Molecular and Cellular Endocrinology 443 (2017) 163–174

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Molecular features of the L-type amino acid transporter 2 determine different import and export profiles for thyroid hormones and amino acids

Katrin M. Hinz [a], Dominik Neef [a], Claudia Rutz [a], Jens Furkert [a], Josef Köhrle [b], Ralf Schülein [a], Gerd Krause [a, *]

[a] Leibniz-Institut für Molekulare Pharmakologie (FMP), Berlin, Germany
[b] Institut für Experimentelle Endokrinologie, Charité-Universitätsmedizin Berlin, Berlin, Germany

ARTICLE INFO

Article history:
Received 5 July 2016
Received in revised form
11 January 2017
Accepted 17 January 2017
Available online 18 January 2017

Keywords:
Thyroid hormone transport
Molecular import and export
Xenopus laevis oocytes
Homology model
Site-directed mutagenesis
Competitive inhibition studies

ABSTRACT

The L-type amino acid transporter 2 (LAT2) imports amino acids (AA) and also certain thyroid hormones (TH), e.g. 3,3′-T_2 and T_3, but not rT_3 and T_4. We utilized LAT2 mutations (Y130A, N133S, F242W) that increase 3,3′-T_2 import and focus here on import and export capacity for AA, T_4, T_3, BCH and derivatives thereof to delineate molecular features.

Transport studies and analysis of competitive inhibition of import by radiolabelled TH and AA were performed in *Xenopus laevis* oocytes. Only Y130A, a pocket widening mutation, enabled import for T_4 and increased it for T_3. Mutant F242W showed increased 3,3′-T_2 import but no import rates for other TH derivatives. No export was detected for any TH by LAT2-wild type (WT). Mutations Y130A and N133S enabled only the export of 3,3′-T_2, while N133S also increased AA export. Thus, distinct molecular LAT2-features determine bidirectional AA transport but only an unidirectional 3,3′-T_2 and T_3 import.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

The transport of TH by TH transmembrane transporters (THTT) is essential for their cell availability (Friesema et al., 1999, 2004; Heuer and Visser, 2009). THTT are members of the solute carrier transporters and belong to two subfamilies, the major facilitator superfamily (MFS) (Kleinau et al., 2011) and the amino acids, polyamines and organocations (APC) transporter superfamily (Kanai et al., 1998). Their structure is very similar, they typically exhibit a common 12 transmembrane helix (TMH) structure (Kinne et al., 2011) and they show antiporter characteristics, due to their import and export capacity of TH (Zevenbergen et al., 2015).

One of the most recently found THTT is LAT2 discovered by Pineda et al. in 1999 (Pineda et al., 1999). LAT2 belongs to the APC-superfamily (Meury et al., 2014) and is a secondary active antiporter that uses chemical potential to exchange AA (Forrest et al., 2011) and imports specific TH like 3,3′-T_2 and T_3 (Kinne et al., 2015).

LAT2 is expressed in several human tissues such as kidney, placenta, brain, liver, spleen, skeletal muscle, heart, small intestine and lung (Rossier et al., 1999), (Braun et al., 2011). LAT2's main properties and characteristics are: transport of zwitterionic AA and import of specific TH. LAT2 is co-expressed with CD98 and substrate transport is sodium-independent and ATP-independent (Forrest et al., 2011; Pineda et al., 1999). Zevenbergen et al. recently demonstrated that the import of different substrates (3,3′-T_2 and 3-monoiodotyrosin (MIT)) by LAT2 is pH dependent (Zevenbergen et al., 2015). The import of TH can be inhibited by the common LAT inhibitor 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH) (Sebastianelli et al., 2008). Until now, no crystal structure of LAT2 has been published. Due to sequence similarity, LAT2 shares a common architecture with the bacterial APC transmembrane transporters (Meury et al., 2014; Rosell et al., 2014). We recently published a homology model of murine LAT2 (Hinz et al., 2015) based on two crystal structures, namely the arginine-agmatine-antiporter (AdiC, PDB: 3L1L) (Gao et al., 2009) and the amino acid polyamine cation transporter (ApcT, PDB: 3GIA) (Shaffer et al., 2009). Both templates are members of the APC superfamily. Based on the sequence similarity, it is assumed that LAT2

* Corresponding author. Leibniz-Institut fuer Molekulare Pharmakologie (FMP), Robert-Roessle Str. 10, 13125, Berlin, Germany.
E-mail address: gkrause@fmp-berlin.de (G. Krause).

http://dx.doi.org/10.1016/j.mce.2017.01.024
0303-7207/© 2017 Elsevier B.V. All rights reserved.

not only has a similar fold but also a similar binding mode for the AA moiety of 3,3′-T_2 (Hinz et al., 2015). In initial structure-function studies, we previously identified functional features of compounds that are mandatory for TH inhibition and for a potential substrate transport by LAT2. One assumption for the substrate properties is that the AA moiety of a TH is of great importance and necessary to be recognized by LAT2. Apart from distinct substrate features on aromatic rings, a certain flexibility of the molecule is necessary for substrate translocation, while steric hindrance (like in T_4) restricts the flexibility and thus its translocation through the traversing pathway of LAT2 (Hinz et al., 2015). Mutagenesis of AA next to the substrate recognition site by shortening the side chains led to an increased import of 3,3′-T_2. For F242W, the opposite effect is observed, indicating a gate function for TH import in LAT2 (Hinz et al., 2015).

Although initial molecular determinants of LAT2 that are involved in cellular import of 3,3′-T_2 have been identified, the molecular mechanism of import is still not fully understood. Moreover, determinants and mechanisms, particularly for the export of TH and AA, are still unknown. Therefore, it is of great interest to gain further knowledge into transport mechanisms of LAT2 and its role in TH transport.

Here we investigated the transport mechanisms by transporter and substrate variations to clarify whether there are differences in import or export between TH and AA by murine LAT2.

In focus are three LAT2 homology model-guided mutations of residues lining the traversing cavity and which are relevant for 3,3′-T_2 import. It was studied whether the expanded space in the traversing pathway leads to the import or export of those TH, which were not at all (e.g. T_4) or only minorly (e.g. T_3) imported by LAT2-WT. Moreover, to gain further knowledge about LAT2 and simultaneously about substrate characteristics for import, in competitive inhibition studies we combined selected LAT2 variants with substrate variants of TH and BCH.

Using the *Xenopus laevis* oocytes as an expression system, we analysed the import and export of TH and their derivatives.

2. Materials and methods

2.1. Materials

Iodo-L-thyronines (3,3′-T_2, T_3, T_4), L-AA (Leu, Phe) and 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH) were obtained from Sigma (St. Louis, MO, USA). 3′-isopropyl-3,5-dimethyl-L-thyronine (S17), 3′-isopropyl-3,5-diiod-L-thyronine (S18) and 3,5-diiod-3′,5′-dimethyl-L-thyronine (S19) were kindly provided by Dr. H. Rokos, formerly Henning Berlin and Dr. R. Thoma, Formula GmbH, Berlin, Germany. All iodo-L-thyronines were dissolved in DMSO. [^{125}I]-3,3′-T2, [^{125}I]-T3, [^{125}I]-T4, [^{3}H]-Leu and [^{3}H]-Phe were purchased from Hartmann Analytic GmbH (Braunschweig, Germany). Compounds for competitive inhibition studies were purchased as previously indicated (Hinz et al., 2015). All other reagents were purchased from Carl Roth or Sigma Aldrich.

2.2. Plasmids

Murine LAT2 with C-terminal FLAG-tag and CD98 with C-terminal His-tag were cloned into the pTLB expression vector as previously described (Kinne et al., 2015). In order to show the expression of the transporter on the plasma membrane, LAT2 was cloned into the pEGFP-N1 expression vector thereby replacing the stop codon of EGFP and CD98 was cloned into the pmCherry-N1 expression vector thereby replacing the stop codon of mCherry (details of the cloning procedure upon request). The resulting construct, LAT2-GFP, encodes LAT2 with a C-terminal GFP tag and CD98mCherry encodes CD98 with a C-terminal mCherry tag.

2.3. Transfection of HEK293 cells

Cells were seeded in poly-L-lysine coated plates (200,000 or 400,000 cells/well) and transiently single- or co-transfected with 0.8 μg LAT2-GFP or LAT2-variants-GFP and CD98 or CD98-mCherry, respectively. For laser scanning microscopy, glass slides were added in the wells before cell seeding. The functionality and expression was ascertained 48 h later by laser scanning microscopy and flow cytometry. Transporter functionality of the fluorescence labelled constructs were checked by 3,3′-T_2 import.

2.4. cRNA preparation and injection in isolated Xenopus laevis oocytes

Plasmids containing CD98-His, LAT2-FLAG or LAT2-FLAG-mutants in pTLB were transcribed using the mMESSAGE mMACHINE® SP6 RNA Transcription Kit (Life Technologies GmbH, Darmstadt, Germany). Oocyte isolation and preparation as well as the injection procedure with 11,5 ng CD98-His and/or 11,5 ng LAT2-FLAG or variant cRNAs was performed as previously described (Hinz et al., 2015).

2.5. TH transport and inhibition assays

After two days of expression, 10–15 oocytes were rinsed with washing buffer (100 mM choline chloride (ChCl), 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 10 mM Tris, pH 7.5). For the TH import assay, oocytes were incubated in 600 μl transport buffer (washing buffer containing 100 nM iodo-L-thyronines and 0.1 nM of the corresponding ^{125}I-labelled iodothyronine) for 60 min. Afterwards oocytes were washed six times with washing buffer on ice. Radioactivity was determined using a gamma counter (Wizard 1470, Perkin Elmer). For competitive inhibition studies, BCH- or AA-like compounds (1 mM) or TH-like compounds (100 μM) were dissolved in transport buffer. [^{125}I]-3,3′-T_2 import was measured in absence and presence of them.

To determine the export of TH, we preloaded co-injected oocytes (LAT2-WT or mutants/CD98) as previously described (Hinz et al., 2015). After 60 min of preloading at RT, cells were washed and further incubated in transport buffer supplemented with AA, 1 mM of L-Phe or L-Leu, respectively, to stimulate the export of preloaded TH. Tracer concentration in the export buffer was determined. Results of not injected oocytes were selected as background (bg). The export calculation is described by the following equation:

$$rel.Export\ (x) = \left(\frac{100\ \times\ Export\ (x)}{avg.Import\ (\overline{x}}\right) = \left(\frac{100 \times rel.Export\ (x)}{avg.rel.Export\ (\overline{x}}\right) - avg.bg\ (\overline{x} \quad (1)$$

Two days prior to the experiments with transiently transfected HEK293 cells (LAT2-GFP-WT or mutants/CD98 or CD98-mCherry), 400,000 cells per well were seeded into 12-well plates. For import measurements, cells were washed and incubated for 20 min with 600 μl transport buffer (125 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgCl_2$, 25 mM HEPES, 5.6 mM D-Glucose, pH 7.4) supplemented with 0.1 nM 3,3′-T_2. After two washing steps with ice-cold PBS, cells were lysed in 0.1 N NaOH, and radioactivity was measured by a gamma counter. All experiments were performed at least twice.

Characteristics of substrate transport of 3,3′-T_2, T_3 or T_4 in LAT2-WT or mutants/CD98 co-injected oocytes were analysed after

Download English Version:

https://daneshyari.com/en/article/5534205

Download Persian Version:

https://daneshyari.com/article/5534205

Daneshyari.com