Neutral amino acid transporter ASCT2 displays substrate-induced Na^+ exchange and a substrate-gated anion conductance

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The neutral amino acid transporter ASCT2 mediates electroneutral obligatory antiport but at the same time requires Na^+ for its function. To elucidate the mechanism, ASCT2 was expressed in *Xenopus laevis* oocytes and transport was analysed by flux studies and two-electrode voltage clamp recordings. Flux studies with ^{22}NaCl indicated that the uptake of one molecule of glutamine or alanine is accompanied by the uptake of four to seven Na^+ ions. Similarly to the transport of amino acids, the Na^+ uptake was mediated by an obligatory Na^+ exchange mechanism that depended on the presence of amino acids but was not stoichiometrically coupled to the amino acid transport. Other cations could not replace Na^+ in this transport mechanism. When NaCl was replaced by NaSCN in the transport buffer, the superfusion of oocytes with amino acid substrates resulted in large inward currents, indicating the presence of a substrate-gated anion channel in the ASCT2 transporter. The K_m for glutamine derived from these experiments is in good agreement with the K_m derived from flux studies; it varied between 40 and 90 μM at holding potentials of -60 and -20 mV respectively. The permeability of the substrate-gated anion conductance decreased in the order $SCN^- \gg NO_3^- > I^- > Cl^-$ and also required the presence of Na^+.

Key words: antiporter, glutamate transport, glutamine transport, structure–function relationships.

INTRODUCTION

The EAAT/ASCT family of transporters is involved in the transport of small neutral and acidic amino acids in mammalian cells [1]. This family can be subdivided into the EAAT-type aspartate/glutamate transporters and the ASC-type transporters for small neutral amino acids. Although they have significant sequence similarity, the transport mechanisms of the two subfamilies differ considerably. Glutamate uptake by EAAT-type transporters is accompanied by the co-transport of three Na^+ ions and one H^+ ion; the return of the carrier is facilitated by the binding of a K^+ ion [2]. As expected from this stoichiometry, the uptake of glutamate is electrogenic. Besides the coupled co-transport/antiport of Na^+, K^+ and H^+, the binding of glutamate to EAAT-type transporters also opens an anion channel, which allows the uncoupled flux of Cl^-, I^- and SCN^- probably through the transport pore [3]. The extent of the glutamate-gated ion fluxes varies with the isoform of the transporter [4].

In contrast with the EAAT-type transporters, substrate uptake via the ASCT1, ASCT2 and ATB^0 transporters obeys an obligatory antiport mechanism [5–7]. The uptake of neutral amino acids occurs only in exchange with intracellular amino acids. Nevertheless, the binding of Na^+ is strictly required for the transport mechanism. Although expected to be electrogenic, amino acid transport via ASCT2 is electroneutral [5,8], whereas the binding of substrate to the ASCT1 and ATB^0 transporter induced inward currents [7,9]. For the ASCT1 transporter it could be demonstrated that these currents were not generated by the co-transport of Na^+ ions but by an associated anion conductance, with similar properties to those displayed by the EAAT-type transporters [7].

The close evolutionary relationship between ASCT-type and EAAT-type transporters allows us to address the following questions: (1) What structural elements are necessary for the ion channel pore formation in a transporter? (2) What are the structural requirements for antiporters and symporters? To this end we investigated the mechanism of the rat ASCT2 transporter, which differs in a number of characteristics from ASCT1, ATB^0 and the EAAT-type transporters.

In this study we show that ASCT2 mediates not only an obligatory exchange of amino acids but also an obligatory exchange of Na^+ ions; however, the ratio between Na^+ exchange and amino acid exchange is not fixed. K^+ ions and protons do not participate in the transport mechanism. Similarly to the other transporters of the family we could detect substrate-induced anion currents when Cl^- was replaced by SCN^-, NO_3^- or I^-.

MATERIALS AND METHODS

Materials

[U-^{14}C]glutamine (9.36 Gbq/mmol), [U-^{14}C]alanine, ^{22}NaCl and ^{86}RbCl were purchased from Amersham/Pharmacia (Freiburg, Germany). Unlabelled amino acids (all L-enantiomers) were purchased from Sigma/Fluka (Deisenhofen, Germany). The cap analogue m^{7}G(5′)ppp(5′)G was obtained from New England Biolabs (Schwalbach, Germany). Collagenase (EC 3.4.24.3; 0.6–0.8 unit/mg) was purchased from Roche (Mannheim, Germany). All other chemicals were of analytical grade and were bought from E. Merck (Darmstadt, Germany), Roth (Karlsruhe, Germany) and Roche (Mannheim, Germany).

Oocytes and injections

Xenopus laevis females were purchased from the South African *Xenopus* facility (Knysna, Republic of South Africa). Oocytes (stages V and VI) were isolated by treatment with collagenase as described [10] and left to recover overnight.

Abbreviation used: cRNA, complementary RNA.
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The cloned ASCT2 cDNA [5] was linearized with *Not*I and transcribed *in vitro* with T7 RNA polymerase in the presence of the cap analogue $m^7G(5')ppp(5')G$ (1 mM). Template plasmid was removed by digestion with RNase-free DNase I. The complementary RNA (cRNA) was purified by extraction with phenol/chloroform followed by precipitation with 0.5 vol. of 7.5 M ammonium acetate and 2.5 vol. of ethanol to remove unincorporated nucleotides. The integrity of the transcript was checked by agarose-gel electrophoresis under denaturing conditions. Oocytes were microinjected with 10 nl of ASCT2 cRNA (1 μg/μl in water) by using a microinjection device (World Precision Instruments, Berlin, Germany) or remained uninjected in the controls.

Flux measurements

For each determination, groups of seven cRNA- or non-injected oocytes were washed twice with 4 ml of ND96 buffer [96 mM NaCl/2 mM KCl/1.8 mM $CaCl_2$/1 mM $MgCl_2$/5 mM Hepes, titrated with NaOH to pH 7.4]. They were then incubated at room temperature in a 5 ml polypropylene tube containing 100 μl of the same buffer and 30 kBq of ^{22}NaCl, 30 kBq of ^{86}RbCl or 5 kBq of [U-^{14}C]glutamine or [U-^{14}C]alanine plus unlabelled substrates as required. To increase the specific radioactivity of ^{22}NaCl, a modified ND96 buffer (ND10) was used in which 86 mM NaCl were replaced by 86 mM *N*-methyl-D-glucaminium chloride. Transport was stopped after the appropriate interval by washing oocytes three times with 4 ml of ice-cold ND96 buffer. Single oocytes were placed in scintillation vials and lysed by addition of 200 μl of 10 % (w/v) SDS. After lysis, 3 ml of scintillation fluid was added and the radioactivity was determined by liquid-scintillation counting. For efflux experiments, seven oocytes were preloaded with 50 μM [U-^{14}C]glutamine or 50μM [U-^{14}C]alanine for 10 min or with 10 mM ^{22}NaCl for 30 min, and subsequently washed three times with 4 ml of ice-cold incubation buffer. Efflux was initiated by replacing the ice-cold incubation buffer by 1 ml of buffer at room temperature. Aliquots of 50 or 100 μl were removed at intervals for counting. The efflux curves were calculated by integration of the measured radioactivity in the supernatant over time, corrected for the number of oocytes incubated.

Electrophysiological measurements

Two-electrode voltage-clamp recordings were performed at a holding potential of -50 and -20 mV. To test for ion selectivity, voltage ramps from -110 to $+60$ mV were performed from a holding potential of -40 mV. The data were filtered at 10 Hz and recorded with a MacLab digital-to-analogue converter and software for data acquisition and analysis (AD Instruments, Castle Hill, Australia). ND96 buffer was used as the superfusing solution. For experiments studying anion selectivity, Cl^- was replaced by gluconate, iodide, nitrate or thiocyanate. In some experiments Na^+ was replaced by Li^+, guanidinium or K^+ ions. L-Glutamine and L-alanine were added to the solutions at the indicated concentrations. The final solutions were titrated to the pH indicated with HCl or KOH. The flow rate of the superfusion was 20 ml/min; a complete exchange of the bath solution was reached within approx. 10 s. The currents given are the maximal values measured during a 30 s substrate superfusion.

pH-sensitive electrodes

pH-sensitive electrodes were made and calibrated as described previously [11,12]. In brief, borosilicate electrodes were pulled, silanized with hexamethyldisilazane (Fluka Chemicals) and baked at 200 °C for 15 min. A column of H^+ cocktail (hydrogen ionophore II-cocktail A; Fluka Chemicals) approx. 300 μm in length, was established at the tip of the electrode. The electrode was back-filled with a solution of 100 mM KCl buffered with 10 mM Hepes, pH 7.0. The electrode was calibrated with solutions of pH 6.0, 7.0 and 8.0. Only electrodes with a linear slope of more than 50 mV/pH unit and stable calibration before and after the experiment were used. Signals were recorded with an electrometer (WPI model FD223). On the basis of the calibration curve for the pH-sensitive electrode, the intracellular pH of oocytes was calculated as the difference between the membrane potential (in mV) measured simultaneously with a 3 M KCl microelectrode and with the pH-sensitive electrode.

Calculations

Kinetic parameters were estimated by fitting uptake data to the equation $v = V_{max}[S]/(K_m + [S])$, or by using the Hill equation $I = I_{max}[S]^h/([S]^h + K_m{}^h)$, where h, [S] and v are the Hill coefficient, the substrate concentration and the uptake velocity respectively, I_{max} is the extrapolated maximal current and K_m is the apparent concentration needed for half-maximal current or uptake. For radioactive flux measurements each data point represents the difference between the mean uptake activity ($\pm$S.E.M.) for n (seven) ASCT2-expressing and n (seven) non-injected oocytes. The S.E.M. of this difference was calculated by using Gauss's law of error propagation. Data derived from electrophysiological experiments are provided as means $\pm$ S.E.M.; n represents the number of oocytes investigated. In all experiments non-injected oocytes were used as controls; these did not show significant differences from water-injected oocytes. The magnitude of the induced currents varied 2–4-fold, depending on the time after cRNA injection and on the batch of oocytes (from different animals). Therefore throughout this paper we show experimental data obtained on the same day for each specific set of experiments. All experiments were repeated with at least two batches of oocytes; in all repetitions qualitatively similar data were obtained. All data were tested for significance by using Student's t test, and only results with $P < 0.05$ were considered to be statistically significant.

RESULTS

Amino acid transport by ASCT2-expressing *Xenopus laevis* oocytes

In agreement with earlier reports, ASCT2-expressing oocytes rapidly took up labelled alanine [5,8]. Determined over an incubation time of 15 min, ASCT2-expressing oocytes had an uptake activity of 1404 ± 164 pmol of alanine/h ($n = 7$) in comparison with non-injected oocytes, which took up 148 ± 10 pmol/h ($n = 7$). The accumulated alanine was only slowly released from the oocytes when these were incubated in amino-acid free transport buffer. In contrast, the addition of unlabelled alanine to the supernatant resulted in a rapid release of alanine from the oocytes (Figure 1A). This result confirmed the exchange mechanism for amino acid substrates by the ASCT2 transporter. In contrast with the transport of glutamine [5], however, a small but significant uniport efflux activity could be detected with the substrate alanine.

Na^+ transport by ASCT2-expressing oocytes

The uptake of neutral amino acids by ASCT2 was Na^+-dependent. At a glutamine concentration of 50 μM, the replacement of NaCl by choline chloride in the transport buffer decreased the

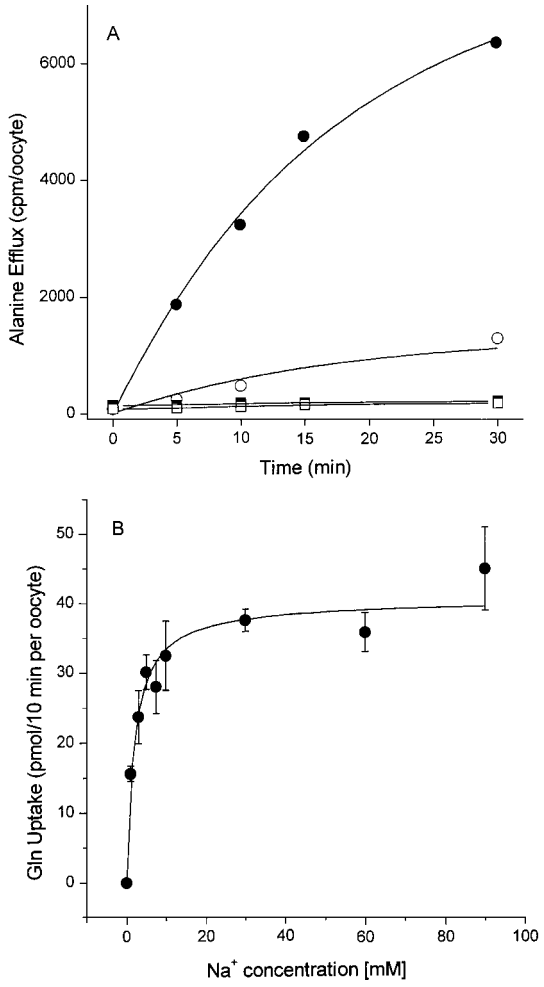


Figure 1 Amino acid transport by rASCT2-expressing oocytes

Oocytes were injected with 10 ng of rASCT2 cDNA (●, ○) or remained uninjected (■, □). (**A**) After an expression period of 2 days, oocytes were preloaded with [^{14}C]alanine (50 μM) for 30 min in ND96 buffer. Oocytes were then washed three times with 4 ml of ice-cold ND96 buffer to remove extracellular alanine. To initiate efflux, this buffer was replaced by ND96 buffer of room temperature. The efflux of alanine was compared in the presence (●, ■) or absence (○, □) of 1 mM extracellular alanine. The combined efflux of seven oocytes was determined and normalized to the efflux of a single oocyte. (**B**) After an expression period of 2 days, the uptake of [^{14}C]glutamine (50 μM) was determined at different Na^+ concentrations. Choline chloride was used to replace NaCl. The uptake activity of non-injected oocytes has been subtracted ($n = 7$).

transport rate from 373 ± 4 to 17.8 ± 0.5 pmol/h in ASCT2-expressing oocytes and from 45 ± 3 to 8.1 ± 0.7 pmol/h in non-injected oocytes. Recently we determined a V_{max} for glutamine of 1080 pmol/h [5], which is similar to an uptake activity of 1252 pmol/h for alanine under saturating conditions (1 mM). Assuming the co-transport of one Na^+ ion with each substrate molecule, a transport current of more than 30 nA would be expected (1 A = 1 C/s). However, the superfusion of oocytes with 1 mM alanine or 1 mM glutamine did not elicit significant transport-associated currents under voltage-clamp conditions (at -50 mV). To investigate the mechanism of the Na^+ dependence of amino acid transport, both uptake activities were compared under identical conditions. At a Na^+ concentration of 96 mM, ^{22}Na uptake was 5–6-fold higher than the corresponding amino acid uptake (Table 1). When the Na^+ concentration was decreased

Table 1 Co-transport of amino acids and $^{22}Na^+$ in ASCT2-expressing oocytes

The uptake of labelled amino acids and $^{22}Na^+$ was compared in the same oocyte batch under identical conditions. Uptake was determined over an incubation period of 15 min in either ND96 buffer, containing 96 mM NaCl, or ND10 buffer, containing 10 mM NaCl. For amino acid and Na^+ uptake, the difference between the transport activity of seven ASCT2-expressing oocytes and that of seven non-injected oocytes was calculated. Amino acids, where added, were at 1 mM.

Buffer	Amino acid	Amino acid uptake (pmol/15 min)	$^{22}Na^+$ uptake (pmol/15 min)	Ratio of Na^+ uptake to amino acid uptake
ND96	Gln	289 ± 32	1730 ± 410	4.8
ND96	Ala	289 ± 28	1990 ± 300	5.7
ND10	Gln	278 ± 41	910 ± 80	3.1
ND10	Ala	280 ± 22	1230 ± 190	4.2
ND96	–	–	340 ± 200	–
ND10	–	–	62 ± 30	–

Table 2 Efflux of Na^+ from rASCT2-expressing oocytes

Oocytes were injected with 10 ng of rASCT2 cDNA. After an expression period of 2 days, oocytes were preloaded with ^{22}NaCl for 30 min in ND10, which contained only 10 mM NaCl to increase the specific activity, in the presence of 1 mM glutamine (first row). Oocytes were then washed three times with 4 ml of ice-cold ND96 buffer to remove extracellular ^{22}NaCl. To initiate efflux, this buffer was replaced by Na^+-containing or Na^+-free ND96 buffer at room temperature. The efflux of $^{22}Na^+$ was compared in the presence or absence of 1 mM glutamine ($n = 7$, rows 2–4).

Experimental conditions	Glutamine	$[Na^+]$ (mM)	^{22}Na content (pmol per oocyte)
Preloading for 30 min	+	10	648 ± 45
Efflux for 30 min	–	96	548 ± 157
Efflux for 30 min	+	0	634 ± 46
Efflux for 30 min	+	96	214 ± 60

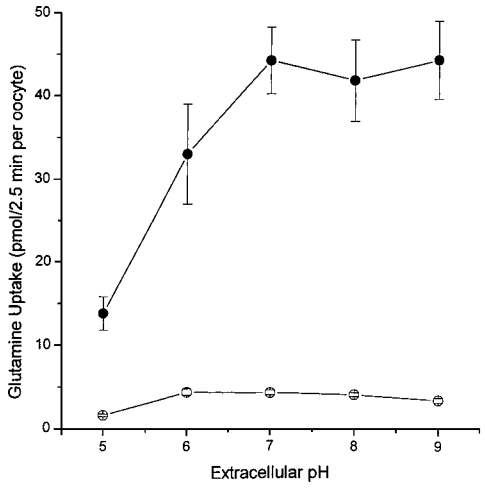


Figure 2 pH dependence of glutamine transport in rASCT2-expressing oocytes

Oocytes were injected with 10 ng of rASCT2 cDNA (●) or remained uninjected (○). After an expression period of 2 days, the uptake of [^{14}C]glutamine (50 μM) was determined over an incubation period of 2.5 min at different pH values. Oocytes were then washed three times with 4 ml of ice-cold ND96 buffer to terminate the uptake. The uptake activity (mean ± S.E.M.) of seven oocytes was calculated for every data point.

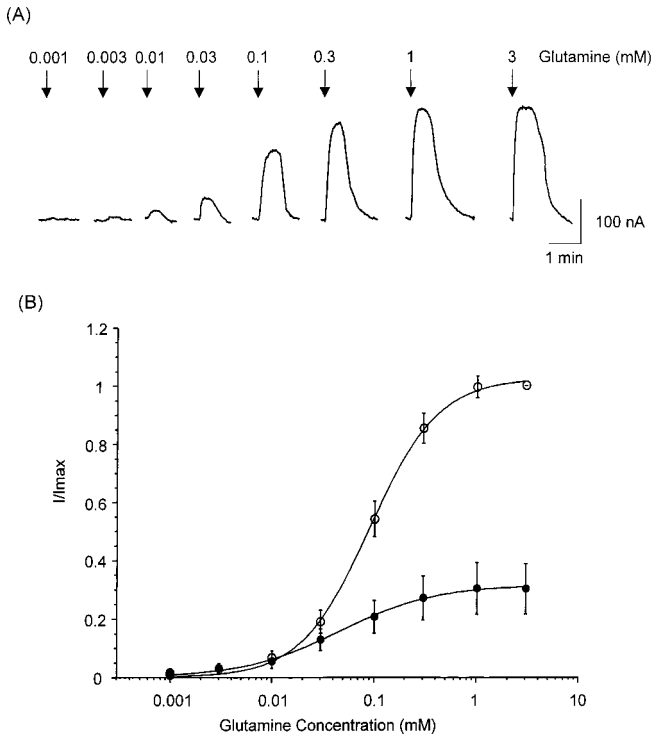


Figure 3 Transport-associated currents in rASCT2-expressing oocytes

Oocytes were injected with 10 ng of rASCT2 cDNA. After an expression period of 2 days, oocytes were superfused with ND96 buffer in which NaCl had been replaced by NaSCN. The glutamine-induced currents were compared at different substrate concentrations. (**A**) Original traces; (**B**) derived curves of the concentration dependence of glutamine-induced currents at holding potentials of −20 mV (●) and −60 mV (○).

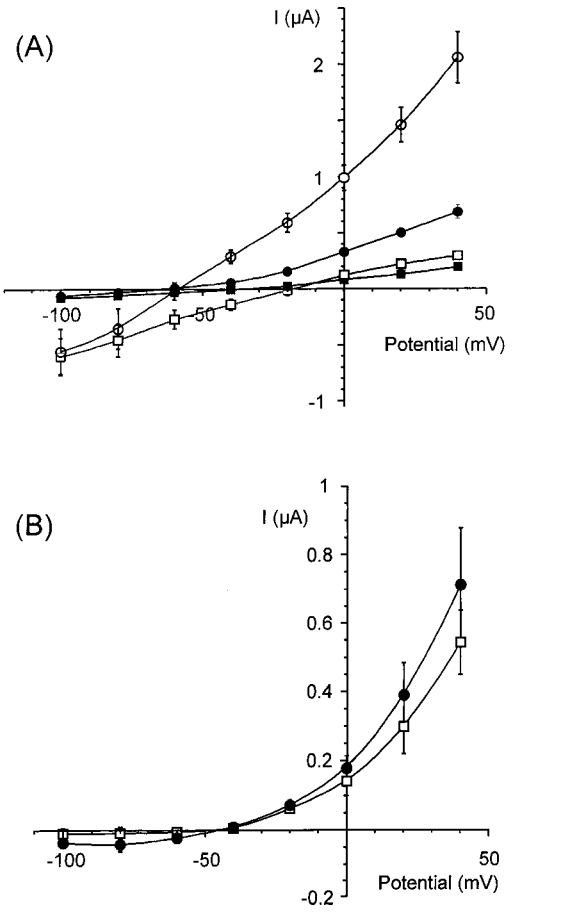


Figure 4 Transport-associated currents in rASCT2-expressing and non-injected oocytes

(**A**) Oocytes were injected with 10 ng of rASCT2 cDNA (○, □) or remained uninjected (■, ●). After an expression period of 2 days, substrate-induced currents (1 mM glutamine) were determined in oocytes superfused with ND96 buffer (■, □) or ND96 buffer in which NaCl had been replaced by NaSCN (●, ○). (**B**) Oocytes were injected with 10 ng of rASCT2 cDNA. After an expression period of 2 days, oocytes were superfused with ND96 buffer in which NaCl had been replaced by NaSCN and which contained 1 mM glutamine (□) or 1 mM alanine (●). The currents in non-injected oocytes, determined under identical conditions, have been subtracted.

to 10 mM, the ratio between Na^+ and amino acid uptake decreased to 3–4 (Table 1). Therefore the uptake of amino acids via ASCT2 is accompanied by the uptake of Na^+ ions but with a variable stoichiometry. The number of Na^+-binding sites on the ASCT2 transporter could therefore not be derived from these experiments. An activation analysis of amino acid transport activity at different Na^+ concentrations resulted in a hyperbolic curve with an apparent K_m of 2.0 ± 0.5 mM and a Hill coefficient of 1.4 ± 0.1 (Figure 1B). To elucidate the discrepancy between the co-transport stoichiometry determined by ^{22}Na fluxes and the activation analysis, we investigated whether Na^+ transport might occur, in a manner similar to amino acid transport, as an exchange process with other cations. To this end, oocytes were preloaded with labelled Na^+. The specific radioactivity was increased by using a transport buffer with a lower Na^+ concentration (10 mM). Under these conditions, approx. 2000 c.p.m. could be preloaded into an oocyte. Release of $^{22}Na^+$ was slow either in the absence of extracellular amino acids or in the absence of extracellular Na^+ but increased rapidly when both transporter substrates where added to the incubation buffer (Table 2). Potassium ions could not replace Na^+ in the transport cycle. Even in transport buffer with lower Na^+ concentration, the addition of glutamine did not induce the transport of K^+, as determined by $^{86}Rb^+$ uptake. At acidic pH the transport rate of ASCT2-expressing oocytes was strongly decreased (Figure 2). We therefore used pH-sensitive microelectrodes to investigate whether intracellular protons competed with Na^+ in the exchange process. However, no changes in the intracellular pH were detected during amino acid transport. The ASCT2 could therefore be described as transporter that obligatorily exchanges Na^+ ions as well as amino acids.

ASCT2 displays activity as a substrate-gated anion channel

In agreement with the ion-transport mechanism elucidated above, ASCT2 behaved as an electroneutral amino acid transporter in NaCl-based buffers. For the related neutral amino acid transporter ASCT1 and the glutamate transporters of the EAAT family it has been shown that the binding of substrates results in the opening of an anion channel that is permeable to halides and other small anions such as NO_3^- and SCN^- [3,4,7]. The permeability of this channel for SCN^- was severalfold higher than the permeability for Cl^-. When NaCl was replaced by NaSCN, large alanine- or glutamine-induced outward currents could also be detected in ASCT2-expressing oocytes (Figure 3A) but not in non-injected oocytes (Figure 4A). The currents increased with increasing glutamine concentrations. K_m values of 46 ± 4 and 90 ± 4 μM were derived from the activation of the SCN^- current

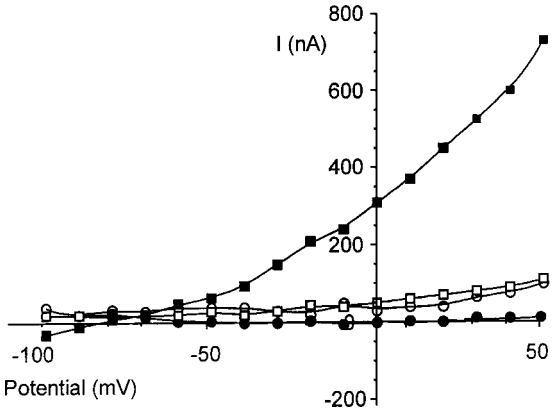
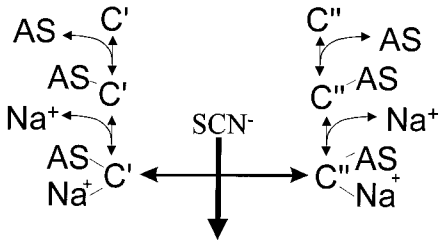


Figure 5 Anion selectivity of substrate-induced transport currents in rASCT2-expressing oocytes

Oocytes were injected with 10 ng of rASCT2 cDNA. After an expression period of 2 days, substrate-induced currents (1 mM glutamine) were determined in oocytes superfused with ND96 buffer (●) or ND96 buffer in which NaCl had been replaced by NaSCN (■), NO_3^- (□) or I^- (○).



Scheme 1 Proposed transport mechanism for ASCT2

In the kinetic scheme, C′ and C″ represent the two conformations of the carrier facing the different sides of the membrane. In accordance with the activation analysis, only one Na^+-binding site is shown. The substrate binds before Na^+ to allow the variable exchange of Na^+ in the presence of amino acid. The vertical arrow indicates the direction of anion conductance.

by increasing glutamine concentrations at holding potentials of −60 and −20 mV respectively (Figure 3B). These values corresponded well to the K_m of 70 μM determined by using labelled glutamine in current-clamped oocytes, which usually have a resting potential of approx. −40 mV [5]. This also demonstrated that the binding of substrate was voltage-dependent. As pointed out above, only marginal substrate-induced currents could be detected in chloride-based buffers (Figure 4A), demonstrating again the electroneutral exchange of amino acids and Na^+. Both glutamine and alanine elicited similar outward rectified currents in ASCT2-expressing oocytes (Figure 4B), which is in agreement with the similar transport rates of both substrates in the flux studies. The substrate-gated anion channel was permeable for small anions, decreasing in the order $SCN^- \gg NO_3^- > I^- > Cl^-$ (Figure 5). Larger anions such as gluconate did not permeate the channel (results not shown). ASCT2-expressing oocytes had a larger background anion conductance, even in the absence of substrate, than non-injected oocytes. Opening of the anion channel required the presence of not only amino acid substrates but also Na^+. When NaSCN was replaced by guanidinium thiocyanate, no substrate-induced currents could be detected (results not shown).

DISCUSSION

The results of this study show that the ASCT2 transporter, similarly to the other members of the EAAT/ASCT family of transporters, displays two different transport functions, a Na^+-dependent amino acid transport activity and a Na^+- and substrate-dependent anion conductance. In the EAAT subfamily of glutamate transporters, three Na^+ ions are co-transported with glutamate and generate the major driving force for the accumulation of glutamate in the cytosol [2]. This co-transport generates significant inward currents in oocytes expressing glutamate transporters [4]. Although ASCT-type transporters are Na^+-dependent as well, no currents associated with the co-transport of Na^+ are detectable. This can now be explained by the Na^+ exchange mechanism of the ASCT2 transporter. The activation analysis of alanine transport suggests that, in contrast with the glutamate transporters, most probably only one or (perhaps) two Na^+-binding sites are present on the transporter but that different stoichiometries are possible. Because of the very high affinity for Na^+, exact transport rates below the K_m were difficult to determine; we therefore could not distinguish accurately between one or two binding sites. The flux stoichiometry suggests that several Na^+ ions can be exchanged before the amino acid dissociates from its binding site. A decrease in the Na^+ concentration from 96 to 10 mM resulted in a significant decrease in Na^+ transport, whereas the amino acid transport activity remained constant (Table 1). This points to a limitation of the amino acid transport rate by the dissociation of the substrate from the transporter.

Both substrate and the co-transported ion depended on each other. Na^+ exchange required the presence of amino acids; amino acid exchange required the presence of Na^+. The characteristics of the ASCT2 amino acid transporter strongly resembled the mechanism of Na^+-dependent neutral amino acid transport in pigeon erythrocytes [13]. In this cell preparation, Koser and Christensen detected highly variable co-transport stoichiometries depending on the substrate. They similarly proposed a scheme in which both the Na^+ ion and the amino acid bind to the transporter but might be released independently [14]. Although we could not detect significant differences between alanine and glutamine transport in respect to Na^+ transport, ASCT2 contains two obligatory antiport activities that are not stoichiometrically coupled. A kinetic scheme in which the substrate binds before Na^+ could account for the observations made in this study (Scheme 1). In this scheme, no conformational changes of the free carrier or the carrier loaded only with amino acid are allowed, to ensure the exchange of amino acids and Na^+. The ASCT-type transporters display a much simpler mechanism than the related glutamate transporters. Glutamate transport is accompanied by a co-transport of three Na^+ ions and one H^+ and an antiport of one K^+ [2]. Mutation of Glu-404 to an aspartic residue in the glutamate transporter GLT-1 [15] results in an inability of the transporter to perform a conformational change in the K^+-loaded form [16]. However, the mutated transporter is still able to perform a Na^+-dependent glutamate exchange similar to the amino acid exchange of the ASCT2 transporter. In agreement with this observation, E404 is well conserved in all members of the EAAT subfamily, whereas it is replaced by a glutamine residue in the ASCT subfamily. In contrast to the glutamate transporters [16] we have to assume that binding

of the substrate has to precede binding of the single Na^+ ion to explain the variable stoichiometry of the transporter.

In addition to this electroneutral transport mechanism, we could detect a substrate-gated anion conductance. The outward currents are generated by the influx of SCN^-, NO_3^- or I^- through a substrate-gated anion channel, whereas the permeation of Cl^- is virtually excluded. Because neither SCN^-, NO_3^- nor I^- are present in the cytosol of the oocytes, no inward currents are detectable at negative membrane potentials. The permeability for Cl^- was too low for an investigation of the voltage dependence in this range. Three observations suggest that this anion conductance is an integral part of the ASCT2 transporter: (1) the K_m values for glutamine agree very well with those of flux studies; (2) substrates of the transporter are able to elicit the anion conductance, e.g. glutamine induces anion currents in ASCT2-expressing oocytes but not in ASCT1-expressing oocytes [7], which is in agreement with the substrate specificity of both transporters; (3) the anion conductance is Na^+-dependent, as is the amino acid transport. A similar Na^+ dependence has also been observed for members of the EAAT family [4] but has been interpreted as a requirement for Na^+ as a counterion for the movement of Cl^- through the transport channel or pore [4]. Although these results shed a light on the transport pathway in the ASCT2 molecule, the low permeability for Cl^- renders a physiological role for this anion conductance unlikely.

The ASCT2 amino acid transporter, although Na^+-dependent, does not use the electrochemical gradient of Na^+ but acts as a Na^+ exchanger. The high affinity for Na^+ allows it to function as an obligatory amino acid exchanger even at low intracellular concentrations of Na^+. Similarly to the glutamate transporters, ASCT2 also constitutes an anion channel that is gated by substrate binding. These results confirm that the isoforms ASCT2 and ATB^0 are most probably orthologous isoforms from different species and not different transporters. The substrate-induced currents, which have been observed in ATB^0 transporters [9], are most probably identical with the anion conductance described in this report and might have been more conspicuous because of small differences in the preparation of the oocytes or longer expression times. Both anion channel activity and Na^+ exchange are most probably evolutionary reminiscences of the glutamate transporter family but have become functionally obsolete during evolution into an amino-acid exchanger.

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