the small intestine, the concentrations of free amino acids in the large intestine may be low and thus the competition for the transport of the prodrugs via $ATB^{0,+}$ may be small. This might enhance the contribution of $ATB^{0,+}$ to the intestinal absorption of these amino-acid–based prodrugs. In addition, $ATB^{0,+}$ is far more concentrative than PEPT1 due to the differences in the driving forces for the two transporters. Therefore, $ATB^{0,+}$ may be more efficient than PEPT1 in mediating the uphill absorption of valacyclovir and valganciclovir in the intestine. However, the relative contributions of PEPT1 and $ATB^{0,+}$ to the absorption of these prodrugs in the intestinal tract *in vivo* remain speculative at present because of lack of information on the concentrations of these prodrugs in the small intestine vs. colon after oral administration under *in vivo* conditions. Another point relevant to the therapeutic potential of $ATB^{0,+}$ as a delivery system for amino-acid–based prodrugs is the expression of the transporter in the airway epithelium and in ocular tissues (7). The expression pattern of $ATB^{0,+}$ suggests that the transporter may be amenable for use as a drug delivery system not only in the intestine but also in the lung (possibly as an aerosol) and in the eye (possibly as eye drops).

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