ELSEVIER

Biochimica et Biophysica Acta 1467 (2000) 338–346

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

www.elsevier.com/locate/bba

Functional significance of N- and C-terminus of the amino acid transporters EAAC1 and ASCT1: characterization of chimeric transporters

J. Li [a,b], J. Fei [b], F. Huang [b], L.H. Guo [b,1], W. Schwarz [a,*]

[a] *Max-Planck-Institute for Biophysics, Kennedyallee 70, 60596 Frankfurt/Main, Germany*
[b] *Shanghai Institute of Cell Biology, Chinese Academy of Sciences, 320 Yue Yang Lu, 200031 Shanghai, China*

Received 6 March 2000; received in revised form 1 May 2000; accepted 9 May 2000

Abstract

To localize functionally significant domains in the amino acid transporters of mouse brain mEAAC1 and mASCT1, cRNA encoding for wild-type and chimeric transporters was injected into *Xenopus* oocytes. Activity of expressed transporters was investigated by measurements of uptake of ^{3}H-labeled glutamate and serine and of glutamate- and serine-induced currents under voltage clamp. Though all transporters accept glutamate and serine as substrate, the central part of the protein (Ala_{94}-Met_{418} of mEAAC1 and Ala_{119}-Ile_{393} of mASCT1) determines substrate selectivity. The C-terminus rectifies the interaction with the respective substrate. A channel mode of the glutamate transporter can be activated by glutamate and serine, and the N- and C-termini of the mEAAC1 seem to be essential for the channel formation.

Keywords: Amino acid transporters; Chimeric transporters; Functional significance

1. Introduction

The inwardly directed electrochemical gradient for Na^+, which is maintained by the ATP-consuming Na^+,K^+ pump [1], serves as driving force for a large variety of neurotransmitter transporters in nerve terminals and glial cells. In a superfamily of acidic amino acid transporters (for a review see [2]), the uptake of substrate is not only coupled to the downhill movement of Na^+, but also coupled to the counter transport of K^+ and co-transport of H^+ [3]. A representative of these transporters is the excitatory amino acid carrier 1 (EAAC1, also named EAAT3), which accepts glutamate as a common substrate. The transporters of the ASC family that commonly accept alanine, serine and cysteine, therefore named ASC, have about 40% identity in amino acid sequence with the glutamate transporters, and hence are assumed to belong to the same superfamily with putative 8–10 transmembrane domains.

For EAAC1, it has been proposed that the transport of one glutamate is coupled to the co-transport of 2 or 3 Na^+ ions [3,4]. As a consequence, 1 or 2 positive net charges are translocated across the membrane per transport cycle generating an inward-directed current. In electrophysiological experiments, it has been shown that the transporter indeed gener-

* Corresponding author. Fax: +49-69-630-3340; E-mail: schwarz@22biophys.mpg.de
[1] Also corresponding author.

0005-2736/00/$ – see front matter
PII: S0005-2736(00)00232-7

ates substrate-activated currents, but evidence has accumulated that this only in part results from the unbalanced charge movements and in part also originates from a 'channel mode' [5]. This mode of the transporter allows ion passage without being coupled to substrate translocation, most likely through a Cl^--permeable channel (see e.g. [6,7]). For ASCT1, an electroneutral amino acid exchange mode coupled to the translocation of one Na^+ has been suggested [8] that does not depend on K^+; a small alanine-induced current has been attributed to an uncoupled flow of Cl^-.

EAAC1 and ASCT1 share more than 40% of identity in their amino acid sequence and have nearly 70% of similarity (compare Fig. 1A for the transporters of mouse brain [9,10]). Hence, it is not surprising that ASCT1 to some extent also transports glutamate, and EAAC1, e.g. serine. Nevertheless, there are clear differences in sensitivity to these two substrates and in the current signals (see also [2,7]). To localize domains that are functionally significant, we constructed chimeras between EAAC1 and ASCT1 of mouse brain (see also [11]) and measured substrate uptake and substrate-induced current after expression in *Xenopus* oocytes.

2. Materials and methods

2.1. Construction of chimeras

Chimeric transporters were constructed from mEAAC1 and mASCT1 using recombinant PCR (compare Fig. 1B), where N- or C-terminal parts were from mEAAC1 (chimeras 1 and 4) with the remainder from mASCT1, and vice versa (chimeras 2 and 3). After checking the sequences, chimeras were cloned into the PNWP vector, which was modified from PNSK2, and has the polyA^+ tail and ribosome binding sites of *Xenopus* oocytes to ensure high transcription in vitro and expression in the oocytes. The following primers were used (bold letters indicate homologous sequence sections):

For chimera 1

E-5P: 5'-gtca ctcgag tgc tca cca tgg gga ag-3'
XhoI mEAAC1: 67-85

Chimera1-3P: 5'-*gca aac cac* **cag cgg ca** aaa tga cca gct-3'
Complementary to mASCT1:399-383 mEAAC1: 286-269

Chimera1-5P: 5'-*ggtcattt* **tgc cgc tg** gtg gtt tgca-3'
Complementary to mEAAC1:271-286 mASCT1:383-400

A-3P: 5'-caga gaattc aga ccc agc cca atc acgg-3'
EcoR.I mASCT1: 1733-1714

For chimera 2

A-5P: 5'-caga ctcgag cgt ggg caa cga catg-3'
XhoI mASCT1: 96-114

Chimera2-3P: 5'-*gga tac gat* **cag cgg ca** gga tga tcat-3'
Complementary to mEAAC1:295-279 mASCT1: 390-373

Chimera2-5P: 5'-*gat cat cc* **tgc cgc tg** a tcg tatcca-3'
Complementary to mASCT1:375-390 mEAAC1: 279-296

E-3P: 5'-atct gaattc agg cat cta gaa ctg tg-3'
EcoR.I mEAAC1: 1664-1646

For chimera 3

E-5P: 5'-gtca ctcgag tgc tca cca tgg gga ag-3'
XhoI mEAAC1: 67-85

Chimera3-3P: 5'-*cgg cac acc tgct* **gct cc** aat gct ggc agc ggt-3'
Complementary to mASCT1:1383-1366 mEAAC1: 1296-1277

Chimera3-5P: 5'-*gct gcc agc att* **gga gc** agc agg tgt gccg-3'
Complementary to mEAAC1:1280-1296 mASCT1:1366-1383

A-3P: 5'-caga gaattc aga ccc agc cca atc acgg-3'
EcoR.I mASCT1: 1733-1714

For chimera 4

A-5P: 5'-caga ctcgag cgt ggg caa cga catg-3'
XhoI mASCT1:96-114

Chimera4-3P: 5'-*ggg cac ccc agca* **gctcc** aac act gga tgc cgt-3'
Complementary to mEAAC1:1309-1292 mASCT1: 1370-1351

Chimera4-5P: 5'-*gca tcc agt gtt* **gga gc** tgc tgg ggt gccc-3'
Complementary to mASCT1:1354-1370 mEAAC1: 1292-1309

E-3P: 5'-atct gaattc agg cat cta gaa ctg tg-3'
EcoR.I mEAAC1: 1664-1646

2.2. Oocytes and cRNA injection

To obtain the oocytes for injection of the respective cRNAs, females of the clawed toad *Xenopus laevis* were anesthetized with tricaine (MS222, Sandoz, Basle (Switzerland) 1 g/l). Parts of the ovary were dissected and treated with collagenase (1.5–3 mg/ml) to remove follicular cells. Full-grown prophase-arrested oocytes were selected for experiments. For expression of transporters, oocytes were injected with 50 nl cRNA for wild-type or chimeric proteins (1 ng/nl). The cells were stored at 19°C in oocyte Ringer's solution (ORi, see below) containing antibiotics (in mg/l: 70 gentamicin or 25 streptomycin plus 20 penicillin). Experiments were performed at room temperature (22°C) after 1–5 days of incubation.

2.3. Western blot

Yolk-free homogenates of oocytes were prepared as described by Vasilets [12] 3 days after injecting cRNA, and by passing the oocytes through 200-μl Eppendorf pipette tips in homogenization buffer (HBO, see solution). Twenty-microliter aliquots of yolk-free homogenates were electrophoresced on PAGE gels. Proteins from non-stained gels were electrophoretically transferred on nitrocellulose membrane for a Western blot (overnight at room temperature, 0.4 mA/cm^2). The preparations expressing mEAAC1, chimera 2 and chimera 4 were immunostained with anti-mEAAC1 C-terminus (462–523 aa) antibody, and mASCT1, chimera1 and chimera3 with anti-mASCT1 C-terminus (475–532 aa) antibody as the primary antibodies (prepared by Shanghai Institute of Cell Biology, Chinese Academy of Sciences), and visualized by the secondary alkaline-phosphatase conjugated antibody (Promega).

2.4. Flux measurements

To determine the maximum transport activity, uptake by single oocytes of ^{3}H-labeled glutamate or serine (Amersham, Braunschweig) was measured at 90 mM external Na^+ and 1 mM total concentration of the respective substrate. Oocytes (about 10 each) were incubated for 20 min in a ORi solution (volume 200 μl) containing 1 mM cold and 15 nM ^{3}H-labeled substrate (9.25 kBq/200 μl). To determine unspecific uptake, some measurements were performed in Na^+-free incubation solution. The rate of uptake was similar to that of oocytes not injected with cRNA. Therefore, the uptake of non-injected control oocytes was normally used for subtraction of the background. To exclude oocytes with high membrane leakage, 1 mM sucrose with 18 μM [^{14}C]sucrose (16 kBq/200 μl, Du Pont NEN, Bad Homburg) was added to the incubation medium (see [13]).

2.5. Electrophysiology

The electrophysiological experiments were performed with conventional two-electrode voltage clamp (TEVC) (see [14]). For characterization of transporter-mediated current, steady-state membrane currents were recorded at the end of 200-ms, rectangular voltage-clamp pulses (from −150 to +30 mV in 10-mV increments) that were applied from a holding potential of −60 mV, and voltage dependencies of steady-state membrane currents were obtained. The transporter-mediated current was determined as the current that can be activated by glutamate or serine.

2.6. Solutions

The standard ORi solution had the following composition (in mM): 90 NaCl, 2 KCl, 2 $MgCl_2$, 5 MOPS (adjusted to pH 7.4 with Tris). In the Na^+-free solution NaCl was replaced by tetramethylammonium chloride. The HBO contained (in mM): Tris–HCl 20 (pH 7.4), $MgCl_2$ 5, NaH_2PO_4 5, EDTA 1, NaCl 100, KCl 10, DTT 1 and PMSF 1 and 5μg/ml of each of leupeptin, pepstatin and antipain.

3. Results

To localize functionally significant domains, chimeras between mEAAC1 and mASCT1 were constructed, where the center parts of wild-type (Ala_{94}-Met_{418} and Ala_{119}-Ile_{393}, respectively) were combined with the N- and/or C-terminal sections of the other transporter (see Fig. 1A,B). Function was investigated by measurements of uptake of glutamate and serine and by measurements of substrate-activated currents.

3.1. Uptake measurements

To test functional expression of wild-type and chimeric transporters in the oocytes, uptake of ^{3}H-labeled glutamate and serine was measured in cRNA-injected and non-injected control oocytes applying saturating concentrations of 1 mM, which gave similar uptake rates as 0.5 mM. Interestingly, in contrast to the activation of uptake, 1 mM is not sufficient for maximum activation of transporter-mediated currents (see below). The uptake rates, nevertheless, differed considerably (Fig. 2) though the Western blots show (Fig. 1C) that all transporters were expressed to about the same extent. The data for uptake were normalized to the rate of glutamate uptake by mEAAC1, which corresponds to 741 ± 43 fmol/s; the rate of serine uptake by mASCT1 corresponds to 540 ± 47 fmol/s. The relative rates of uptake, corrected for unspecific uptake, are summarized in the first rows of Tables 1 and 2, respectively. As expected, mEAAC1 shows highest uptake for glutamate, mASCT1 for serine. Glutamate is also well accepted by chimera 2 and, though to a smaller extent, by chimera 3. Glutamate is also transported by mASCT1 and chimeras 1 and 4, but at an even further reduced rate (Fig. 2A and Table 1). In turn, serine is well accepted by mASCT1 and chimeras 1 and 4 while mEAAC1 and chimeras 2 and 3 transport serine at an efficacy of only about 40% compared to mASCT1 (see Fig. 2B and Table 2).

A

```
mEAAC1   - MGKP--------------------------------TSSGCDWRRFLRNHWLLLSTVAAVVLGIVLGVVVRGH----SELSNLDKFYFAFPGEILMRMLKL -65
mASCT1   - MEKSGETNGYLDGTQAEPAAGPRTPETAMGKSQRCAS-------FFRRHALVLLTVSGVLVGAGMGAALRGLQLTPTQIT-----YLAFPGEMLLRMLRM -88

mEAAC1   - VILPLIVSSMITGVAALDSNVSGKIGLRAVVYYFSTTVIAVILGIVLVVSIKPG--------------------VTQKVNDINRTGKTPEVSTMDAMLDL -145
mASCT1   - IILPLVVCSLVSGAASLDASSLGRLGGIAVAYFGLTTLSASALAVALAFIIKPGAGAQTLQSSSLGLENSGPPPVSKE--------------TVDSFLDL -174

mEAAC1   - IRNMFPENLVQACFQQYKTKREEVKPVGDPGGNATEVSVTTAMT--------TMSENKTKEYKIVGLYSDGINVLGLIIFCLVFGLVIGKMGEKGQILVD -237
mASCT1   - LRNLFPSNLV-------------------------VAAFTTSATDYTVVTHNTSSGNVTKEKIPVVTDVEGMNILGLVLFALVLGVALKKLGPEGEDLIR -249

mEAAC1   - FFNALSDATMKIVQIIMCYMPIGILFLIAGKIIEVEDWEIFRKLGLYMATVLSGLA----------IHSLIVLPLLYFIVVRKNPFRFALGMAQALLTA -326
mASCT1   - FFNSFNEATMVLVSWIMWYVPIGIMFLIGSKIVEMKDI----------VMLVTSLGKYIFASMLGHVIHGGIVLPLVYFAFTRKNPFTFLLGLLTPFATA -339

mEAAC1   - LMISSSSATLPVTFRCAEEKNQVDKRITRFVLPVGATINMDGTALYEAVAAVFIAQLNGLDLSIGQIVTISITATAASIGAAGVPQAGLVTMVIVLSAVG -426
mASCT1   - FATCSSSATLPSMMKCIEENNGVDKRISRFILPIGATVNMDGAAIFQCVAAVFIAQLNNVDLNAGQIFTILVTATASSVGAAGVPAGGVLTIAIILEAIG -439

mEAAC1   - LPAEDVTLIIAVDWLLDRFRTMVNVLGDAFGTGIVEKLSKKELEQ-----------------------------------MDVSSEVNIVNPFALEP -488
mASCT1   - LPTHDLSLILAVDWIVDRTTTVVNVEGDALGAGILNHLNQKVVKKGEQELQEVKVEAIPNSKSEEETSPLVTHQNPAGPVAIAPELESKESVL        -532

mEAAC1   - TTLDNEDSDTKKSYVNGGFAVDKSDTISFTQTSQF -523
```

Fig. 1. (A) Alignment of amino acid sequence of mEAAC1 and mASCT1. The dashes indicate identical amino acids. The gray underlaid sections represent putative transmembrane domains according to [9,20]. (B) Chimeric patterns of transporters and principle of their construction. (C) Western blots.

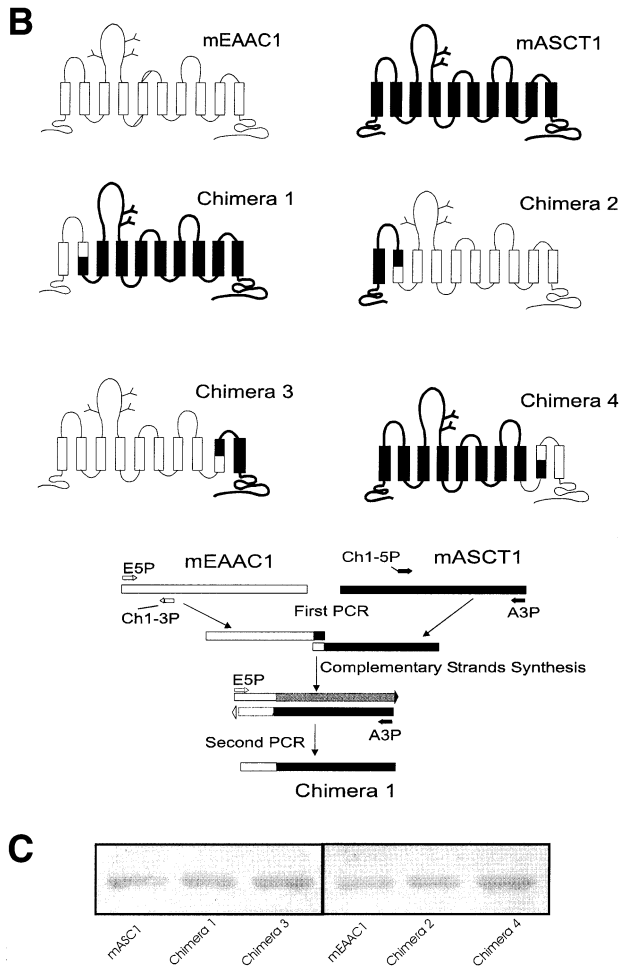

Fig. 1 (*continued*).

3.2. Voltage-clamp experiments

Glutamate transport by EAAC1 involves unbalanced charge movements, and hence, is electrogenic. Assuming that one positive net charge is transported into the cell per one glutamate, a current of −70 nA can be expected from the rate of glutamate uptake (see Table 1). In the oocytes with expressed mEAAC1, 1 mM glutamate can induce a current of even several μA (see Fig. 3), which supports the view of an uncoupled channel mode that exceeds the electrogenicity of the transport mode by more than one order of magnitude. A reversal potential for the direction of the current could not be detected up to +30 mV, but this is in line with the findings for the EAAC1 of rat [15] and the human EAAT3 [16]. On this scale of transmitter-mediated current, mASCT1 and the chimeras do not generate any significant current in response to glutamate. But at higher resolution (see inset in Fig. 3A) transporter-mediated steady-state currents can clearly be resolved. Particularly chimeras 2 and 3, and to some extent also chimera 1, exhibit currents (for −100 mV see Table 1). For better comparison with the currents expected from the flux measurements, Table 2 also lists the currents measured at −40 mV, which is close to the resting potential of the oocytes. These measured currents activated by glutamate are in the range one would expect for the electrogenic transport mode. At least there seems to be no major contribution from a channel mode in mASCT1 and the chimeras as seen for mEAAC1.

Also the currents activated by serine in mASCT1 and the chimeras are smaller than or within the range expected for electrogenic transporters. Interestingly, serine as the substrate for mASCT1 (Fig. 2) could activate a pronounced current signal in mEAAC1 of several hundred nA (Table 2). All the other transporters produce at −40 mV negligible current. Nevertheless, at −100 mV, signals of inward current can clearly be detected; at potentials less negative than −40 mV the current reverses direction indicating that it is mediated by a channel mode [3].

The dependency of transporter-mediated current on substrate concentration was measured at different membrane potentials; the estimated concentrations

Table 1
Characteristics of glutamate-activated transport by wild-type and chimeric transporters

	mEAAC1	mASCT1	Chimera 1	Chimera 2	Chimera 3	Chimera 4
Flux (normalized)	1	0.19	0.13	0.80	0.56	0.25
Expected current (nA)	−70	−13	−9	−56	−39	−18
Current (nA) (at −100/−40 mV, and 1 mM)	−2558/−747	−10/−6	−45/−18	−67/−20	−88/−31	−19/ns
$K_{1/2}$ (mM) (at −100 mV)	0.25	1.25	0.24	0.20	2.05	0.71

Expected currents were determined from rate of glutamate uptake on the basis of one net charge being transported per glutamate. ns, no significant current detectable. $K_{1/2}$ values are for current activation by glutamate according to Eq. 1 given in the legend to Fig. 4.

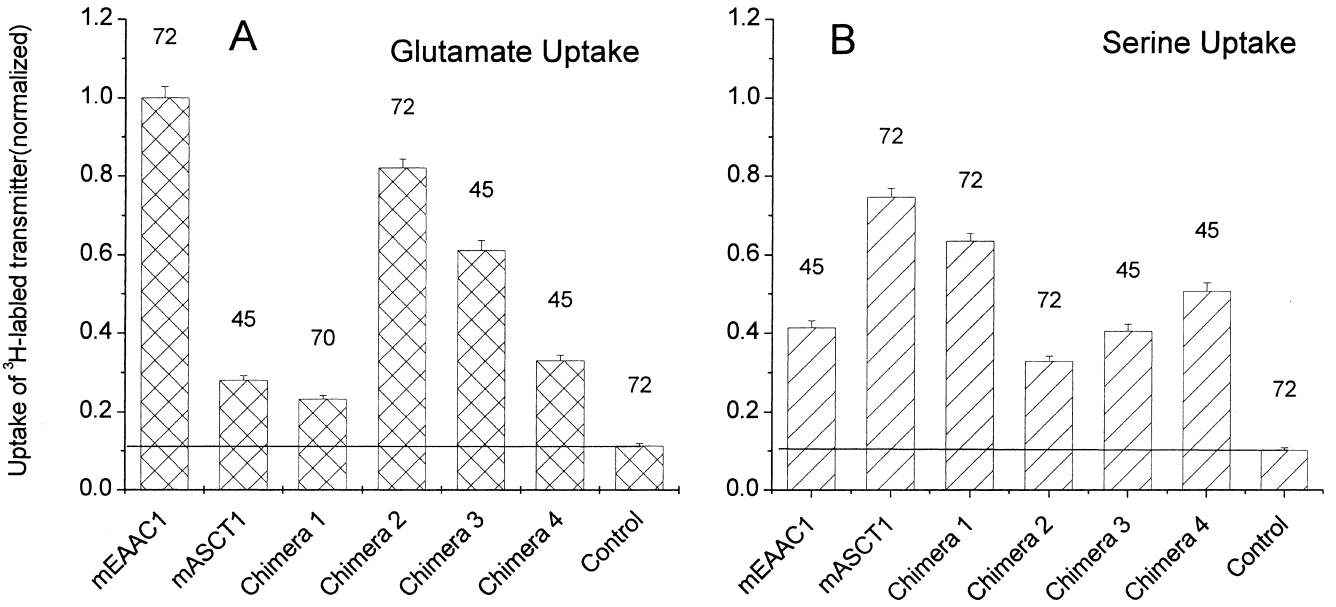

Fig. 2. Uptake of ^{3}H-labeled glutamate (A) or serine (B) by mEAAC1, mASCT1 and the chimeric transporters in comparison to non-injected control oocytes. Total concentration of neurotransmitters in the incubation medium was 1 mM. The horizontal lines represent unspecific uptake, which corresponds to the uptake seen in non-injected control oocytes. Data are normalized to glutamate uptake by EAAC1 and are averages ± S.E.M., numbers of oocytes are given above each column.

for half-maximum stimulation were voltage-independent. This is in line with the observation for EAAC1 of rat which has a $K_{1/2}$ value for glutamate that is voltage-dependent only for non-saturating Na^+ concentrations [15] though the values we found for mEAAC1 are by a factor of about 5 larger. As an example, Fig. 4A and B show the dependencies at −100 mV for current stimulation by glutamate and serine, respectively. The data for dependency on glutamate concentration could be fitted with a Hill coefficient of $n = 1$, while for serine a Hill coefficient of $n = 2$ was used for the fits. This was independent of the transporter studied. The corresponding $K_{1/2}$ values are listed in Tables 1 and 2. These values are all much higher than those found for stimulation of uptake, which are in the range of tens of μM for EAAC1 and ASCT1 [17]. Also in our flux measurements, we found a $K_{1/2}$ value of about 30 μM for stimulation of mEAAC1 by glutamate, and all the transporters show maximum rate of uptake at 500 μM.

4. Discussion

mEAAC1 and mASCT1 expressed in *Xenopus* oocytes both transport at a high rate glutamate and serine. Glutamate is favored by mEAAC1 with an uptake rate of more than 700 fmol/s, serine by mASCT1 with an uptake rate of about 550 fmol/s. These values are by a factor of about 5 higher than what we found previously for the GABA transporter

Table 2
Characteristics of serine-activated transport by wild-type and chimeric transporters

	mEAAC1	mASCT1	Chimera 1	Chimera 2	Chimera 3	Chimera 4
Flux (normalized)	0.48	1	0.83	0.35	0.47	0.63
Expected current (nA)	−25	−52	−43	−18	−24	−33
Current (nA) (at −100/−40 mV, and 1 mM)	−643/−203	−19/ns	−113/−17	−32/−12	−28/ns	−159/ns
$K_{1/2}$ (mM) (at −100 mV)	1.35	1.37	1.06	1.35	1.96	1.53

Expected currents were determined from rate of serine uptake on the basis of one net charge being transported per serine. ns, no significant inward current detectable. $K_{1/2}$ values are for current activation by serine according to Eq. 1 given in the legend to Fig. 4.

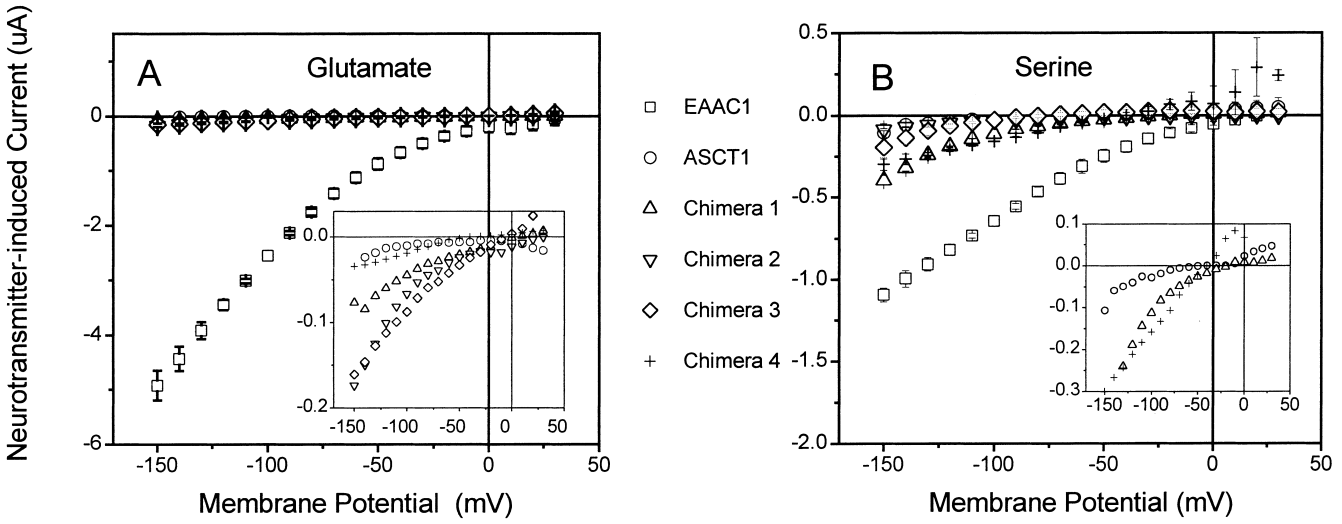

Fig. 3. Voltage dependence of current activated by 1 mM glutamate (A) or serine (B) mediated by mEAAC1, mASCT1 and the chimeric transporters. Data are averages from 3–5 oocytes (±S.E.M.). In the inset in A, the data for mASCT1 and the chimera are redrawn at a higher current resolution, in B, the data for mASCT1 and chimeras 1 and 4.

GAT1, which was expressed at a density of about 2×10^{11} transporters per oocyte [18] corresponding to about 10,000 per μm^2. The non-common substrates, glutamate for mASCT1 and serine for mEAAC1, are transported at rates of 140 and 260 fmol/s, respectively. Western blots indicate similar degree of expression of the transporters, but it is of course hard to judge whether the differences in rate of uptake between different transporters result from differences in the transport rate of a transport molecule or in number of transporters targeted to the oocyte membrane (compare e.g. [19]).

Glutamate induces in mEAAC1 huge currents that are too large to be assigned to pure electrogenic transport, and the existence of channel mode has been proposed (see e.g. [7]). Comparison of the currents expected from translocation of 1 or 2 charges per glutamate with the currents detected at −40 mV yields that the measured currents are by a factor of 5 to 10 larger (see Table 1). We made a similar observation with activation of mEAAC1 by serine (Table 2). Though the inward currents reach several hundred nA at the negative potentials, no reversal potential could be detected within the covered potential

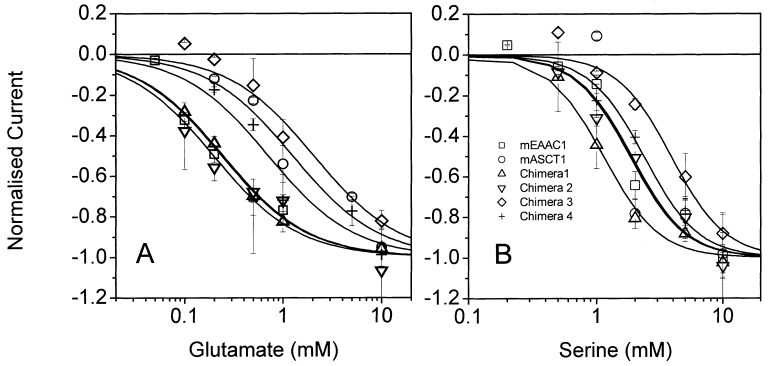

Fig. 4. Dependence of substrate-activated current on glutamate (A) and serine concentration (B) at −100 mV. Data are normalized to saturate at $I_{max}=1$ and are averages from 3 to 5 oocytes (±S.E.M.). Solid lines are fits of

$$I = I_{max}\frac{[S]^n}{[S]^n + K_{1/2}^n} \quad (1)$$

to the data with $n=1$ in A and $n=2$ in B.

range up to +30 mV, but it was shown by others [6] that a reversal potential can be detected under optimized ionic conditions and the changes of the reversal potential with external Cl^- follow Nernst dependency. The absence of a reversal potential could be due to an inward-rectifying gating mechanism, and that the current at only slightly negative and at positive potentials is governed by the normal transport mode. For the formation of the channel mode, the entire mEAAC1 protein seems to be essential since all the other transporters, mASCT1 and the chimeras, generate currents that are similar to or even less than the currents expected from pure electrogenic substrate transport. Serine is nearly as effective as glutamate in activation of this channel mode in mEAAC1.

An uncoupled electric current is also associated with the ASCT1, which has a reversal potential close to the equilibrium potential for Cl^- [8]. Though the serine-activated currents are small, a reversal at potentials of about −30 mV can clearly be detected (Fig. 3). It was suggested that this current is carried by Cl^- and mediated by a channel mode or an endogenous channel coupled to the transporter (see e.g. [20]); in its transport mode ASCT1 operates in an electrically silent exchange mode and does not contribute to the electrical signal. The channel mode of mASCT1 must have a different molecular basis than the channel mode in EAAC1 since it can also be detected in the chimeras 1 and 4 (inset Fig. 3B), but the current can only be activated by serine not by glutamate. While for the formation of the EAAC1-channel mode the presence of the N- and C-terminal domains in the mEAAC1 protein are necessary, the ASCT1-channel mode can still be formed if either the N- or the C-terminus of the mASCT1 protein are replaced by the corresponding domains of mEAAC1. Whether N- and C- terminal domains are sufficient for EAAC1-channel formation needs further investigations that are under progress.

Another manifestation of channel and transport modes in the two amino acid transporters are the different dependencies of uptake and current on substrate concentration (see Tables 1 and 2). While rate of glutamate and serine uptake are saturated at 1 mM, $K_{1/2}$ values for activation of the current are in the range of 1 mM. Only mEAAC1 and chimeras 1 and 2 exhibit $K_{1/2}$ values of about 200 μM, which is, nevertheless, by a factor of 5 higher than the $K_{1/2}$ values for activation of glutamate uptake. This may reflect different sensitivities for the substrate of transport and channel mode.

Both wild-type and the chimeric transporters accept glutamate as well as serine as substrate, but transport occurs at different rates. Glutamate is transported by mEAAC1 and chimera 2 at comparably high rate, and slightly less by chimera 3 (Table 1). They all have in common the central part of the EAAC1 protein (Fig. 1) and it seems that the C-terminal domain of mEAAC1 stabilizes the sensitivity for glutamate. The center domain between residue 331 and 411 (numbering of mEAAC1) shows a high degree of identity conserved. In the glutamate transporter family, Glu_{373} and Tyr_{372} (Glu_{404} and Tyr_{403} in EAAT2 of rat) are conserved, but are not present in ASCT1. It has been suggested that these residues are located in the vicinity of the substrate-translocation pathway [21,22], and a pore loop-like structure has been recognized [23]. N- and C-termini are not essential for substrate recognition with respect to uptake as well as current stimulation, but the presence of the C-terminus improves substrate transport.

Serine is transported at comparably high rate by mASCT1 and chimera 1 and slightly less by chimera 4 (Table 2). In analogy to the transporters of glutamate, the transporters for serine share the central part of mASCT1 (Fig. 1), and the C-terminal domain of mASCT1 seems to stabilize the sensitivity for serine.

5. Conclusion

Though all the investigated wild-type and chimeric transporters accept glutamate as well as serine as substrate, the central part is essential for the discrimination between glutamate and serine. The C-terminus of EAAC1 supports the affinity for glutamate and of ASCT1 for serine. Activation of mEAAC1 and mASCT1 leads to a current component uncoupled from the transport mode that seems to have different molecular basis. Activation of the EAAC1-type channel mode needs the presence of both the N- and C-terminus of EAAC1.

Acknowledgements

We are very grateful to Drs. Christof Grewer and Jürgen Rettinger for their helpful comments on the manuscript. We thank Heike Biehl, Heike Fotis and Eva-M. Gärtner for their assistance in oocyte handling and flux measurements. The work was supported on the basis of an Research Agreement between the Chinese Academy of Sciences and Max-Planck Gesellschaft.

References

[1] J.C. Skou, Biochim. Biophys. Acta 23 (1957) 394.
[2] Y. Kanai, Curr. Opin. Cell Biol. 9 (1997) 565.
[3] N. Zerangue, M.P. Kavanaugh, Nature 383 (1996) 634.
[4] M. Bouvier, M. Szatkowski, A. Amato, D. Attwell, Nature 360 (1992) 471.
[5] L.J. Defelice, R.D. Blakely, Biophys. J. 70 (1996) 579.
[6] J.I. Wadiche, M.P. Kavanaugh, J. Neurosci. 18 (1998) 7650.
[7] M.S. Sonders, S.G. Amara, Curr. Opin. Neurobiol. 6 (1996) 294.
[8] N. Zerangue, M.P. Kavanaugh, J. Biol. Chem. 271 (1996) 27991.
[9] Y. Kanai, M.A. Hediger, Nature 360 (1992) 467.
[10] J.L. Arriza, M.P. Kavanaugh, W.A. Fairman, Y.N. Wu, G.H. Murdoch, R.A. North, S.G. Amara, J. Biol. Chem. 268 (1993) 15329.
[11] J. Li, J. Peng, J. Fei, F. Huang, Q. Gu, L. Guo, Chin. Sci. Bull. 44 (1999) 524.
[12] L.A. Vasilets, Cell. Physiol. Biochem. 7 (1997) 1.
[13] G. Schmalzing, H. Omay, S. Kröner, S. Gloor, H. Appelhans, W. Schwarz, Biochem. J. 279 (1991) 329.
[14] A.V. Lafaire, W. Schwarz, J. Membr. Biol. 91 (1986) 43.
[15] Y. Kanai, S. Nussberger, M.F. Romero, W.F. Boron, S.C. Hebert, M.A. Hediger, J. Biol. Chem. 270 (1995) 16561.
[16] J.I. Wadiche, S.G. Amara, M.P. Kavanaugh, Neuron 15 (1995) 721.
[17] N. Utsunomiyatate, H. Endou, Y. Kanai, FEBS Lett. 416 (1997) 312.
[18] Y. Liu, U. Eckstein-Ludwig, J. Fei, W. Schwarz, Biochim. Biophys. Acta 1415 (1998) 246.
[19] J.C. Garcia, M. Strube, K. Leingang, K. Keller, M.M. Mueckler, J. Biol. Chem. 267 (1992) 7770.
[20] M. Palacin, R. Estevez, J. Bertran, A. Zorzano, Phys. Rev. 78 (1998) 969.
[21] G. Pines, Y. Zhang, B.I. Kanner, J. Biol. Chem. 270 (1995) 17093.
[22] R. Zarbiv, M. Grunewald, M.P. Kavanaugh, B.I. Kanner, J. Biol. Chem. 273 (1998) 14231.
[23] M. Grunewald, A. Bendahan, B.I. Kanner, Neuron 21 (1998) 623.