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A novel mode of operation of SLC22A11: Membrane insertion of estrone sulfate versus translocation of uric acid and glutamate

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ABSTRACT

Estrone sulfate alias estrone-3-sulfate (E3S) is considerably larger and much more hydrophobic than typical substrates of SLC22 transporters. It is puzzling that many otherwise unrelated transporters have been reported to transport E3S. Here we scrutinized the mechanism of transport of E3S by SLC22A11 (alias OAT4), by direct comparison with uric acid (UA), an important physiological substrate. Heterologous expression of SLC22A11 in human 293 cells gave rise to a huge unidirectional efflux of glutamate (Glu) and aspartate, as determined by LC–MS/MS. The uptake of E3S was 20-fold faster than the uptake of UA. Yet, the outward transport of Glu was inhibited by extracellular E3S, but not by UA. The release of E3S after preloading was *trans*-stimulated by extracellular dehydroepiandrosterone sulfate (DHEAS), but neither by UA nor 6-carboxyfluorescein (6CF). The equilibrium accumulation of E3S was enhanced 3-fold by replacement of chloride with gluconate, but the opposite effect was observed for UA. These results establish that SLC22A11 provides entirely different transport mechanisms for E3S and UA. Therefore, E3S must not be used as a substitute for UA to assay the function of SLC22A11. In equilibrium accumulation experiments, the transporter-mediated uptake was a linear function of the concentration of UA and 6CF. By contrast, in the same concentration range the graph for E3S was hyperbolic. This suggests that SLC22A11 inserts E3S into a small volume with limited capacity, the plasma membrane. Our data support the notion that the reverse process, extraction from the membrane, is also catalyzed by the carrier.

1. Introduction

In humans, normal blood serum levels of uric acid (UA) are much higher than in other mammals (e.g. 30–50 μM in mice) [1]. The upper end of the normal range is 360 μM for women and 400 μM for men. Chronic hyperuricemia promotes the deposition of sodium urate crystals within joints which then causes gout. In developed countries, gout is the most common inflammatory arthritis, affecting 1–2% of adults [2]. With prior serum urate levels ⩾535 μM, the annual incidence rate of gouty arthritis in men was 4.9%, compared with 0.1% for urate levels below 415 μM [3]. Hyperuricemia is not only associated with gout, but also with hypertension, diabetes, and renal and cardiovascular diseases. For example, there is a strong correlation between hyperuricemia and children with primary hypertension [4]. Underexcretion of UA by the kidney is the primary cause of hyperuricemia in about 90% of cases [2].

Renal excretion accounts for more than 70% of the daily uric acid disposal. Glomerular filtration is followed by reabsorption and secretion; these transport processes occur in parallel in the proximal tubule [5]. The overall effect is that in healthy adults only 7–12% of the filtered urate is excreted into urine. At the normal blood pH (= 7.4), UA (effective pK_a in blood = 5.75) is present almost completely (98%) as urate anion (see Fig. 1). Urate therefore cannot pass through cell membranes passively, but requires assistance from urate transporters. Clearly, these carriers are important, since altered function (by mutation or drug interaction) can cause hyperuricemia. Indeed, the main causal factors of primary gout seem to be diet and genetic polymorphisms of renal urate transporters [2]. Drugs that raise serum UA levels (= antiuricosuric) include diuretics, pyrazinoate, pyrazinamide, ethambutol, and the NSAIDs aspirin and diclofenac.

Abbreviations: 6CF, 6-carboxyfluorescein; Asp, aspartate; DHEAS, dehydroepiandrosterone sulfate; E3S, estrone-3-sulfate; ETT, ergothioneine transporter; Glu, glutamate; LC, liquid chromatography; MS, mass spectrometry; PAH, *p*-aminohippuric acid; UA, uric acid.

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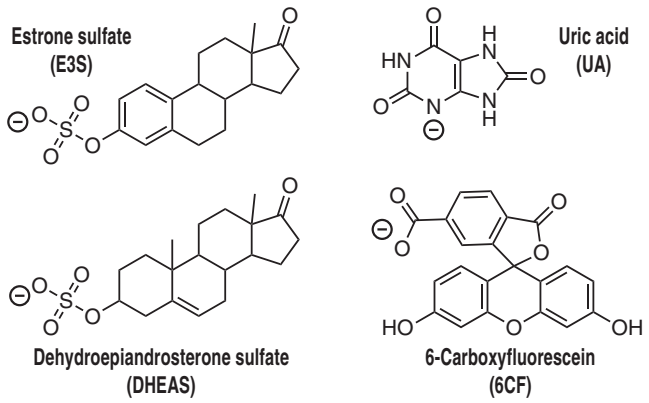

Fig. 1. Structures of substrates of SLC22A11. Charges are drawn to indicate the dominating species at pH = 7.4.

Prominent transporters for the reabsorption of urate in the proximal tubule are SLC22A12 (functional name URAT1) [6] in the apical and SLC2A9 (URATv1, GLUT9) [7] in the basolateral domain; however, several other carriers seem to be involved, both in secretion and reabsorption [5,8]. Among these, SLC22A11 (OAT4) [9] stands out since it exists only in humans and higher primates; this matches exactly the species with high basal UA serum levels. Even more importantly, SLC22A11 must be relevant for serum UA levels, since an association between genetic variants of SLC22A11 and serum UA levels was detected in genome-wide association studies [10,11].

SLC22A11 mRNA was confined on a Northern blot to kidney and placenta [12]. The protein was detected by immunohistochemistry in the apical membrane of proximal tubules [13] and on the basolateral membrane of the syncytiotrophoblast [14]. A transport function was first demonstrated for SLC22A11 by heterologous expression in *Xenopus* oocytes where the uptake of radiotracer, compared to control oocytes, was increased about 10-fold for estrone sulfate (E3S) and dehydroepiandrosterone sulfate (DHEAS) [12]. The K_m values were very low, at 1 and 0.6 μM, respectively. Later, uptake of ^{14}C-uric acid via SLC22A11 was demonstrated based on the expression in oocytes (5-fold increase; the specific uptake relative to URAT1 was at 38%) and in 293 cells (1.7-fold increase; specific clearance = 0.013 μl min^{-1} mg protein^{-1}) [9]. Also, 6-carboxyfluorescein (6CF) was introduced as a substrate for uptake here. Uptake of *p*-aminohippuric acid (PAH) and glutarate into mouse cells stably expressing SLC22A11 [13] was not confirmed in subsequent studies [9] including our own experiments (PAH uptake, radiotracer and LC–MS/MS assays; not shown); however, efflux of PAH and glutarate via SLC22A11 was inferred from *trans*-stimulation (293 cells) and radiotracer efflux (oocytes) experiments [9]. Because of the much higher transport efficiency and the better signal-to-background ratio, E3S and its congener DHEAS (see Fig. 1 for structures) are usually favored over uric acid as model uptake substrates of SLC22A11.

Because of its vastly hydrophobic structure (log *P* = 3.1), estrone is hardly soluble in water (limit: 50 or 110 μM). Attachment of sulfate improves solubility about 100 times to 10 mM. Compared to other typical substrates of SLC22 transporters like 1-methyl-4-phenylpyridinium (M_r = 170), PAH (193), ergothioneine (229), carnitine (161), and uric acid (167), E3S is considerably larger (349) and much more hydrophobic. It is puzzling that besides SLC22A11 so many (= 18) transporters from separate families have been reported to take up E3S (Table 1). The aim of this study was to scrutinize the mechanism of transport of E3S by SLC22A11, by direct comparison with uric acid. Amazingly, our results indicate that SLC22A11 provides entirely different transport mechanisms for E3S and UA. We arrive at the unprecedented conclusion that E3S is not translocated into the cytosol. Instead, SLC22A11 catalyzes both the insertion into and the extraction from the plasma membrane of E3S.

Table 1
List of putative E3S uptake transporters of the plasma membrane. Heterologous expression of these carriers resulted in accumulation of E3S in vesicles, oocytes, or mammalian cells to at least twice the control value.

#	Transporter	Gene symbol	Reference
1	MRP1	*ABCC1*	[31]
2	BCRP	*ABCG2*	[32]
3	NTCP	*SLC10A1*	[33]
4	SOAT	*SLC10A6*	[34]
5	NPT1	*SLC17A1*	[35]
6	OAT2	*SLC22A7*	[36]
7	OAT3	*SLC22A8*	[37]
8	OAT7	*SLC22A9*	[38]
9	OAT6	*SLC22A20*	[39]
10	MATE1	*SLC47A1*	[40]
11	MATE2K	*SLC47A2*	[40]
12	OSTα + OSTβ	*SLC51A + SLC51B*	[41]
13	OATP1A2	*SLCO1A2*	[42]
14	OATP1B1	*SLCO1B1*	[43]
15	OATP1B3	*SLCO1B3*	[44]
16	OATP2B1	*SLCO2B1*	[43]
17	OATP4A1	*SLCO4A1*	[45]
18	OATP4C1	*SLCO4C1*	[29]

2. Materials & methods

2.1. Plasmid constructs

The cDNA coded by the *SLC22A11* gene from human was generated by RT-PCR, cloned into pUC19, fully sequenced, and inserted into expression vector pEBTetLNC, a derivative of pEBTetD. pEBTetD is an episomal Epstein-Barr plasmid vector for doxycycline-inducible protein expression in human cell lines based on the simple tetracycline repressor [15]. pEBTetLNC contains, in addition, UCOE0.7 (ubiquitous chromatin opening element) [16] upstream of the CMV enhancer/promoter to prolong plasmid maintenance in transfected cell lines. The amino acid sequence of SLC22A11h corresponds to GenBank entry NM_018484. The 5′-interface between pEBTetLNC and the cDNA is **GTTTAAACTT AAGCTT** GCCACC ATGGCGTTCTCGAAG (polylinker in bold, cDNA underlined); the 3′-interface is AGTACCTCGCTCTAG **CTCGAG CGATCGC**. Plasmid pEBTetD/SLC22A13h has been described previously [17].

2.2. Cell culture

293 cells (ATCC CRL-1573; also known as HEK-293 cells), a transformed cell line derived from human embryonic kidney, were grown at 37 °C in a humidified atmosphere (5% CO_2) in plastic culture flasks (Falcon 353112, Becton Dickinson, Heidelberg, Germany). The growth medium was Dulbecco's Modified Eagle Medium (Life Technologies 31885-023, Invitrogen, Karlsruhe, Germany) supplemented with 10% fetal bovine serum (Biochrom, Berlin, Germany), 100 U/ml penicillin, and 0.1 mg/ml streptomycin (P4458, Sigma-Aldrich, Munich, Germany). Medium was changed every 2–3 days and the culture was split every 5 days.

Stably transfected cell lines were generated as reported previously [15]. As there is no integration of pEBTet vectors into the genome, clonal isolation of transfected cells is not necessary; we thus use cell pools rather than single cell clones. Cell culture medium always contained 3 μg/ml puromycin (P-600, Gold Biotechnology, St. Louis, MO, USA) to maintain plasmids. To turn on protein expression, cells were cultivated for at least 20 h with 1 μg/ml doxycycline (195044, MP Biomedicals, Eschwege, Germany) in