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1 **Study of the transport of thyroid hormone by transporters of the SLC10 family**

2

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21 **Key words**

22 SLC10 family; SLC10A1; Ntcp; transporter; thyroid hormone; iodothyronine (sulfates);
23 taurocholate; CRYM, mu-crystallin

24

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28

29 **Disclosure statement**

30 The authors have nothing to disclose.

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32

1 **Abstract**

2 Transport of (sulfated) iodothyronines across the plasma membrane is required for their
3 intracellular metabolism. Rat Na⁺/taurocholate cotransporting polypeptide (Ntcp; Slc10a1) has been
4 identified as an important transporter protein. We demonstrate that among the 7 members of the solute
5 carrier family SLC10, only human SLC10A1 mediates sodium-dependent transport of the
6 iodothyronine T4 and iodothyronine sulfates T3S and T4S. In contrast to SLC10A2-7, cells co-
7 expressing SLC10A1 and the deiodinase D1 demonstrate a dramatic increase in T3S and T4S
8 metabolism. The SLC10A1 substrates taurocholate, DHEAS and E3S inhibit T3S and T4S transport.
9 Furthermore, co-transfection of SLC10A1 with CRYM, a well-known intracellular iodothyronine-
10 binding protein, results in an enhanced intracellular accumulation of T3S and T4S, indicating that
11 CRYM binds iodothyronine sulfates.

12 The present findings indicate that the liver-specific transporter SLC10A1 transports (sulfated)
13 iodothyronines, thereby increasing their intracellular availability. Therefore, SLC10A1 may fulfill a
14 critical step in providing liver D1 with iodothyronine sulfates for rapid degradation.

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Introduction

Thyroid hormone (TH) is essential for numerous metabolic processes. A principal regulatory role to achieve appropriate intracellular TH concentrations is reserved for the 3 deiodinating enzymes. The deiodinases D1 and D2 activate TH by converting the prohormone T4 to T3, whereas D3 has an inactivating function by deiodination of T4 and T3 to transcriptionally inactive rT3 and 3,3'-T2, respectively [1]. Although deiodination is regarded as the major pathway for TH metabolism, a number of alternate pathways have physiological relevance [2]. Sulfoconjugation is catalyzed by sulfotransferases, which transfer a sulfate group to the phenolic ring of iodothyronines. Although the thyromimetic activity of iodothyronine sulfates is limited, these compounds may have biological relevance [3,4]. It is known that sulfation accelerates degradation of the iodothyronines by D1 [5]. On the other hand, biologically inactive sulfated TH may serve as a pool contributing to the availability of TH, because, for example, bioactive T3 may be recovered from T3S by sulfatases in selective tissues [2].

Deiodination of iodothyronines and their sulfates takes place intracellularly. Therefore, transport of (sulfated) iodothyronines across the plasma membrane, which is facilitated by transporter proteins, is required [6]. However, only a few proteins are known to be active and specific iodothyronine transporters [6]. Previously, we found that *Xenopus laevis* oocytes injected with cRNA coding for rat Na⁺/taurocholate cotransporting polypeptide (Ntcp), currently known as Slc10a1, facilitated uptake of iodothyronines and their sulfates [7]. In addition to the known human ileal apical sodium-dependent bile acid transporter (ASBT; SLC10A2), five new members of the SLC10 family were identified recently [8-10]. However, SLC10A3, 4, 5 and 7 are orphan transporters for which no substrates have been identified.

For several TH transporters, it has been shown that different members of the same transporter family, facilitate TH transport, such as monocarboxylate transporter (MCT) 8 and MCT10 of the MCT family, and multiple members of the organic anion transporting polypeptide (OATP) family, including OATP1A2, 1C1, 1B1 and 1B3 [11-14]. Therefore, in the present study we investigate in transfected HEK293 and COS1 cells which members of the SLC10 family, in addition to SLC10A1, may transport (sulfated) iodothyronines, whether or not in addition to already known substrates.

Materials and Methods

Materials

[¹²⁵I]T3, [¹²⁵I]T4, [¹²⁵I]T3S and [¹²⁵I]T4S were synthesized as described [15]. [³H]taurocholate ([³H]TC) and [³H]estrone-1-sulfate ([³H]E1S) were purchased from Perkin Elmer (Boston, MA). Unlabeled iodothyronines were obtained from Henning (Berlin, Germany). All other compounds were purchased from Sigma (St. Louis, MO).

1 Cloning

2 Human (h) SLC10A1 (kindly provided by prof. dr. P.J. Meier, Zurich, Switzerland), hSLC10A2
3 (obtained from Open Biosystems, Huntsville, AL) and hSLC10A3 (kindly provided by dr. T. Abe,
4 Japan) were subcloned in pcDNA3 (Invitrogen, Carlsbad, CA). The coding regions of rat (r) Slc10a4
5 and hSLC10A5-7 were cloned in pcDNA5 (Invitrogen, Carlsbad, CA) as described [9,10,16,17].
6 rSlc10a4 was used as the hSLC10A4 clone was not available at the time of our experiments. Human
7 μ -crystallin (hCRYM) was cloned in the pSG5 vector (Stratagene, La Jolla, CA) [12] and rD1 in
8 pcDNA3 [18].

9

10 Transport studies

11 For transport experiments equal numbers of HEK293 and COS1 cells were seeded in 6-well
12 culture dishes in DMEM/F12 medium supplemented with 9% heat-inactivated FBS and 100 nM
13 sodium selenite. This results in very similar amounts of cellular protein as determined by a standard
14 Bradford assay. At 75% confluency, cells were transfected with 500 ng pcDNA3.hSLC10A1-3,
15 pcDNA5.rSlc10a4, or pcDNA5.hSLC10A5-7 alone or co-transfected with 500 ng pSG5.hCRYM. 48
16 h after transfection, cells were washed with incubation medium (Dulbecco's PBS containing 0.1% D-
17 glucose with or without 0.1% BSA). Uptake was tested by incubation of the cells for 5-30 min at
18 37°C with 1 nM (1×10^5 cpm) [125 I]T3, [125 I]T4, [125 I]T3S or [125 I]T4S in 1.5 ml incubation medium, in
19 the absence or presence of taurocholate (TC), dehydroepiandrosterone sulfate (DHEAS) or estrone-3-
20 sulfate (E3S). After incubation, cells were washed with incubation medium, lysed with 0.1 M NaOH,
21 and counted in a gamma counter. Uptake of [3 H]TC and [3 H]E1S by the SLC10 carriers was tested in
22 a similar manner by incubation of cells for 30 min with 1 μ M [3 H]TC or 0.1 μ M [3 H]E1S in 1.5 ml
23 incubation medium. Cells were lysed with 0.1% SDS and subsequently counted for radioactivity.

24 For measurement of efflux, cells were loaded for 30 min with 1.5 ml incubation medium
25 containing 1 nM (1×10^5 cpm) [125 I]T3S or [125 I]T4S. After removal of the medium, cells were briefly
26 washed and incubated for 5-30 min with incubation medium (containing 1% BSA) without ligand.
27 Finally, medium was removed, and cells were washed with incubation medium, lysed with 0.1 M
28 NaOH and counted in a gamma counter.

29 To evaluate Na^+ -dependence of transport, 1 nM [125 I]T4, [125 I]T3S, [125 I]T4S or 1 μ M [3 H]TC
30 were incubated with the cells in a medium containing 142.9 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO_4 ,
31 1.2 mM KH_2PO_4 , 1.8 mM CaCl_2 , 20 mM HEPES and 0.1% BSA (pH 7.4) or in medium where Na^+
32 was replaced with an equimolar amount of choline.

33

34 Metabolism studies

35 COS1 cells were cultured in 24-well culture dishes and transfected with 100 ng pcDNA3.rD1 and
36 100 ng pcDNA3, pcDNA3.hSLC10A1-3, pcDNA5.rSlc10a4 or pcDNA5.hSLC10A5-7. After 48 h
37 transfection, cells were incubated for 24 h at 37°C with 1 nM (5×10^5 cpm) [125 I]T3S or [125 I]T4S in

1 0.25 ml DMEM/F12. After incubation, medium was harvested and analyzed by HPLC as described
2 previously [18].

3

4 Statistical analysis

5 Results are presented as the means and ranges of duplicate determinations in a representative
6 experiment (n=2-4). Statistical significance was determined using the Student's *t* test.

7

8 **Results**

9 Previously, HEK293 cells as well as COS1 cells have been used to study various members of the
10 SLC10 family [9,10,16,17,19]. Therefore, we used both cell lines in our experiments. We first
11 assessed uptake of (sulfated) iodothyronines in HEK293 cells. No induction of T3 uptake was
12 observed in cells transfected with any of the SLC10 transporters (Fig. 1A and *data not shown*),
13 whereas an insignificant increase in T4 uptake was seen if SLC10A1 was expressed (Fig. 1B). T3S
14 and T4S uptake was largely induced by SLC10A1 in contrast to the other SLC10 transporters (Fig.
15 1C,D and *data not shown*). Figure 1E shows a clear time-dependent increase in the uptake of T3S and
16 T4S in cells expressing SLC10A1. As transporters may facilitate not only substrate uptake but also
17 efflux, we investigated whether SLC10A1 mediates T3S and T4S export. No difference in efflux of
18 T3S and T4S was observed between cells expressing low *versus* high levels of SLC10A1 (Fig. 1F,G),
19 suggesting that SLC10A1 does not facilitate the efflux of these substrates. Re-uptake of T3S and T4S
20 does not play a major role, as 1% BSA in the efflux medium efficiently blocks uptake of sulfated
21 iodothyronines (*data not shown*). We confirmed that SLC10A1 transports TC in a Na⁺-dependent
22 manner (*data not shown*). As anticipated, uptake of T4 (*data not shown*), T3S (Fig. 1H) and T4S (Fig.
23 1I) is also a Na⁺-dependent process.

24 Because HEK293 cells demonstrate a considerable endogenous transport activity of (sulfated)
25 iodothyronines, we also tested transfected COS1 cells for uptake of different ligands. To confirm that
26 COS1 cells transfected with cDNA coding for each of the SLC10 family members are appropriate to
27 study transport function, uptake was studied of TC, a known substrate for SLC10A1 and 2. Fig. 2
28 shows a 69-fold and 6-fold increase of TC uptake in cells transfected with SLC10A1 and 2,
29 respectively, whereas the other SLC10 members did not demonstrate any transport activity.
30 Furthermore, E1S was only transported by SLC10A1 and 6 (*data not shown*). These results agree with
31 the existing evidence, indicating that this cell expression system is appropriate to study transporter
32 characteristics. Because functional expression can only be demonstrated for SLC10A1, 2 and 6,
33 transport studies are only shown for these transporters.

34 Then, we examined the uptake of the iodothyronines T3 and T4 by transfected COS cells. Fig.
35 3A,B clearly shows that only SLC10A1 is modestly capable of transporting T4, whereas T3 is not
36 transported by any of the SLC10 proteins. In contrast to the unconjugated iodothyronines, the T3S and
37 T4S cellular uptake is largely stimulated if SLC10A1 is expressed (Fig. 3C,D). The induction of

1 uptake is more pronounced if cells are co-transfected with CRYM, a cytosolic binding protein with
2 high affinity for iodothyronines. In contrast to SLC10A1, SLC10A2-7 do not transport the tested
3 iodothyronine sulfates (Fig. 3C,D and *data not shown*).

4 To assess whether SLC10A1-mediated transport of iodothyronine sulfates facilitates their
5 intracellular metabolism by D1, cells were co-transfected with SLC10A1-7 and D1. Subsequently,
6 cells were incubated for 24 h with T3S or T4S and medium radioactivity was analyzed. Intracellular
7 T3S and T4S metabolism dramatically increased in cells co-expressing SLC10A1 and D1 (*data not*
8 *shown*). In contrast, in cells co-expressing any of the other SLC10 proteins with D1, negligible
9 metabolite production was seen. In the analyzed medium, only the substrate (T3S or T4S) or I⁻ was
10 observed, but no intermediate metabolites.

11 In an attempt to calculate kinetic parameters for T3S and T4S uptake by SLC10A1, we
12 incubated cells transfected with SLC10A1 with 0.01-5 μM of each substrate. No clear saturation was
13 observed for T3S and T4S uptake at concentrations of up to 5 μM (*data not shown*). It was not
14 possible to study the effects of higher concentrations of T3S and T4S due to the limited availability of
15 these compounds caused by the low solubility of the iodothyronines used for their synthesis.

16 The previous experiments indicate that of all SLC10 transporters only SLC10A1 specifically
17 transports T3S and T4S. Because SLC10A1 also facilitates uptake of bile acids and steroid sulfates,
18 we studied the inhibitory effects of TC, DHEAS and E3S on SLC10A1-mediated transport of T3S and
19 T4S. As the tested compounds may interfere with T3S or T4S binding to BSA, we used incubation
20 medium without BSA to directly test the interactions with SLC10A1. At 50 μM concentrations,
21 SLC10A1-mediated T3S and T4S transport is reduced to ~20% by TC, whereas weaker inhibitory
22 effects are observed by DHEAS and E3S (Fig. 4).

23 Finally, we pursued the interesting observation that co-transfection of CRYM, which has
24 affinity for the iodothyronines T4, T3, 3,5-T2 and, less so, rT3 [20,21], enhanced net cellular uptake
25 of iodothyronine sulfates (Fig. 3). Therefore, we tested the hypothesis that CRYM binds intracellular
26 T3S and T4S, resulting in a diminished T3S and T4S efflux. This mechanism would explain the
27 increased accumulation of the iodothyronine sulfates in cells co-expressing SLC10A1 and CRYM.
28 We studied efflux of T3S and T4S after 30 min incubation with these ligands in cells transfected with
29 SLC10A1 alone or in combination with CRYM. Fig. 5 demonstrates that in the absence of CRYM,
30 T3S and T4S are rapidly released from the cells. In contrast, T3S and T4S efflux was diminished
31 significantly in cells co-expressing SLC10A1 and CRYM. HPLC analysis of the cell contents detected
32 solely the incubated substrates T3S or T4S, but no metabolites (*data not shown*). This indicates that
33 CRYM binds intracellular T3S and T4S and, thus, largely prevents their efflux.

34

35 Discussion

36 NTCP (SLC10A1) and ASBT (SLC10A2), which were the SLC10 family members discovered
37 initially, are both known as bile acid transporters [8]. Of the 5 recently identified SLC10 proteins,

1 only the sodium-dependent organic anion transporter (SOAT; SLC10A6) has been shown to transport
2 steroid sulfates but not bile acids, whereas SLC10A3-7 remained orphan carriers [8-10]. The
3 hypothesis to test which members of the SLC10 family transport iodothyronines is based on the
4 observation that rSlc10a1 transports (sulfated) iodothyronines and that in different transporter families
5 several members, with varying degrees of similarity, transport iodothyronines [7,22]. Therefore, we
6 investigated which members of this SLC10 transporter family are capable of mediating transport of
7 (sulfated) iodothyronines.

8 We directly compared the TC transport by all SLC10 proteins. As expected, only SLC10A1 and 2
9 facilitated TC uptake. This finding confirms earlier reports that only these two SLC10 proteins
10 transport TC [8,9]. In cells expressing SLC10A1, TC uptake is much higher than in cells transfected
11 with SLC10A2. This is also in agreement with earlier observations, which suggested a higher
12 apparent V_{\max} for TC transport by SLC10A1 compared to SLC10A2 [19].

13 In subsequent experiments the uptake was studied of the iodothyronines T3 and T4 as well as the
14 iodothyronine sulfates T3S and T4S by cells transfected with cDNA of each of the SLC10 members.
15 Our findings clearly demonstrate the absence of any uptake of the tested iodothyronine derivatives by
16 any of the SLC10 proteins, except for SLC10A1. These negative findings were substantiated by
17 metabolism studies, showing no stimulation of T3S and T4S conversion in cells co-expressing
18 SLC10A2-7 and D1 compared to controls. These data are remarkable as at least some SLC10 proteins
19 appeared to be likely transporters for the tested substrates. For example, despite a marked amino acid
20 similarity (62.8%) between SLC10A1 and 2, which even have a common substrate (TC), no transport
21 was observed of the tested iodothyronine derivatives by SLC10A2 [8]. Furthermore, no transport was
22 seen of iodothyronine sulfates by SLC10A6, in spite of the preferential transport of steroid sulfates
23 and the 62.6% amino acid similarity to SLC10A1 [8]. These data suggest a high degree of substrate
24 specificity in this transporter family. Closely agreeing results were obtained for SLC10A1-7 in
25 different cell lines (HEK293, COS1) and for SLC10A1 and 3 in *Xenopus* oocytes (Friesema *et al.*,
26 unpublished observations). However, it cannot be excluded that other factors like ancillary proteins
27 are needed for proper function of the transporters which did not demonstrate transport activity in the
28 present study. It should be noted that substrates are only known for SLC10A1, 2 and 6. Thus, the
29 absence of TH transport has only been demonstrated for SLC10A2 and 6. Functional expression of
30 SLC10A3, 4, 5 and 7 could not be established due to the lack of physiological substrates.

31 Among the SLC10 family, our findings indicate that only SLC10A1 transports the iodothyronine
32 T4 and iodothyronine sulfates T3S and T4S. As expected, this transport was Na^+ -dependent, like that
33 of other substrates such as TC and E3S [8]. Our data suggest that a negative charge is important for
34 iodothyronines to be transported by SLC10A1. The phenolic hydroxyl group of T4 dissociates
35 (becomes negatively charged) above pH 6.5, whereas that of T3 only above pH 8.5. The highly
36 negative charge of the sulfate group substantiates the importance of ionisation for substrate
37 recognition or binding to SLC10A1. If SLC10A1 and D1 are co-expressed, a dramatic increase of

1 T3S and T4S metabolism is observed. This indicates that SLC10A1 increases the intracellular
2 availability of T3S and T4S. It was not possible to calculate kinetic parameters for T3S and T4S
3 transport.

4 The fold increase in uptake induced by hSLC10A1 was larger for iodothyronine sulfates than for
5 the native iodothyronines (due to lower background rather than larger absolute increase). Our findings
6 suggest that hSLC10A1 plays an important role in the uptake of iodothyronine sulfates into the liver
7 *in vivo* where they are rapidly degraded by D1 [5]. This is in line with the observation that SLC10A1
8 mainly facilitates uptake, but not efflux. In our T3S and T4S metabolism assays, we observed only I
9 as a metabolite, which underlines that these compounds and their intermediates (T2S and rT3S) are
10 converted very rapidly. Therefore, SLC10A1 may fulfill a critical step in providing liver D1 with
11 iodothyronine sulfates for rapid degradation, thereby contributing to the low circulating levels of
12 iodothyronine sulfates in adulthood.

13 An opposite situation may exist in fetal life. It is known that serum iodothyronine sulfate levels
14 are much higher in the fetus than in adults, which may serve as an additional regulatory mechanism
15 for optimizing tissue-specific TH bioavailability [23,24]. Several factors, like low D1 activity in fetal
16 life, may contribute to these increased levels of fetal iodothyronine sulfates. However, mouse and rat
17 Slc10a1 expression dramatically increases from low levels before birth to high levels at birth [25,26].
18 In human fetal liver, the expression of SLC10A1 is almost undetectable [27]. The low expression of
19 human SLC10A1 during fetal development may result in a low uptake of iodothyronine sulfates into
20 the liver. This will diminish the supply of iodothyronine sulfates to hepatic D1, resulting in a decrease
21 of clearance of these compounds. This indicates that the metabolism of iodothyronine sulfates is not
22 only regulated by D1 expression but also depends on the level of transporter proteins. This is
23 supported by recent observations in which some members of the OATP family are also capable of
24 transporting T3S and T4S [14,28]. The contribution of the different transporters (SLC10A1 *versus*
25 OATPs) to T3S and T4S transport requires further studies.

26 The SLC10A1 substrates TC, DHEAS and E3S were able to inhibit T3S and T4S transport, of
27 which TC exerted the most potent inhibitory effects. Recently, bile acids were shown to induce TH-
28 mediated metabolism via a signalling pathway involving D2 [29]. Our observations that iodothyronine
29 sulfates are transported by the predominant hepatic bile acid transporter SLC10A1 may add a new
30 dimension to the concept connecting bile acids and TH in the control of energy expenditure. When
31 serum bile acids concentrations increase, for example after a meal [30], uptake of iodothyronine
32 sulfates by SLC10A1 will diminish, thereby preventing hepatic clearance of iodothyronine sulfates.
33 As a consequence, increased serum concentrations of iodothyronine sulfates may be available for
34 desulfation in extrahepatic tissues, resulting in increased bioactive TH concentrations and, thus,
35 increased metabolism in these tissues. These effects would be most effective in cells possessing both a
36 T3S/T4S transporter and a sulfatase. It should be stressed that the physiological importance remains
37 speculative, as serum concentrations of iodothyronine sulfates are generally low in adults [23,31].

1 Therefore, the degree of contribution to biologically active TH via desulfation of iodothyronine
2 sulfates remains uncertain.

3 We performed several experiments in which we studied the effect of co-transfection with CRYM
4 on the uptake and efflux of iodothyronine sulfates. Our observations that CRYM prevents the efflux
5 of the tested substrates may suggest that CRYM binds iodothyronine sulfates. This is substantiated by
6 the finding that in these experiments cellular T3S and T4S remained intact. Originally, CRYM was
7 reported to bind to native iodothyronines in the presence (T3>T4>rT3) and also the absence (3,5-T2)
8 of NADPH [20,21]. Subsequent studies mainly focused on the role of CRYM in the intracellular
9 binding of T3 [32-34]. Our findings may implicate an extension of the role of CRYM to function as a
10 binding protein for iodothyronine sulfates. If so, one would speculate that it may direct the
11 iodothyronine sulfates to intracellular targets, for example D1. However, it should be noted that
12 kinetic properties of CRYM were not directly studied. Therefore, additional studies are required to
13 elucidate the *in vivo* relevance of this apparent novel function of CRYM.

14 In conclusion, we provide evidence that also human SLC10A1 transports iodothyronine sulfates,
15 in contrast with SLC10A2-7. In addition, our results suggest that CRYM is capable of binding
16 iodothyronine sulfates. The present paper supports the notion that TH regulates metabolism via
17 different pathways. Further studies are required to investigate to which extent our findings are
18 relevant for physiology.

19

20

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23

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29 **Disclosure statement**

30 The authors have nothing to disclose.

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1 **References**

- 2 [1] Bianco, A.C. and Kim, B.W. (2006) Deiodinases: implications of the local control of
3 thyroid hormone action. *J Clin Invest* 116, 2571-9.
- 4 [2] Wu, S.Y., Green, W.L., Huang, W.S., Hays, M.T. and Chopra, I.J. (2005) Alternate
5 pathways of thyroid hormone metabolism. *Thyroid* 15, 943-58.
- 6 [3] Spaulding, S.W., Smith, T.J., Hinkle, P.M., Davis, F.B., Kung, M.P. and Roth, J.A.
7 (1992) Studies on the biological activity of triiodothyronine sulfate. *J Clin Endocrinol*
8 *Metab* 74, 1062-7.
- 9 [4] Santini, F., Hurd, R.E., Lee, B. and Chopra, I.J. (1993) Thyromimetic effects of
10 3,5,3'-triiodothyronine sulfate in hypothyroid rats. *Endocrinology* 133, 105-10.
- 11 [5] Otten, M.H., Mol, J.A. and Visser, T.J. (1983) Sulfation preceding deiodination of
12 iodothyronines in rat hepatocytes. *Science* 221, 81-3.
- 13 [6] Visser, W.E., Friesema, E.C., Jansen, J. and Visser, T.J. (2008) Thyroid hormone
14 transport in and out of cells. *Trends Endocrinol Metab* 19, 50-56.
- 15 [7] Friesema, E.C., Docter, R., Moerings, E.P., Stieger, B., Hagenbuch, B., Meier, P.J.,
16 Krenning, E.P., Hennemann, G. and Visser, T.J. (1999) Identification of thyroid
17 hormone transporters. *Biochem Biophys Res Commun* 254, 497-501.
- 18 [8] Geyer, J., Wilke, T. and Petzinger, E. (2006) The solute carrier family SLC10: more
19 than a family of bile acid transporters regarding function and phylogenetic
20 relationships. *Naunyn Schmiedebergs Arch Pharmacol* 372, 413-31.
- 21 [9] Godoy, J.R., Fernandes, C., Doring, B., Beuerlein, K., Petzinger, E. and Geyer, J.
22 (2007) Molecular and phylogenetic characterization of a novel putative membrane
23 transporter (SLC10A7), conserved in vertebrates and bacteria. *Eur J Cell Biol* 86,
24 445-60.
- 25 [10] Geyer, J., Fernandes, C.F., Doring, B., Burger, S., Godoy, J.R., Rafalzik, S.,
26 Hubschle, T., Gerstberger, R. and Petzinger, E. (2008) Cloning and molecular
27 characterization of the orphan carrier protein Slc10a4: Expression in cholinergic
28 neurons of the rat central nervous system. *Neuroscience*.
- 29 [11] Friesema, E.C., Ganguly, S., Abdalla, A., Manning Fox, J.E., Halestrap, A.P. and
30 Visser, T.J. (2003) Identification of monocarboxylate transporter 8 as a specific
31 thyroid hormone transporter. *J Biol Chem* 278, 40128-35.
- 32 [12] Friesema, E.C., Jansen, J., Jachtenberg, J.W., Visser, W.E., Kester, M.H. and Visser,
33 T.J. (2008) Effective cellular uptake and efflux of thyroid hormone by human
34 monocarboxylate transporter 10 (MCT10). *Mol Endocrinol* 22, 1357-69.
- 35 [13] Pizzagalli, F., Hagenbuch, B., Stieger, B., Klenk, U., Folkers, G. and Meier, P.J.
36 (2002) Identification of a novel human organic anion transporting polypeptide as a
37 high affinity thyroxine transporter. *Mol Endocrinol* 16, 2283-96.
- 38 [14] van der Deure, W.M., Friesema, E.C., de Jong, F.J., de Rijke, Y.B., de Jong, F.H.,
39 Uitterlinden, A.G., Breteler, M.M., Peeters, R.P. and Visser, T.J. (2008) OATP1B1:
40 an important factor in hepatic thyroid hormone and estrogen transport and
41 metabolism. *Endocrinology* 149, 1495-701.
- 42 [15] Mol, J.A. and Visser, T.J. (1985) Synthesis and some properties of sulfate esters and
43 sulfamates of iodothyronines. *Endocrinology* 117, 1-7.
- 44 [16] Geyer, J., Doring, B., Meerkamp, K., Ugele, B., Bakhiya, N., Fernandes, C.F., Godoy,
45 J.R., Glatt, H. and Petzinger, E. (2007) Cloning and functional characterization of
46 human sodium-dependent organic anion transporter (SLC10A6). *J Biol Chem* 282,
47 19728-41.
- 48 [17] Fernandes, C.F., Godoy, J.R., Doring, B., Cavalcanti, M.C., Bergmann, M., Petzinger,
49 E. and Geyer, J. (2007) The novel putative bile acid transporter SLC10A5 is highly
50 expressed in liver and kidney. *Biochem Biophys Res Commun* 361, 26-32.

- 1 [18] Friesema, E.C., Kuiper, G.G., Jansen, J., Visser, T.J. and Kester, M.H. (2006) Thyroid
2 hormone transport by the human monocarboxylate transporter 8 and its rate-limiting
3 role in intracellular metabolism. *Mol Endocrinol* 20, 2761-72.
- 4 [19] Craddock, A.L., Love, M.W., Daniel, R.W., Kirby, L.C., Walters, H.C., Wong, M.H.
5 and Dawson, P.A. (1998) Expression and transport properties of the human ileal and
6 renal sodium-dependent bile acid transporter. *Am J Physiol* 274, G157-69.
- 7 [20] Vie, M.P., Evrard, C., Osty, J., Breton-Gilet, A., Blanchet, P., Pomerance, M.,
8 Rouget, P., Francon, J. and Blondeau, J.P. (1997) Purification, molecular cloning, and
9 functional expression of the human nicotinamide-adenine dinucleotide phosphate-
10 regulated thyroid hormone-binding protein. *Mol Endocrinol* 11, 1728-36.
- 11 [21] Moreno, M., Silvestri, E., Lombardi, A., Visser, T.J., Goglia, F. and Lanni, A. (2003)
12 Identification of 3,5-diiodo-L-thyronine-binding proteins in rat liver cytosol by
13 photoaffinity labeling. *Endocrinology* 144, 2297-303.
- 14 [22] Visser, W.E., Friesema, E.C., Jansen, J. and Visser, T.J. (2008) Thyroid hormone
15 transport in and out of cells. *Trends Endocrinol Metab* 19, 50-6.
- 16 [23] Chopra, I.J., Wu, S.Y., Teco, G.N. and Santini, F. (1992) A radioimmunoassay for
17 measurement of 3,5,3'-triiodothyronine sulfate: studies in thyroidal and nonthyroidal
18 diseases, pregnancy, and neonatal life. *J Clin Endocrinol Metab* 75, 189-94.
- 19 [24] Santini, F., Cortelazzi, D., Baggiani, A.M., Marconi, A.M., Beck-Peccoz, P. and
20 Chopra, I.J. (1993) A study of the serum 3,5,3'-triiodothyronine sulfate concentration
21 in normal and hypothyroid fetuses at various gestational stages. *J Clin Endocrinol*
22 *Metab* 76, 1583-7.
- 23 [25] Cheng, X., Buckley, D. and Klaassen, C.D. (2007) Regulation of hepatic bile acid
24 transporters Ntcp and Bsep expression. *Biochem Pharmacol* 74, 1665-76.
- 25 [26] Boyer, J.L., Hagenbuch, B., Ananthanarayanan, M., Suchy, F., Stieger, B. and Meier,
26 P.J. (1993) Phylogenetic and ontogenic expression of hepatocellular bile acid transport.
27 *Proc Natl Acad Sci U S A* 90, 435-8.
- 28 [27] Chen, H.L., Liu, Y.J., Feng, C.H., Wu, C.Y., Shyu, M.K., Yuan, R.H. and Chang,
29 M.H. (2005) Developmental expression of canalicular transporter genes in human
30 liver. *J Hepatol* 43, 472-7.
- 31 [28] van der Deure, W.M., Hansen, P.S., Peeters, R.P., Kyvik, K.O., Friesema, E.C.,
32 Hegedus, L. and Visser, T.J. (2008) Thyroid hormone transport and metabolism by
33 OATP1C1 and consequences of genetic variation. *Endocrinology* 149, 5307-14.
- 34 [29] Watanabe, M., Houten, S.M., Matakai, C., Christoffolete, M.A., Kim, B.W., Sato, H.,
35 Messaddeq, N., Harney, J.W., Ezaki, O., Kodama, T., Schoonjans, K., Bianco, A.C.
36 and Auwerx, J. (2006) Bile acids induce energy expenditure by promoting
37 intracellular thyroid hormone activation. *Nature* 439, 484-9.
- 38 [30] Everson, G.T. (1987) Steady-state kinetics of serum bile acids in healthy human
39 subjects: single and dual isotope techniques using stable isotopes and mass
40 spectrometry. *J Lipid Res* 28, 238-52.
- 41 [31] Eelkman Rooda, S.J., Kaptein, E. and Visser, T.J. (1989) Serum triiodothyronine
42 sulfate in man measured by radioimmunoassay. *J Clin Endocrinol Metab* 69, 552-6.
- 43 [32] Suzuki, S., Mori, J., Kobayashi, M., Inagaki, T., Inaba, H., Komatsu, A., Yamashita,
44 K., Takeda, T., Miyamoto, T., Ichikawa, K. and Hashizume, K. (2003) Cell-specific
45 expression of NADPH-dependent cytosolic 3,5,3'-triiodo-L-thyronine-binding protein
46 (p38CTBP). *Eur J Endocrinol* 148, 259-68.
- 47 [33] Suzuki, S., Suzuki, N., Mori, J., Oshima, A., Usami, S. and Hashizume, K. (2007)
48 micro-Crystallin as an intracellular 3,5,3'-triiodothyronine holder in vivo. *Mol*
49 *Endocrinol* 21, 885-94.

- 1 [34] Suzuki, S., Mori, J. and Hashizume, K. (2007) mu-crystallin, a NADPH-dependent
2 T(3)-binding protein in cytosol. Trends Endocrinol Metab 18, 286-9.
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1 Figure legends

2
3 Fig. 1. Uptake of (sulfated) iodothyronines in HEK293 cells transfected with hCRYM and
4 hSLC10A1, hSLC10A2 and hSLC10A6. Uptake was measured after 30 min incubation with 1 nM T3
5 (A), T4 (B), T3S (C) or T4S (D) in D-PBS containing 0.1% glucose and 0.1% BSA. (E) Uptake of
6 T3S or T4S in HEK293 cells co-transfected with hCRYM and empty vector (circles) or hSLC10A1
7 (triangles). Uptake was measured after 5, 10 and 30 min incubation with 1 nM T3S (empty symbols)
8 or T4S (filled symbols) in D-PBS containing 0.1% glucose and 0.1% BSA. 1% uptake represents 10
9 pmol of incubated substrate. Cellular protein amounts to ~3 mg in all wells. Effects of high (500 ng -
10 filled circles) versus low (100 ng - open circles) expression of SLC10A1 on the efflux of T3S (F) and
11 T4S (G). Cells were incubated for 30 min with 1 nM T3S or T4S in D-PBS containing 0.1% glucose
12 and 0.1% BSA. After brief washing, cells were incubated (10-30 min) with efflux medium, containing
13 1% BSA, and subsequently processed as described in Materials and Methods. Results (mean \pm range)
14 are corrected for uptake in control-transfected cells. Treated as a first order process, the plot of log
15 cellular T3S or T4S vs time is linear, providing efflux rate constants which are independent of the
16 amount of SLC10A1 expressed. Na⁺-dependence of the SLC10A1-mediated uptake of T3S (H) and
17 T4S (I). Cells were incubated for 30 min with 1 nM T3S or T4S in sodium chloride medium or
18 sodium-free choline medium. Significances represent mean \pm range obtained in cells expressing a
19 SLC10 transporter *versus* empty vector. * P < 0.05; ** P < 0.01; *** P < 0.001.

20
21 Fig. 2. Uptake of 1 μ M TC in COS1 cells transfected with cDNA coding for each of the SLC10
22 family members. Cells transfected with empty vector were used as controls. Uptake was measured
23 after 30 min TC incubation in D-PBS containing 0.1% glucose and 0.1% BSA. 1% uptake represents
24 uptake of 10 nmol taurocholate. Cellular protein amounts to ~3 mg in all wells. * P < 0.05; ** P <
25 0.001.

26
27 Fig 3. Uptake of iodothyronines in COS1 cells transfected with cDNA coding for hSLC10A1,
28 hSLC10A2 and hSLC10A6. Uptake was measured after 30 min incubation with 1 nM T3 (A), T4 (B),
29 T3S (C) or T4S (D) in D-PBS containing 0.1% glucose and 0.1% BSA. Significances represent mean
30 \pm range obtained in cells expressing a SLC10 transporter *versus* empty vector, co-transfected with
31 (grey bars) or without (white bars) CRYM. 1% uptake represents uptake of 10 pmol of incubated
32 substrate. Cellular protein amounts to ~3 mg in all wells. * P < 0.05; ** P < 0.01; *** P < 0.001.

33
34 Fig. 4. Influence of 50 μ M concentrations of TC, DHEAS and E3S on the uptake of T3S (A) and T4S
35 (B) in HEK293 cells transfected with SLC10A1. Cells were incubated for 10 min with 1 nM T3S or
36 T4S in D-PBS containing 0.1% glucose in the presence of each of the inhibitors. Results are corrected

1 for uptake in control-transfected cells. 1% uptake represents uptake of 10 pmol of incubated substrate.
2 Cellular protein amounts to ~3 mg in all wells. * P < 0.05.

3

4 Fig. 5. Effects of CRYM on the efflux of iodothyronine sulfates. COS1 cells were transfected with
5 pcDNA3 or pcDNA3.hSLC10A1 with or without pSG5.hCRYM. Cells were incubated for 30 min
6 with T3S (A) or T4S (B). After brief washing, cells were incubated (5-30 min) with efflux medium,
7 containing 1% BSA, and subsequently processed as described in Materials and Methods.
8 Significances represent values obtained in cells expressing hSLC10A1 with hCRYM *versus*
9 hSLC10A1 without hCRYM. 1% uptake represents uptake of 10 pmol of incubated substrate. Cellular
10 protein amounts to ~3 mg in all wells. * P < 0.05; ** P < 0.01; *** P < 0.001.

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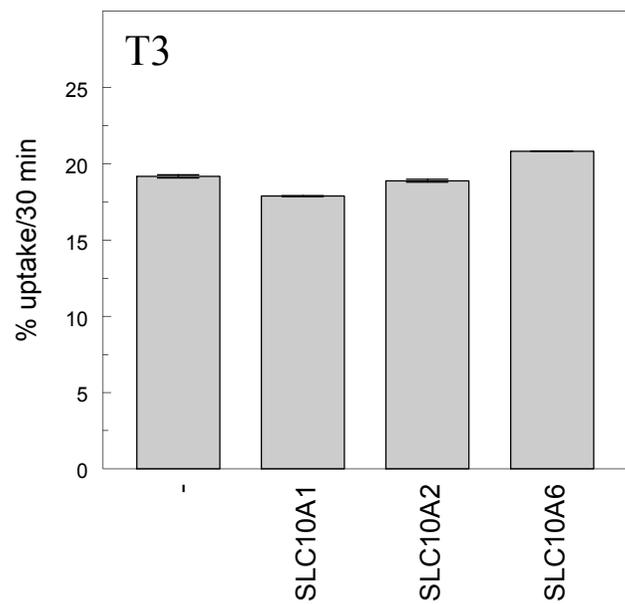


Fig. 1A

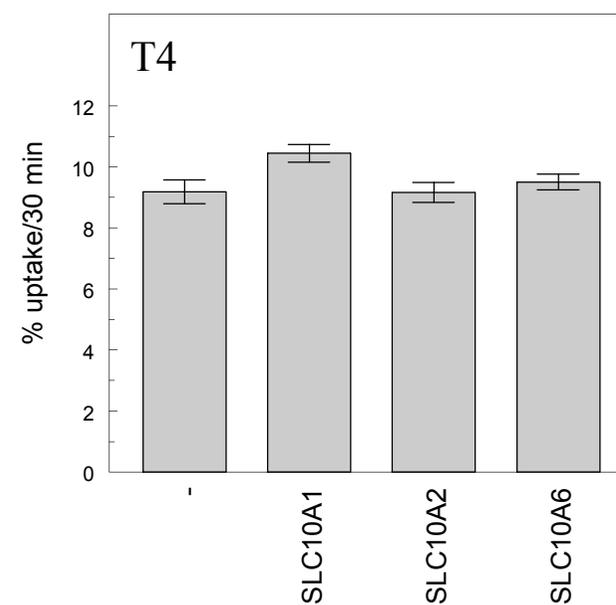


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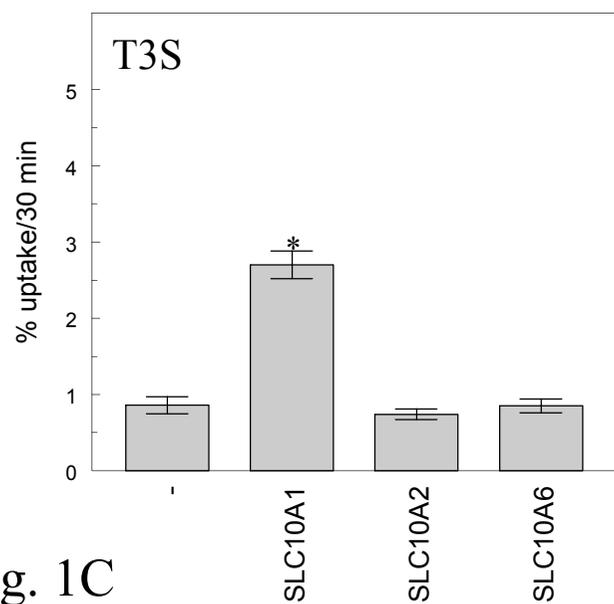


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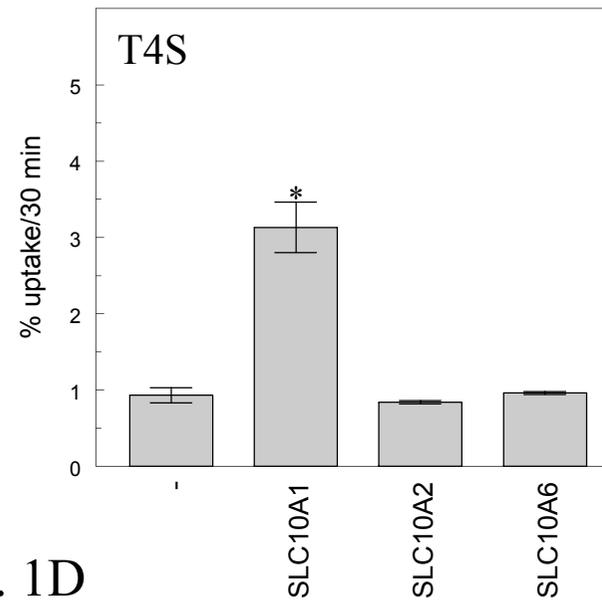


Fig. 1D

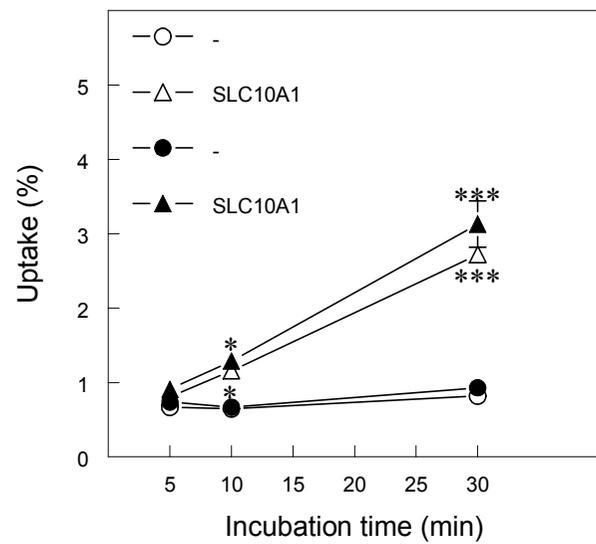


Fig. 1E

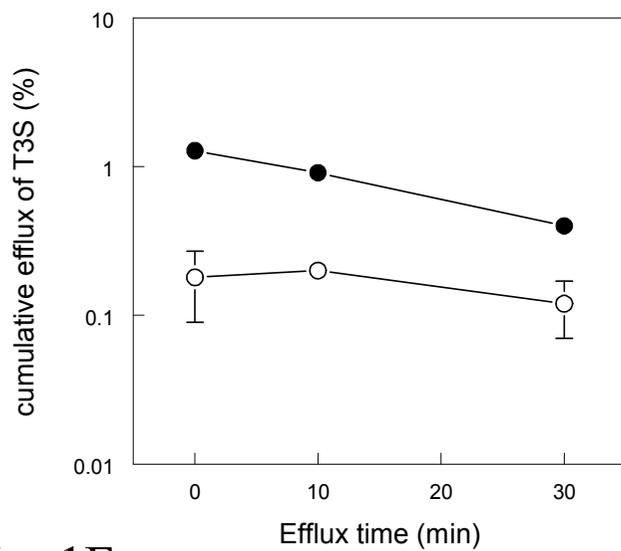


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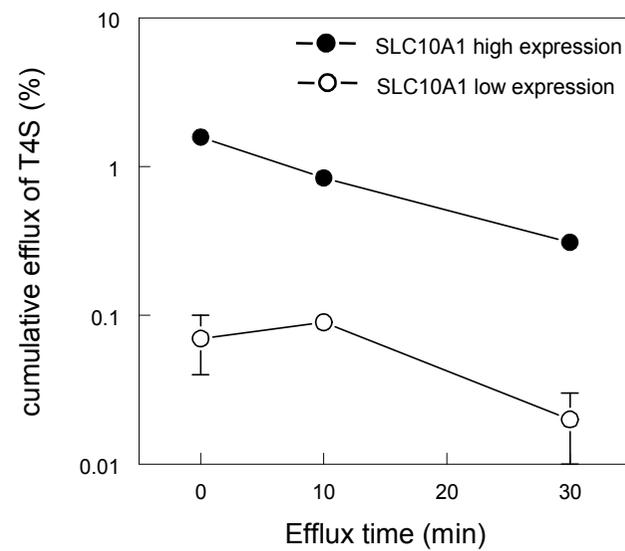


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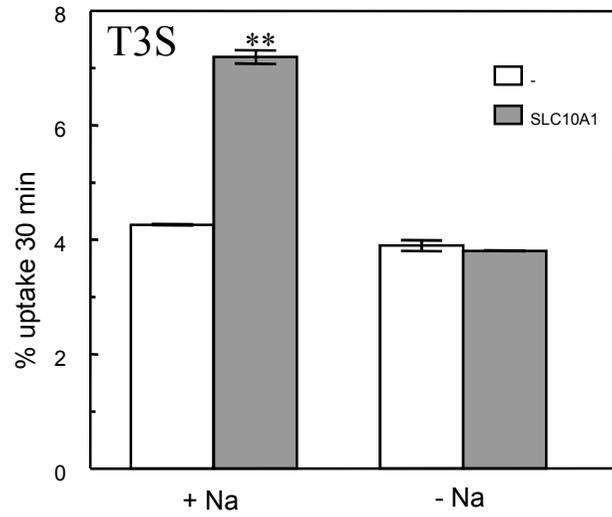


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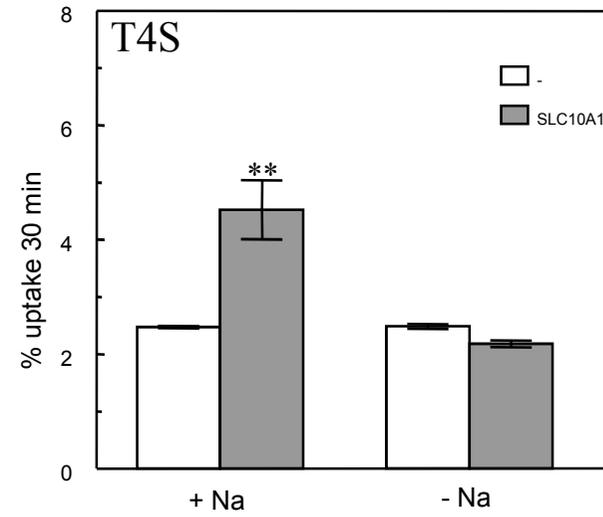


Fig. 1I

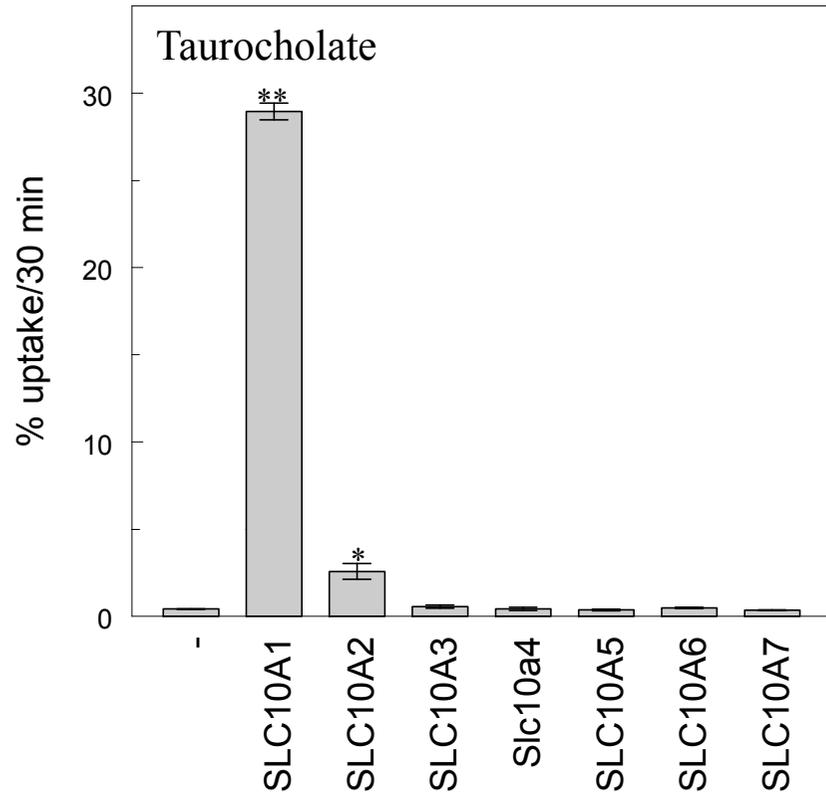
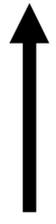


Fig. 2

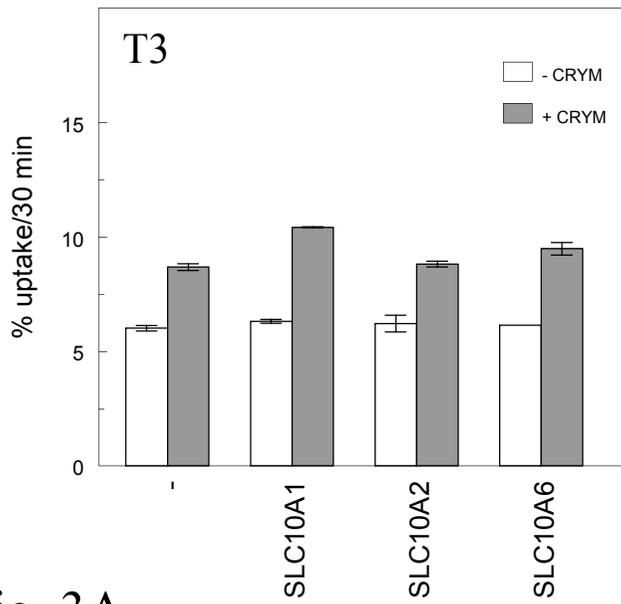


Fig. 3A

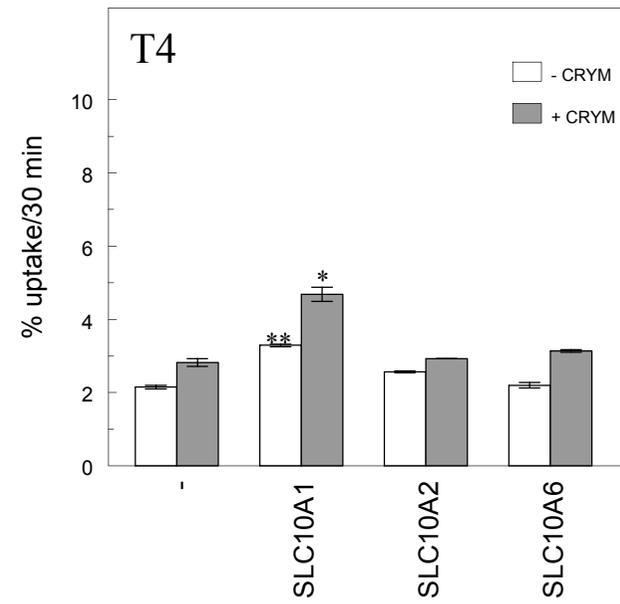


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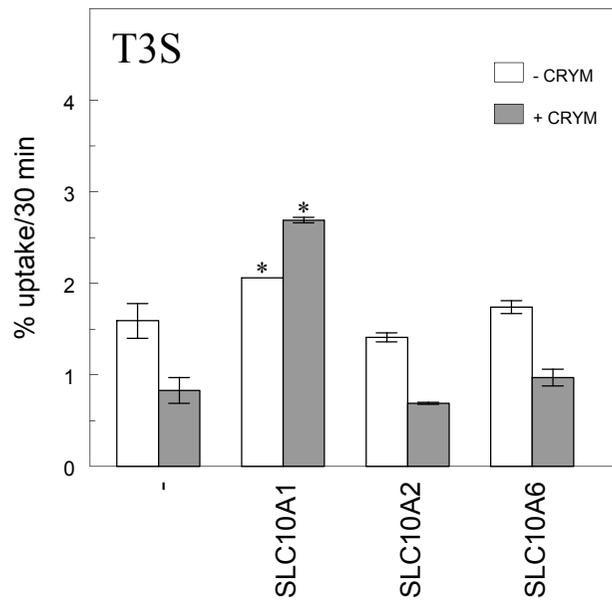


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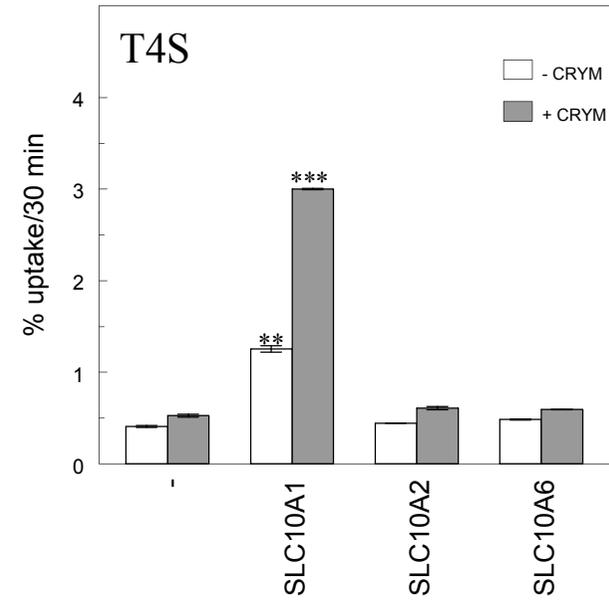


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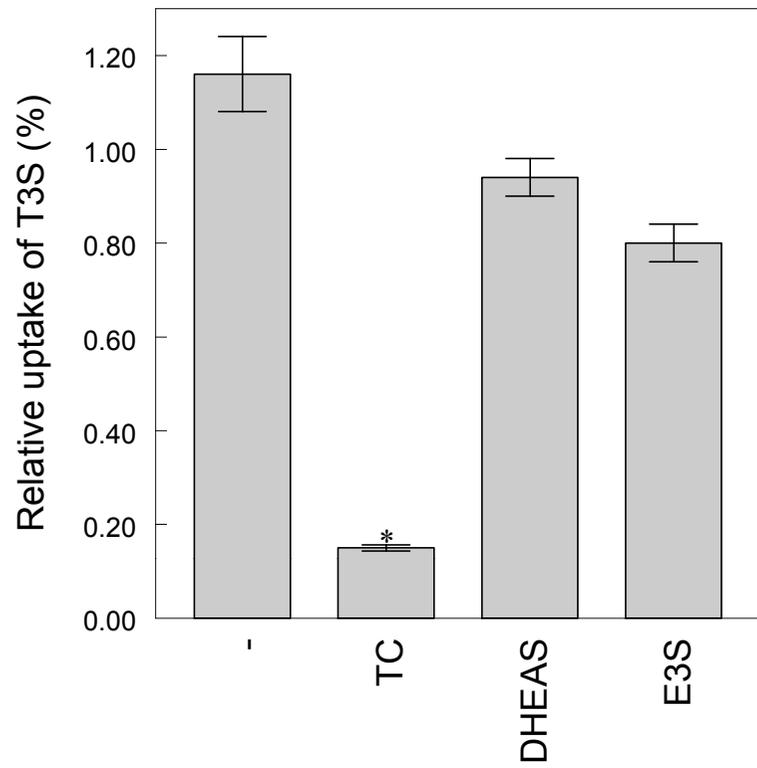


Fig. 4A

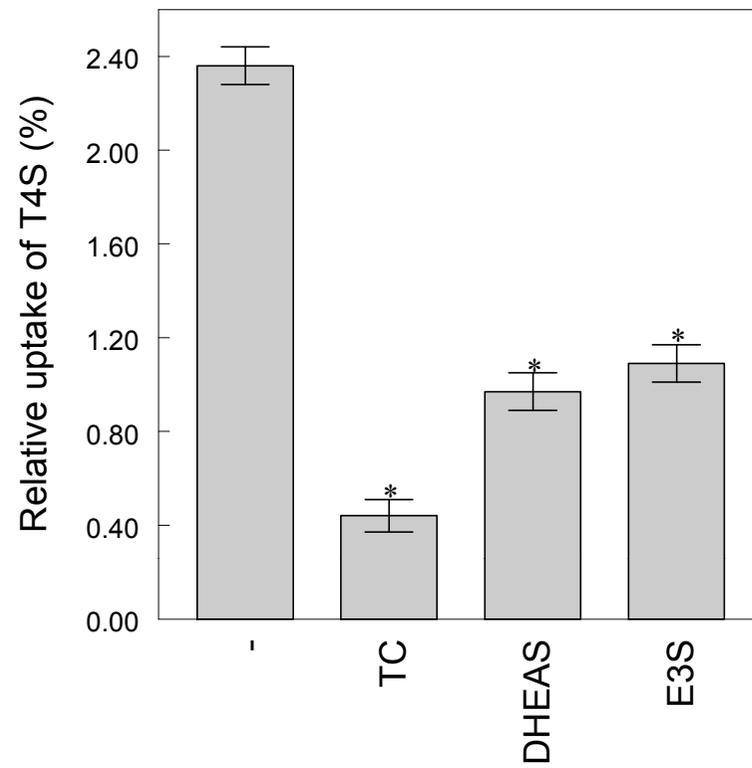


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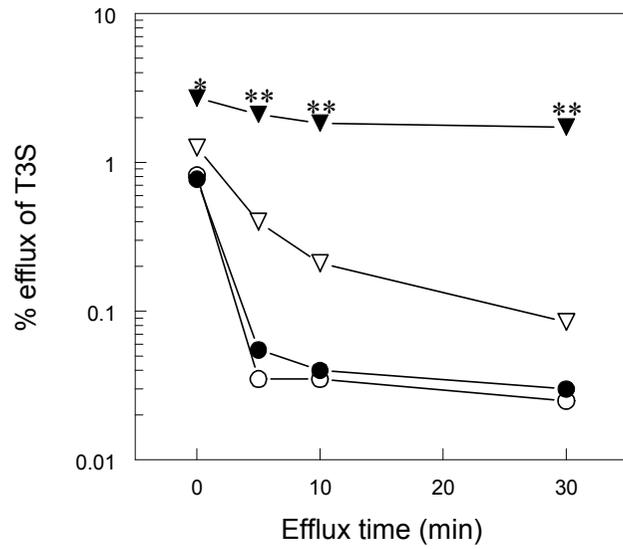


Fig. 5A

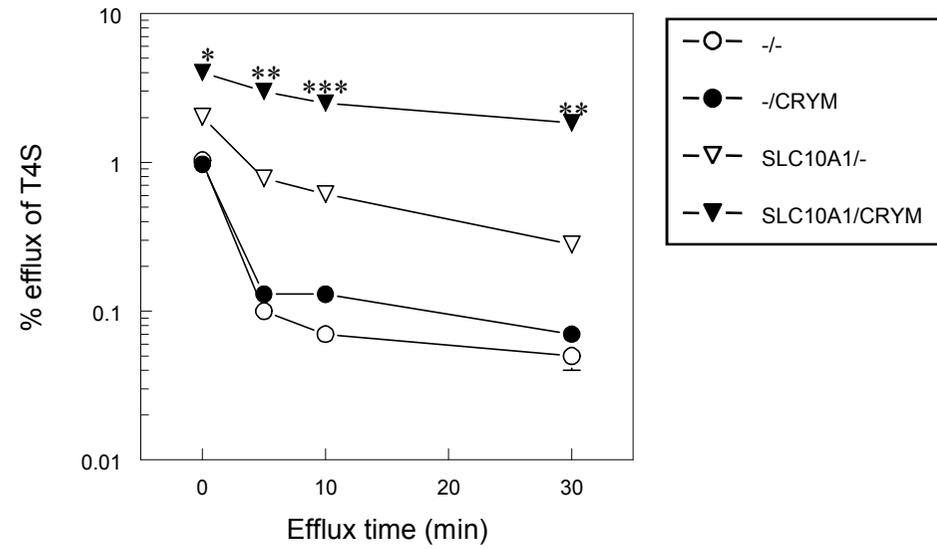


Fig. 5B