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# Stereoselective cell uptake of adrenergic agonists and antagonists by organic cation transporters

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## 1. Introduction

While receptor binding and enzymatic catalysis are widely known to often be highly stereospecific processes, stereoselectivity in membrane transport by polyspecific solute carriers (SLC) is not so evident and often not considered in research. Given the very broad substrate spectrum of many of these transporters, it may predominantly be the physicochemical properties of a substance that determine which transporter is relevant [1]. In this study, we focus on stereospecificity in organic cation transport of adrenergic and antiadrenergic drugs, since many of these have chiral centres and are often administered as racemic mixtures.

The importance of stereospecificity in pharmacodynamics has already been thoroughly studied for several (anti)adrenergic drugs. For

instance, the prototypic adrenergic substance (*R*)-adrenaline is over 20-fold more potent than (*S*)-adrenaline. Also, the spasmolytic actions of salbutamol (albuterol) and formoterol were attributed solely to (*R*)-salbutamol and (*R,R*)-formoterol, while the counterpart enantiomers showed significantly less agonist activity at the beta<sub>2</sub>-adrenergic receptor [2,3]. Stereoselectivity has also been extensively studied with respect to drug metabolism [4], and it was strongly observed in the sulfation of some beta-adrenergic drugs [5]. Before sulfate conjugation or other metabolic reactions can take place in enterocytes, hepatocytes, or renal tubular cells, these relatively hydrophilic drugs must first enter the cell. Organic cation transporters (OCTs) are predominantly responsible for the transport of more hydrophilic cationic substances in the liver and kidneys for metabolism and excretion [6]. Transport via OCT1 and OCT3 may be particularly relevant in the context of hepatic

*Abbreviations:* MATE, multidrug and toxin extrusion protein; OCT, organic cation transporter; SLC, solute carrier

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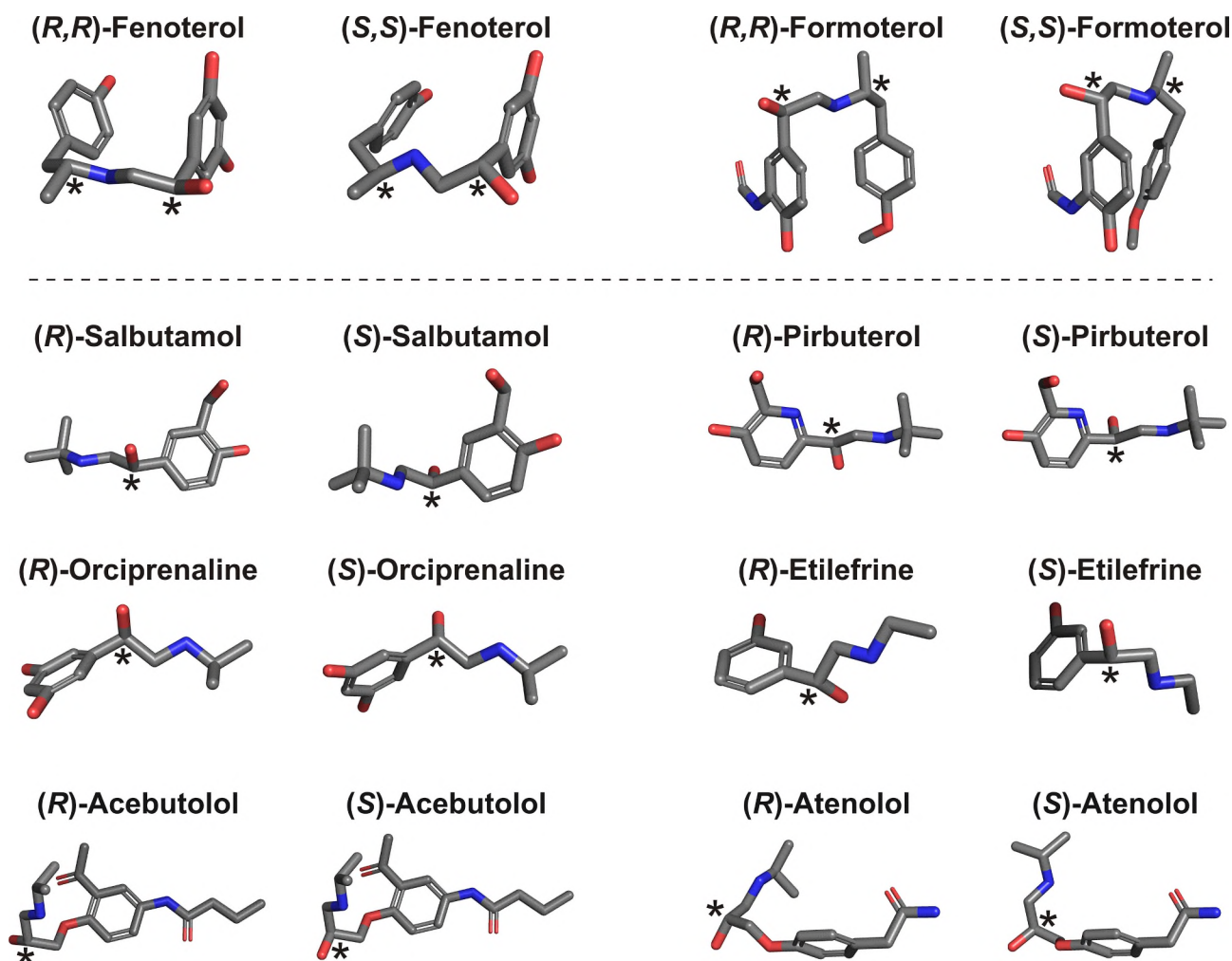


Fig. 1. Beta-adrenergic receptor agonists and antagonists investigated for stereoselective transport by OCTs and transporters of the MATE-family. These were selected for physicochemical properties ( $pK_a > 8.0$  and  $\log D_{pH7.4} < 1$ ) that renders them likely transporter substrates. Chiral centres are indicated by an asterisk.

metabolism [7], whereas OCT2 and MATE2-K are often involved in renal elimination.

Only few studies investigating stereoselectivity in drug membrane transport have been published so far [8]. For example, for the beta<sub>1</sub>-adrenergic receptor partial agonist xamoterol, the (S)-enantiomer was found to be 2-fold preferentially transported by OCT1 [1]. With regard to other substrates of OCT1, literature data on stereoselectivity is scarce. Here, we report a comprehensive characterisation of the extent of stereospecificity in the transport of different beta-adrenergic receptor agonists and antagonists (Fig. 1) by wild-type and genetic variants of OCT1 as well as by the related transporters OCT2, OCT3, MATE1, and MATE2-K.

## 2. Materials and methods

### 2.1. *In vitro* uptake experiments

Transport experiments were performed with HEK293 cells stably transfected to overexpress OCT1\*1 (wild-type), OCT1\*2 (M420del), OCT1\*3 (R61C), OCT1\*4 (G401S), OCT1\*5 (M420del/G465R), OCT1\*6 (C88R/M420del), OCT1\*7 (S14F), OCT1\*8 (R488M), OCT2, OCT3, MATE1, or MATE2-K. All cell lines were generated using the Flp-In system (Thermo Fisher Scientific, Darmstadt, Germany) as previously described [7,9,10], except for the OCT3-overexpressing HEK293 cells that were a kind gift from Drs. Koepsell and Gorbulev (University of Würzburg, Germany). Cells were kept in culture for no more than 30

passages. Tested drugs were purchased as racemates from Sigma-Aldrich (Darmstadt, Germany; catalogue numbers: fenoterol, F1016; formoterol, F9552; salbutamol, S8250; orciprenaline, M2398; acebutolol, A3669; atenolol, A7655) or Santa Cruz Biotechnology (Heidelberg, Germany; pirbuterol, sc-476485; etilefrine, sc294579A). Internal standards were purchased from Sigma-Aldrich (desvenlafaxine, D-2069; metoprolol, 80337), Santa Cruz Biotechnology (tulobuterol, sc-213131; (S)-propranolol, sc-294579A), or Biozol Diagnostica (Eching, Germany; fenoterol-d6, TRC-F248852).

Cells were seeded on 12-well plates coated with poly-D-lysine 48 h before the transport experiments and incubated at 37 °C, 95% relative humidity, and 5% CO<sub>2</sub>. Cell lines overexpressing MATE1 and MATE2-K were incubated with 30 mM NH<sub>4</sub>Cl in HBSS + (10 mM HEPES in HBSS, pH 7.4; Thermo Fisher Scientific, Darmstadt, Germany) for 30 min prior to the assay to invert the direction of transport. All cell lines were washed with 37 °C HBSS + and subsequently incubated with the pre-warmed substrate in HBSS + for one (MATE1, MATE2-K) or two (OCTs) minutes at 37 °C. The reaction was stopped by adding ice-cold HBSS +, and the cells were washed twice with ice-cold HBSS + before lysis with 80% acetonitrile (LGC Standards, Wesel, Germany). Subsequently, the intracellular substrate accumulation was determined using LC-MS/MS.

### 2.2. Stereoselective concentration analyses

Cell uptake was quantified by stereoselective HPLC and tandem mass spectrometric detection using a Shimadzu Nexera™ HPLC system

**Table 1**  
HPLC settings for the separation of (anti)adrenergic drug enantiomers.

Drug	Column <sup>a</sup>	Mobile phase <sup>b</sup>	Flow rate [ $\mu\text{l} \times \text{min}^{-1}$ ]	Retention time A [min]	Retention time B [min]
Fenoterol	Chiral-CBH	10 mM NH <sub>4</sub> Ac, pH 5.8, 5% IPA	500	5.3 (R,R)	6.1 (S,S)
Formoterol	Chiral-CBH	10 mM NH <sub>4</sub> Ac, 10% ACN	300	15.4 (R,R)	17.0 (S,S)
Salbutamol	Chirobiotic T	20 mM NH <sub>4</sub> Ac, pH 4.5, 96% MeOH	1000	6.8 (R)	7.7 (S)
Pirbuterol	Chiral-CBH	10 mM NH <sub>4</sub> Ac, pH 5.8, 5% IPA	300	3.3 (P1)	3.5 (P2)
Orciprenaline	Chirobiotic T	20 mM NH <sub>4</sub> Ac, pH 4.5, 93% MeOH	500	9.2 (P1)	10.7 (P2)
Etilefrine	Chirobiotic T	20 mM NH <sub>4</sub> Ac, pH 4.5, 93% MeOH	500	10.8 (P1)	11.6 (P2)
Acebutolol	Chiral-CBH	10 mM NH <sub>4</sub> Ac, pH 5.8, 10% ACN	500	2.9 (R)	4.8 (S)
Atenolol	Chiral-CBH	10 mM NH <sub>4</sub> Ac, pH 5.8, 5% IPA	300	3.8 (R)	5.1 (S)

<sup>a</sup> CBH, cellobiohydrolase

<sup>b</sup> ACN, acetonitrile; IPA, isopropanol; MeOH, methanol; NH<sub>4</sub>Ac, ammonium acetate

**Table 2**  
Chemical properties pK<sub>a</sub> and logD<sub>pH7.4</sub> and relevant data for mass-spectrometric detection of the substrates and analytical internal standards.

Drug	pK <sub>a</sub>	logD <sub>pH7.4</sub>	Q1 Mass <sup>a</sup> [Da]	Q3 Mass <sup>b</sup> [Da]	DP <sup>c</sup> [V]	CE <sup>d</sup> [V]	CXP <sup>e</sup> [V]
<b>Substrates</b>							
Fenoterol <sup>1</sup>	9.63	0.33	304.1 (304.1)	107.1 (135.2)	70 (70)	44 (24)	12 (12)
Formoterol <sup>1</sup>	9.81	0.04	345.2 (345.2)	149.1 (121.1)	70 (70)	28 (42)	15 (15)
Salbutamol <sup>2</sup>	9.40	-1.32	240.2 (240.2)	148.2 (222.2)	60 (60)	24 (24)	15 (15)
Pirbuterol <sup>1</sup>	9.59	-1.78	241.3 (241.3)	149.1 (167.2)	65 (65)	30 (24)	15 (15)
Orciprenaline <sup>3</sup>	9.70	-0.94	212.1 (212.1)	152.0 (107.0)	56 (56)	23 (39)	10 (8)
Etilefrine <sup>4</sup>	9.73	-1.07	182.1 (182.1)	164.0 (91.0)	51 (51)	17 (37)	10 (6)
Acebutolol <sup>2</sup>	9.65	-0.68	337.2 (337.2)	116.0 (98.1)	91 (91)	31 (29)	8 (8)
Atenolol <sup>5</sup>	9.67	-1.80	267.2 (267.2)	145.2 (74.0)	130 (1 3 0)	38 (35)	10 (14)
<b>Internal standards</b>							
Fenoterol-d6	-	-	310.3 (310.3)	109.1 (141.0)	70 (70)	40 (26)	12 (12)
Desvenlafaxine	-	-	264.3 (264.3)	58.1 (107.2)	60 (60)	47 (50)	8 (8)
Metoprolol	-	-	268.2 (268.2)	116.1 (74.0)	86 (86)	27 (35)	8 (14)
(S)-Propranolol	-	-	260.2	116.2	85	30	10
Tulobuterol	-	-	228.1 (228.1)	153.9 (119.1)	60 (60)	23 (41)	10 (8)

<sup>a</sup> Q1, first quadrupole (qualifiers below in parentheses);

<sup>b</sup> Q3, third quadrupole (qualifiers in parentheses);

<sup>c</sup> DP, declustering potential;

<sup>d</sup> CE, collision energy;

<sup>e</sup> CXP, collision cell exit potential;

<sup>1</sup> quantified with internal standard fenoterol-d6;

<sup>2</sup> quantified with internal standard desvenlafaxine;

<sup>3</sup> quantified with internal standard metoprolol;

<sup>4</sup> quantified with internal standard (S)-propranolol;

<sup>5</sup> quantified with internal standard tulobuterol

that included a LC-30AD pump, a SIL-30AC autosampler, a CTO-20AC column oven, and a CBM-20A controller (Shimadzu, Kyoto, Japan). A Chiral-CBH column (100 × 3 mm, 4.6  $\mu\text{m}$ ; Sigma-Aldrich, Darmstadt, Germany) with a corresponding 10 × 3 mm guard column or an Astec Chirobiotic T (15 cm × 2.1 mm, 5  $\mu\text{m}$ ; Sigma-Aldrich, Darmstadt, Germany) column with a corresponding 2 cm × 1 mm guard column was used (Table 1). Oven temperature was 25 °C for all methods, and separation was achieved by isocratic elution. The order of enantiomer elution was inferred from available literature, where the investigated substrates had been separated by HPLC using identical columns and similar mobile phases [11–15]. However, no reference literature was found for etilefrine, orciprenaline, and pirbuterol. In these cases, the enantiomers were only named by the order of elution, as the identification of the enantiomers was not the focus of this study. Tested substrates and suitable internal standards were detected using an API 4000 tandem mass spectrometer (AB SCIEX, Darmstadt, Germany) with the parameters listed in Table 2.

### 2.3. Calculations

The net active transport by overexpressed transporters was calculated by subtracting the uptake measured in an empty vector control cell line. The parameters  $K_m$  and  $v_{\text{max}}$  were estimated by regression analysis using the Michaelis-Menten equation. Means and standard

errors were calculated from individual  $K_m$  and  $v_{\text{max}}$  values of at least three independent experiments. The intrinsic clearance  $Cl_{\text{int}}$  was calculated as the ratio of  $v_{\text{max}}$  over  $K_m$ . The kinetic parameters  $v_{\text{max}}$ ,  $K_m$ , and  $Cl_{\text{int}}$  were tested for statistical significance using Student's *t*-test with an alpha-value of 0.05.

## 3. Results

### 3.1. Stereoselective OCT1-mediated cellular uptake of adrenergic drugs

First, we analysed the extent of stereoselectivity in the OCT1-mediated transport of eight clinically relevant beta-adrenergic receptor agonists and antagonists. The test compounds were selected based on their physicochemical properties ( $\text{pK}_a > 8.0$  and  $\text{logD}_{\text{pH7.4}} < 1$ ), because more lipophilic or acidic substances are mostly not transported by OCTs to a relevant extent.

We observed a 1.9- and 1.7-fold (calculated as the ratio of the larger over the smaller parameter) higher maximum transport velocity ( $v_{\text{max}}$ ) for the (R,R)-enantiomers of fenoterol and formoterol in comparison to the corresponding (S,S)-enantiomers (Figs. 2, 3, Table 3). The  $K_m$  values were also higher for (R,R)-fenoterol and (R,R)-formoterol. We observed a 1.1- to 1.7-fold difference in  $v_{\text{max}}$  between the enantiomers of salbutamol, pirbuterol, orciprenaline (metaproterenol), etilefrine, acebutolol, and atenolol (Figs. 2, 3, Table 3). However, no notable differences

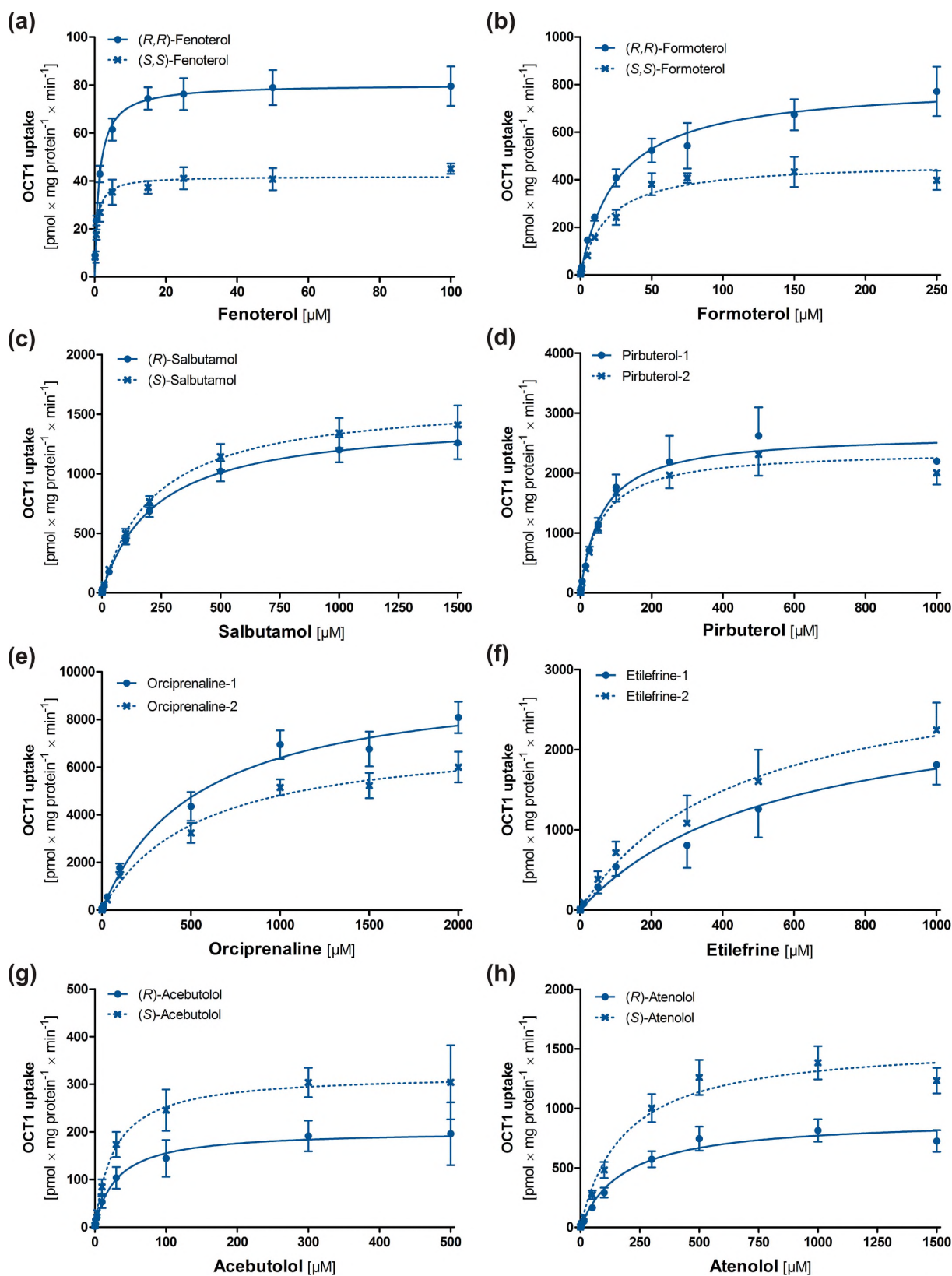
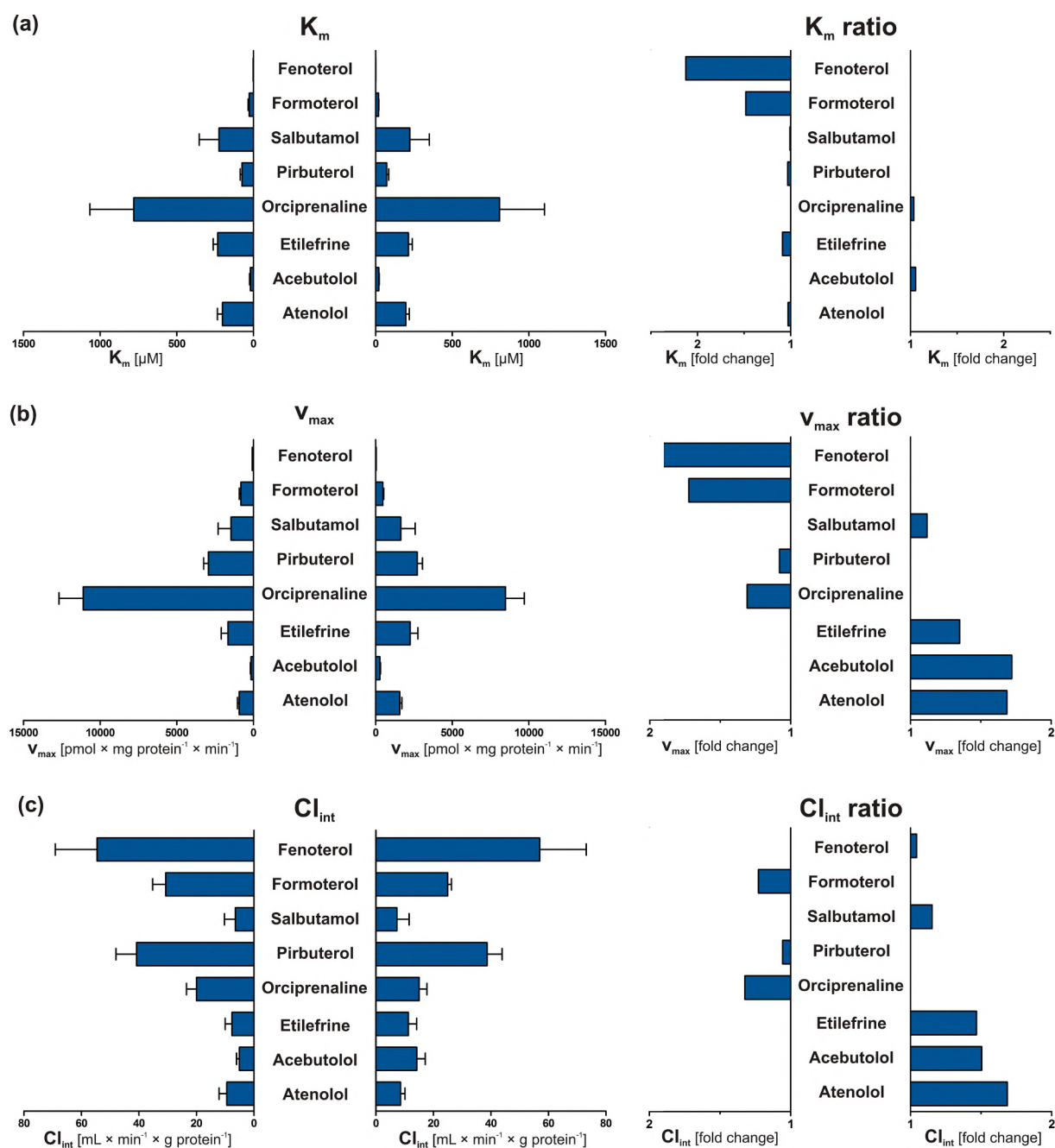


Fig. 2. Transport of (anti)adrenergic drug enantiomers by wild-type OCT1, determined using stably transfected HEK293 cells. Shown are the mean  $\pm$  SEM of at least three independent experiments for each drug. Enantiomers that could not be identified were numbered according to the order of HPLC elution.

in  $K_m$  between the enantiomers of these drugs were seen. The intrinsic clearance differed 1.1- to 1.7-fold between the enantiomers of salbutamol, orciprenaline, etilefrine, acebutolol, and atenolol, whereas no significant difference was seen between the enantiomers of pirbuterol.

To summarise, remarkable differences, particularly in the maximum uptake velocity, were seen between the enantiomers of structurally related (anti)adrenergic drugs, which is notably indicative of stereospecificity in the molecular interaction between substrate and



**Fig. 3.** Stereoselectivity in the OCT1-mediated transport of sympathomimetic and sympatholytic drugs. The ratios on the right were calculated as the quotients of the higher and the lower values.

transporter. However, general conclusions about stereoselective uptake by OCT1 cannot be deduced at present, and stereoselectivity must be determined for every substrate individually.

### 3.2. Differential stereoselective transport among genetic variants of OCT1

Next, we investigated possible differences in stereospecific membrane transport of a subset of (anti)adrenergic drugs with particular clinical importance, namely fenoterol, formoterol, salbutamol, and atenolol, between common naturally occurring variants of OCT1 (OCT1\*2 to OCT1\*8). With the exception of fenoterol uptake via OCT1\*4, a reduction in the transport velocity was observed for all substrates in the variants OCT1\*2, \*3, \*4, and \*7 (Table 4). No transport activity was detected in OCT1\*5 and \*6 (data not shown), which are known to be non-functional [7]. In contrast, OCT1\*8 showed a

transport capacity similar to wild-type (for salbutamol) or higher (for fenoterol, formoterol, and atenolol). Generally, the stereoselectivity of transport did not differ strongly between any of these common OCT1 variants, with a few notable exceptions further outlined below.

For fenoterol, the observed  $v_{max}$  but also the  $K_m$  were approximately twice as high for the pharmacologically active (*R,R*)-fenoterol in comparison to (*S,S*)-fenoterol in wild-type OCT1. Interestingly, for the worldwide most common variant OCT1\*2, the enantioselectivity was completely opposite: the  $v_{max}$  was 1.6-fold higher and the  $K_m$  almost 5-fold higher for (*S,S*)-fenoterol. Notable is also the switch in affinity from wild-type (2.1-fold lower  $K_m$  for (*S,S*)-fenoterol) to OCT1\*7 (3.1-fold lower  $K_m$  for (*R,R*)-fenoterol). There is little difference in the intrinsic clearance between the two enantiomers of fenoterol for wild-type OCT1 and all studied variants, except for OCT1\*4, where it is 1.7-fold higher for (*R,R*)-fenoterol.

**Table 3**

Kinetic parameters for the transport of racemic (anti)adrenergic drugs by wild-type OCT1.

Transporter	Substrate	$K_m$ (± SEM) [μM]	$v_{max}$ (± SEM) [pmol × mg protein <sup>-1</sup> × min <sup>-1</sup> ]	$Cl_{int}$ (± SEM) [ml × min <sup>-1</sup> × g protein <sup>-1</sup> ]	Stereoselectivity		
					$K_m$	$v_{max}$	$Cl_{int}$
<b>OCT1</b>	(R,R)-Fenoterol	1.7* (± 0.3)	81.5** (± 2.6)	54.6 (± 14.6)	2.13-fold for (R,R)	1.94-fold for (R,R)	1.04-fold for (S,S)
	(S,S)-Fenoterol	0.8* (± 0.2)	42.0** (± 3.5)	57.0 (± 16.2)			
	(R,R)-Formoterol	28.3 (± 6.2)	820.4* (± 102.8)	30.7 (± 4.5)	1.48-fold for (R,R)	1.72-fold for (R,R)	1.23-fold for (R,R)
	(S,S)-Formoterol	19.1 (± 2.0)	476.1* (± 54.5)	25.0 (± 1.3)			
	(R)-Salbutamol	224.2 (± 18.4)	1464.3* (± 157.6)	6.5** (± 3.8)	1.01-fold for (R)	1.12-fold for (S)	1.12-fold for (S)
	(S)-Salbutamol	222.5 (± 20.5)	1637.3* (± 192.6)	7.3** (± 4.2)			
	Pirbuterol-1	75.3 (± 11.4)	2942.7 (± 307.2)	40.9 (± 7.2)	1.03-fold for (1)	1.08-fold for (1)	1.06-fold for (1)
	Pirbuterol-2	72.9 (± 12.3)	2724.3 (± 337.4)	38.7 (± 5.3)			
	Orciprenaline-1	780.5 (± 285.9)	11106.3 (± 1579.8)	20.0* (± 3.5)	1.04-fold for (2)	1.31-fold for (1)	1.32-fold for (1)
	Orciprenaline-2	808.8 (± 292.6)	8482.0 (± 1224.2)	15.1* (± 2.7)			
	Etilefrine-1	232.9 (± 29.8)	1667.1** (± 432.9)	7.7* (± 2.3)	1.08-fold for (2)	1.35-fold for (2)	1.47-fold for (2)
	Etilefrine-2	214.0 (± 24.9)	2253.8** (± 506.8)	11.3* (± 2.9)			
	(R)-Acebutolol	19.9 (± 5.7)	161.5*** (± 41.9)	9.5** (± 2.7)	1.05-fold for (S)	1.72-fold for (S)	1.51-fold for (S)
	(S)-Acebutolol	21.0 (± 2.5)	277.9*** (± 45.1)	14.3** (± 2.9)			
	(R)-Atenolol	201.9 (± 33.1)	929.7*** (± 115.3)	5.1** (± 1.0)	1.03-fold for (R)	1.69-fold for (S)	1.69-fold for (S)
	(S)-Atenolol	196.4 (± 23.1)	1567.4*** (± 143.6)	8.6** (± 1.5)			

SEM, standard error of the mean; asterisks indicate statistical significance of the differences between the two enantiomers (Student's *t*-test; \* *p* < 0.05, \*\* *p* < 0.01, and \*\*\* *p* < 0.001).**Table 4**

Kinetic parameters for the transport of racemic (anti)adrenergic drugs by genetic variants of OCT1.

Transporter	Substrate	$K_m$ (± SEM) [μM]	$v_{max}$ (± SEM) [pmol × mg protein <sup>-1</sup> × min <sup>-1</sup> ]	$Cl_{int}$ (± SEM) [ml × min <sup>-1</sup> × g protein <sup>-1</sup> ]	Stereoselectivity		
					$K_m$	$v_{max}$	$Cl_{int}$
<b>OCT1*2</b>	(R,R)-Fenoterol	11.4 (± 5.9)	49.6 (± 14.4)	28.9* (± 25.3)	4.85-fold for (S,S)	1.55-fold for (S,S)	1.09-fold for (R,R)
	(S,S)-Fenoterol	55.3 (± 36.8)	77.0 (± 34.0)	26.4* (± 25.0)			
	(R,R)-Formoterol	22.3* (± 5.6)	278.5 (± 107.3)	12.1 (± 1.8)	2.53-fold for (R,R)	2.51-fold for (R,R)	1.08-fold for (S,S)
	(S,S)-Formoterol	8.8* (± 5.1)	111.1 (± 61.6)	13.1 (± 0.4)			
	(R)-Salbutamol	338.2 (± 139.2)	597.6 (± 216.0)	2.2* (± 1.2)	1.30-fold for (R)	1.03-fold for (S)	1.23-fold for (S)
	(S)-Salbutamol	260.8 (± 94.6)	614.7 (± 204.0)	2.7* (± 1.6)			
	(R)-Atenolol	410.1 (± 256.6)	536.0 (± 264.3)	1.7* (± 0.4)	1.83-fold for (R)	1.28-fold for (S)	2.12-fold for (S)
	(S)-Atenolol	223.8 (± 79.8)	687.8 (± 190.6)	3.6* (± 0.8)			
<b>OCT1*3</b>	(R,R)-Fenoterol	no transport	no transport	no transport	-	-	-
	(S,S)-Fenoterol						
	(R,R)-Formoterol	77.8 (± 27.5)	162.7 (± 43.6)	2.2 (± 0.2)	2.29-fold for (S,S)	1.27-fold for (S,S)	1.05-fold for (R,R)
	(S,S)-Formoterol	178.5 (± 162.1)	205.9 (± 155.4)	2.1 (± 1.0)			
	(R)-Salbutamol	no transport	no transport	no transport	-	-	-
	(S)-Salbutamol						
<b>OCT1*4</b>	(R,R)-Fenoterol	9.6 (± 0.8)	151.7* (± 11.8)	16.3** (± 2.1)	1.07-fold for (S,S)	1.72-fold for (R,R)	1.70-fold for (R,R)
	(S,S)-Fenoterol	10.3 (± 1.6)	88.4* (± 1.5)	9.6** (± 2.2)			
	(R,R)-Formoterol	52.8 (± 7.4)	161.4* (± 35.9)	3.0 (± 0.4)	2.07-fold for (R,R)	1.74-fold for (R,R)	1.60-fold for (S,S)
	(S,S)-Formoterol	24.5 (± 19.7)	92.6* (± 63.3)	4.8 (± 1.3)			
	(R)-Salbutamol	no transport	no transport	no transport	-	-	-
	(S)-Salbutamol						
<b>OCT1*7</b>	(R,R)-Fenoterol	1.1 (± 0.5)	32.9 (± 8.0)	38.7 (± 14.9)	3.09-fold for (S,S)	1.20-fold for (R,R)	1.10-fold for (S,S)
	(S,S)-Fenoterol	3.4 (± 3.0)	27.5 (± 7.4)	42.6 (± 25.8)			
	(R,R)-Formoterol	78.6 (± 42.6)	788.7 (± 229.9)	14.3 (± 4.5)	2.00-fold for (R,R)	2.11-fold for (R,R)	1.28-fold for (R,R)
	(S,S)-Formoterol	39.3 (± 14.9)	373.8 (± 107.3)	11.2 (± 2.2)			
	(R)-Salbutamol	494.7 (± 222.4)	898.8 (± 218.3)	2.8** (± 1.6)	1.35-fold for (R)	1.01-fold for (R)	1.18-fold for (S)
	(S)-Salbutamol	365.8 (± 136.9)	888.4 (± 154.0)	3.3** (± 1.9)			
<b>OCT1*8</b>	(R)-Atenolol	148.4 (± 63.9)	296.1** (± 53.2)	2.9* (± 1.0)	1.01-fold for (R)	2.01-fold for (S)	1.66-fold for (S)
	(S)-Atenolol	146.6 (± 41.0)	596.0** (± 57.6)	4.8* (± 1.3)			
	(R,R)-Fenoterol	3.1* (± 0.5)	204.2* (± 32.7)	68.8 (± 15.1)	1.48-fold for (R,R)	1.95-fold for (R,R)	1.17-fold for (R,R)
	(S,S)-Fenoterol	2.1* (± 0.8)	104.7* (± 20.4)	58.9 (± 16.3)			
	(R,R)-Formoterol	44.5 (± 5.6)	1589.3** (± 188.1)	35.8* (± 1.0)	1.14-fold for (S,S)	1.45-fold for (R,R)	1.64-fold for (R,R)
	(S,S)-Formoterol	50.8 (± 3.4)	1099.0** (± 200.8)	21.8* (± 4.3)			
<b>OCT1*8</b>	(R)-Salbutamol	210.9 (± 21.9)	1424.7** (± 203.9)	6.8* (± 3.9)	1.06-fold for (S)	1.19-fold for (S)	1.12-fold for (S)
	(S)-Salbutamol	223.4 (± 13.7)	1701.3** (± 195.4)	7.6* (± 4.4)			
	(R)-Atenolol	299.3 (± 91.1)	1059.9** (± 79.0)	4.0** (± 0.8)	1.10-fold for (S)	2.05-fold for (S)	1.73-fold for (S)
	(S)-Atenolol	328.4 (± 49.7)	2177.0** (± 60.8)	6.9** (± 0.9)			

SEM, standard error of the mean; asterisks indicate statistical significance of the differences between the two enantiomers (Student's *t*-test; \* *p* < 0.05, \*\* *p* < 0.01, and \*\*\* *p* < 0.001).

With regard to formoterol, allelic OCT1 variants with the exception of OCT1\*3 showed, similar to wild-type OCT1, a preference for the pharmacologically active (*R,R*)-formoterol.

The uptake of salbutamol enantiomers by wild-type OCT1 and allelic variants showed only minor stereoselectivity. It was reduced in OCT1\*2 and \*7, and similar, or marginally higher, to the wild-type in \*8. An interesting observation was the markedly different substrate affinity for OCT1\*3 and \*4: whereas fenoterol was transported to a significant extent by \*4, transport of salbutamol and atenolol was completely absent in \*3 and \*4.

With atenolol, we observed a general preference for the pharmacologically active (*S*)-atenolol, both in terms of maximum transport velocity and lower  $K_m$ .

Our results indicate that stereoselectivity in transport is for the OCT1 variants in most cases relatively similar to the wild-type, but a few notable exceptions were found.

### 3.3. Differences in stereoselectivity between different organic cation transporters

Beside OCT1, other cation transporters may also be involved in cellular uptake and hepatic or renal elimination. We therefore studied the extent of stereoselectivity in the transport of fenoterol, formoterol, salbutamol, and atenolol by the related solute carriers OCT2, OCT3, MATE1, and MATE2-K as well (Fig. 4, Table 5).

Fenoterol transport by OCT2 revealed the most drastic differences in stereoselectivity: OCT1 showed an almost 2-fold higher  $v_{max}$  for (*R,R*)-fenoterol. In contrast, OCT2 transported (*S,S*)-fenoterol with a 20-fold higher  $v_{max}$ , while (*R,R*)-fenoterol transport was, in comparison,

negligibly low. This resulted in a 37-fold higher intrinsic clearance for the presumably inactive (*S,S*)-enantiomer. The strong difference is particularly surprising given the high (70%) protein sequence identity shared between OCT1 and OCT2. For OCT3, the maximum transport velocity for both fenoterol enantiomers was similar to that of OCT1, while the  $K_m$  was about 10-fold higher. The antiporters MATE1 and MATE2-K transported fenoterol with significantly higher capacity but also higher  $K_m$ , whereby they showed a modest preference for the (*R,R*)-enantiomer.

Transport of formoterol by OCT2, OCT3, MATE1, and MATE2-K was completely absent or too low to determine pharmacokinetic parameters reliably.

With salbutamol, a differential enantiopreference was observed for both OCT2 and OCT3: While OCT1 and all OCT1 variants showed no stereoselectivity or only a minor degree of stereoselectivity towards (*S*)-salbutamol, OCT2 and OCT3 displayed significantly higher (3.5-fold and 4.6-fold)  $v_{max}$  values for (*R*)-salbutamol relative to (*S*)-salbutamol. This resulted in 1.9- and 5.9-fold greater intrinsic clearances for the pharmacologically active (*R*)-enantiomer. Comparable to fenoterol, MATE transporters showed a low affinity-high capacity transport of salbutamol, in this case with a preference for (*S*)-salbutamol.

Transport of atenolol by OCT2 was characterised by a lower  $v_{max}$  compared to OCT1 and a similar preference for (*S*)-atenolol. While MATE2-K exhibited OCT1-like atenolol transport, it was particularly surprising that MATE1 transported atenolol with a strongly increased capacity and a preference for the pharmacologically inactive (*R*)-atenolol.

In conclusion, unlike the relatively moderate differences in stereoselectivity among allelic variants of OCT1, a more complex picture

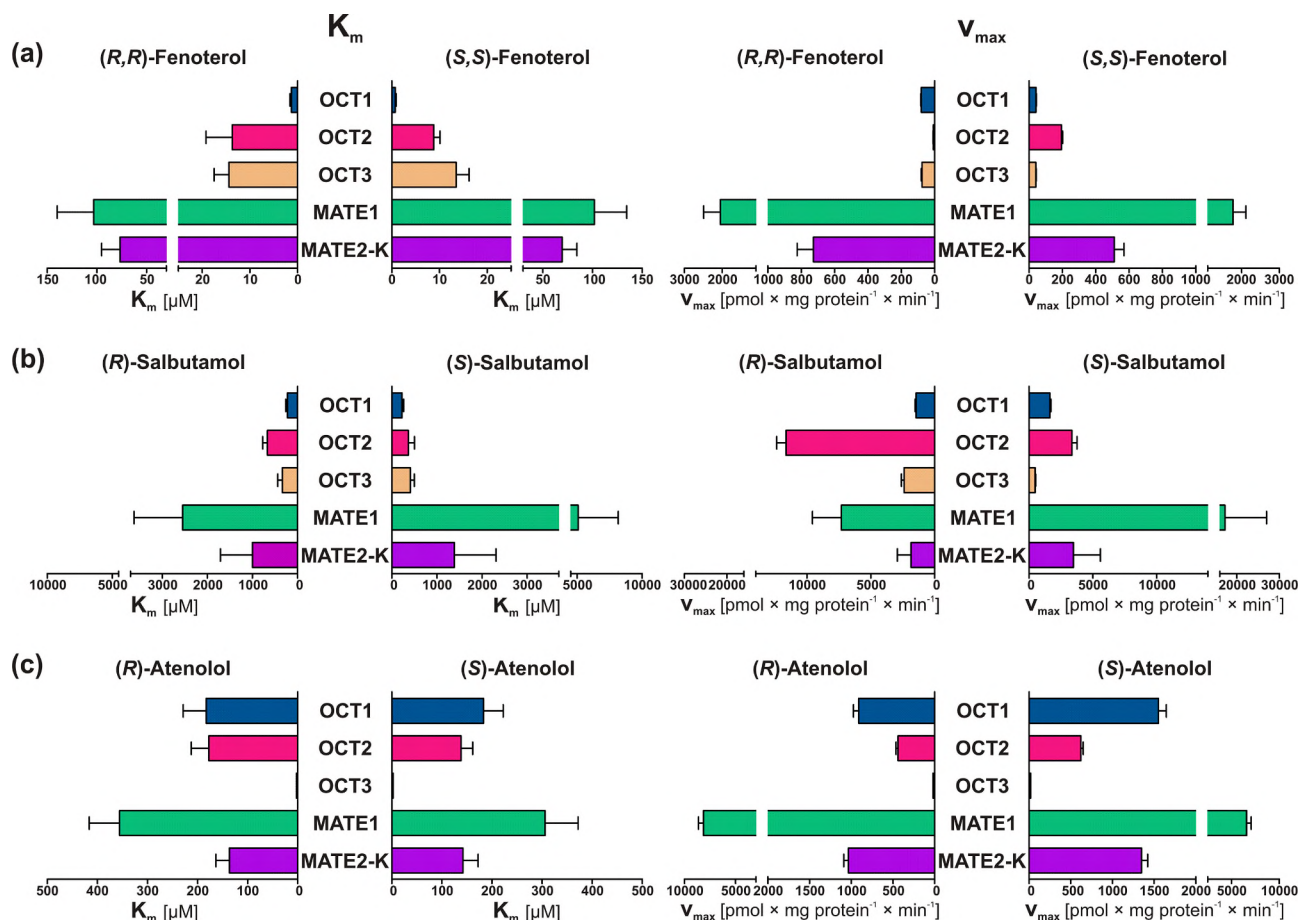


Fig. 4. Comparison of  $K_m$  (left) and  $v_{max}$  (right) between wild-type OCT1 and related cation transporters for (a) fenoterol, (b) salbutamol, and (c) atenolol. Formoterol transport kinetic parameters could not be determined with high precision and are given in Table 5 only.



**Table 5**

Kinetic parameters for the transport of racemic (anti)adrenergic drugs by OCT1-related transporters.

Transporter	Substrate	$K_m$ ( ± SEM) [μM]	$V_{max}$ ( ± SEM) [pmol × mg protein <sup>-1</sup> × min <sup>-1</sup> ]	$Cl_{int}$ ( ± SEM) [ml × min <sup>-1</sup> × g protein <sup>-1</sup> ]	Stereoselectivity		
					$K_m$	$V_{max}$	$Cl_{int}$
<b>OCT2</b>	(R,R)-Fenoterol	21.2 ( ± 10.6)	9.9** ( ± 2.7)	0.6** ( ± 0.2)	2.37-fold for (R,R)	19.6-fold for (S,S)	36.7-fold for (S,S)
	(S,S)-Fenoterol	8.9 ( ± 0.6)	194.1** ( ± 11.6)	22.0** ( ± 2.6)			
	(R,R)-Formoterol	no transport	no transport	no transport			
	(S,S)-Formoterol				-	-	-
	(R)-Salbutamol	679.0* ( ± 92.5)	11766.7** ( ± 1256.3)	17.5** ( ± 0.7)	1.88-fold for (R)	3.45-fold for (R)	1.86-fold for (R)
	(S)-Salbutamol	361.1* ( ± 66.9)	3407.3** ( ± 682.2)	9.4** ( ± 0.2)			
	(R)-Atenolol	290.6 ( ± 92.9)	609.0* ( ± 149.5)	2.4* ( ± 0.6)	1.68-fold for (R)	1.23-fold for (S)	1.96-fold for (S)
	(S)-Atenolol	172.9 ( ± 35.7)	749.3* ( ± 113.8)	4.7* ( ± 0.9)			
	<b>OCT3</b>	(R,R)-Fenoterol	15.2 ( ± 2.5)	76.5** ( ± 7.4)	5.3* ( ± 0.8)	1.07-fold for (R,R)	1.85-fold for (R,R)
(S,S)-Fenoterol		14.2 ( ± 2.9)	41.3** ( ± 4.1)	3.1* ( ± 0.5)			
(R,R)-Formoterol		no transport	no transport	no transport			
(S,S)-Formoterol					-	-	-
(R)-Salbutamol		356.8 ( ± 62.7)	2418.7* ( ± 317.4)	7.1* ( ± 1.3)	1.45-fold for (S)	4.60-fold for (R)	5.92-fold for (R)
(S)-Salbutamol		518.9 ( ± 195.1)	526.3* ( ± 81.0)	1.2* ( ± 0.3)			
(R)-Atenolol		no transport	no transport	no transport	-	-	-
(S)-Atenolol							
<b>MATE1</b>		(R,R)-Fenoterol	111.3 ( ± 16.3)	2091.3 ( ± 133.7)	19.8 ( ± 3.5)	1.08-fold for (R,R)	1.15-fold for (R,R)
	(S,S)-Fenoterol	103.5 ( ± 18.0)	1822.3 ( ± 360.6)	17.4 ( ± 1.4)			
	(R,R)-Formoterol	231.5 ( ± 114.7)	301.5 ( ± 152.3)	2.6 ( ± 1.6)			
	(S,S)-Formoterol	343.8 ( ± 321.0)	447.3 ( ± 363.4)	2.5 ( ± 1.2)	1.49-fold for (S)	1.48-fold for (S,S)	1.04-fold for (R,R)
	(R)-Salbutamol	2545.7 ( ± 1079.7)	7313.3 ( ± 2260.4)	3.1* ( ± 0.4)	2.00-fold for (S)	2.35-fold for (S)	1.19-fold for (S)
	(S)-Salbutamol	5081.0 ( ± 3073.9)	17216.7 ( ± 9812.9)	3.7* ( ± 0.4)			
	(R)-Atenolol	381.5 ( ± 68.7)	8326.3** ( ± 775.1)	22.6 ( ± 2.4)	1.01-fold for (S)	1.19-fold for (R)	1.07-fold for (R)
	(S)-Atenolol	384.7 ( ± 137.9)	7009.3** ( ± 916.5)	21.2 ( ± 4.3)			
	<b>MATE2-K</b>	(R,R)-Fenoterol	76.4 ( ± 4.1)	728.6* ( ± 92.9)	9.5** ( ± 0.8)	1.10-fold for (R,R)	1.42-fold for (R,R)
(S,S)-Fenoterol		69.5 ( ± 1.1)	511.7* ( ± 47.0)	7.4** ( ± 0.7)			
(R,R)-Formoterol		181.7 ( ± 158.1)	77.6 ( ± 38.5)	1.0 ( ± 0.7)			
(S,S)-Formoterol		35.7 ( ± 12.5)	33.7 ( ± 6.1)	1.1 ( ± 0.6)	5.09-fold for (R,R)	2.30-fold for (R,R)	1.10-fold for (S,S)
(R)-Salbutamol		999.4 ( ± 709.7)	1855.8 ( ± 1069.2)	2.2 ( ± 0.5)	1.39-fold for (S)	1.88-fold for (S)	1.23-fold for (S)
(S)-Salbutamol		1386.2 ( ± 921.9)	3487.0 ( ± 2105.0)	2.7 ( ± 0.3)			
(R)-Atenolol		150.1 ( ± 40.5)	1060.9* ( ± 128.7)	7.6* ( ± 1.1)	1.04-fold for (S)	1.30-fold for (S)	1.26-fold for (S)
(S)-Atenolol		155.7 ( ± 45.2)	1383.7* ( ± 200.7)	9.6* ( ± 1.2)			

SEM, standard error of the mean; asterisks indicate statistical significance of the differences between the two enantiomers (Student's *t*-test; \*  $p < 0.05$ , \*\*  $p < 0.01$ , and \*\*\*  $p < 0.001$ ).

with, in some instances, very strong degrees of stereoselectivity was seen with other solute carriers. Particularly interesting was the observed opposite stereoselectivity between the highly homologous (70% shared amino acid sequence identity) transporters OCT1 and OCT2 for some substrates. In addition, large changes in transport activity and stereoselectivity were observed between the two hepatic uptake transporters OCT1 and OCT3. These results indicate a completely non-uniform behaviour among relatively similar transporters with relatively similar chemical compounds.

#### 4. Discussion

In a comprehensive study on stereospecificity in OCT-mediated transport, we have assessed a selection of beta-adrenergic receptor-targeting drugs that are always or often administered as racemates in clinical drug therapy. Our data is indicative of notable stereospecificity in OCT1-mediated transport, with the most common genetic variants of OCT1 showing similar enantiomer preferences to the wild-type in overall but with exceptions. A general trend, however, was not evident, and at present, stereoselective uptake by organic cation transporters must be determined for every substrate individually. Enantiospecificity between different solute carriers differed surprisingly strongly, with the partially opposing enantiomer preferences between the closely related OCT1, OCT2, and OCT3 being of particular interest. As proposed recently, multiple substrate binding sites might contribute to the observed stereoselectivity in transport by organic cation transporters [16,17], and the amino acids in the substrate binding cleft are crucial for OCT substrate recognition and transport [18]. Moreover, pharmacophore

modelling showed that organic cation transporters interact with molecules with pronounced three-dimensional structures [19], compared to organic anion transporters. The stereoselectivity in transport of some of the substrates analysed in our study is compatible with a three-dimensional substrate recognition site.

Generally, the therapeutic effects for almost all beta<sub>2</sub>-adrenergic receptor agonists currently in clinical use are attributed to the (R)-enantiomers, while the (S)-enantiomers were found to be almost inactive at the beta<sub>2</sub>-adrenergic receptor [20]. Accordingly, for the anti-adrenergic drugs atenolol and acebutolol, it is the (S)-enantiomers that function as beta-adrenergic receptor antagonists [21].

As the pharmacokinetics of a drug are dependent on a number of stereoselective processes in the organism, it is of great relevance to compare how our *in vitro* results relate to clinical findings and data on stereoselectivity in the biotransformation of the studied drugs.

For fenoterol, no data was found in the literature regarding stereoselective pharmacokinetics in humans. However, *in vitro* sulfoconjugation of fenoterol was stereoselective, with the preferred enantiomer depending on the sulfotransferase and the site of sulfation [22]. Our data showed that wild-type OCT1 transports the pharmacologically active enantiomer, (R,R)-fenoterol, with almost double the maximum transport velocity compared to the (S,S)-enantiomer but with proportionally lower affinity. Interestingly, this was nearly the opposite for OCT1\*2, a variant that is particularly common in people of Central and South American origin [7]. However, for both wild-type OCT1 and the \*2 variant, no stereoselectivity was seen in the intrinsic clearance. Thus, it is difficult to predict from this data which implication the OCT1 related stereoselectivity will have on clinical pharmacokinetics and

pharmacodynamics. At the very low therapeutic concentrations, the differences in  $K_m$  may have a stronger effect than those in  $v_{max}$  or intrinsic clearance. Very interesting is the large degree of stereospecificity (37-fold higher intrinsic clearance for (S,S)-fenoterol) observed for OCT2, a transporter that is highly expressed in the kidneys. However, given the relatively low renal clearance of fenoterol, this may not be of greater relevance in clinical therapy.

Formoterol plasma concentrations were 1.5-fold higher for the (S,S)-enantiomer than for the (R,R)-enantiomer one hour following inhalative dosing of the 1:1 mixture of (R,R)- and (S,S)-formoterol in humans [23]. Both after inhalative and oral administration, the excretion of unchanged drug in urine was greater for (S,S)- than for (R,R)-formoterol, but excretion as formoterol glucuronide conjugate was greater for (R,R)- over (S,S)-formoterol [14,24,25]. The latter is particularly interesting in light of the fact that glucuronide conjugation in liver microsomes was found to be more than 2-fold higher for the (S,S)-enantiomer [14]. The total excretion (unchanged and as glucuronide conjugate) after oral dosage was greater for (R,R)-formoterol. There appeared to be a significant difference between male and female participants [26]. The enantiopreference for (R,R)-formoterol in metabolism and the 1.2-fold higher OCT1-mediated intrinsic clearance of (R,R)-formoterol could together contribute to higher plasma concentrations of (S,S)-formoterol.

Salbutamol is probably the most widely-used short-acting beta-mimetic drug. Upon administration of the racemate, significant differences in pharmacokinetics were found, with up to 8-fold higher systemic exposure (AUC) for the (S)- versus the (R)-enantiomer after inhalative dosage, and more than 20-fold following oral administration [27–29]. The larger systemic (S):(R) ratio after oral dosage is mostly a result of a stereoselective first-pass metabolism, but pH-dependent chiral inversion of the (R)- to the (S)-enantiomer was also found to occur to a smaller extent (ca. 6%) in the stomach but not following inhalation [27,30–33]. The plasma levels of (R)- and (S)-salbutamol were higher when given in enantiopure form compared to the racemate, suggesting a possible influence of each enantiomer on the clearance of the opposite enantiomer when administered as racemic mixture [28]. With respect to the biotransformation, sulphate conjugation in the intestine and liver was 12-fold higher for (R)-salbutamol [33–36]. Stereospecificity in presystemic metabolism in the lungs was observed *in vitro* but not confirmed in humans [36,37]. A common single nucleotide polymorphism (rs1975350) in *SULT1A3*, the main metabolising enzyme, had no significant effect on the stereospecificity of the pharmacokinetics [33,38]. We observed a small but probably negligible enantiopreference for the (S)-enantiomer by OCT1, MATE1, and MATE2-K, which, hence, do not appear to contribute to the higher systemic exposure for (S)-salbutamol. However, the almost 6-fold higher intrinsic clearance of (R)-salbutamol observed for OCT3, another hepatic uptake transporter, is in line with the clinical observations. In accordance is also the almost 2-fold higher intrinsic clearance of (R)-salbutamol for OCT2, which may very well be of relevance, given the comparatively high renal clearance of unchanged salbutamol (46% for (R)-salbutamol; 55% for (S)-salbutamol [39]).

Acebutolol plasma and urine concentrations were both 1.2-fold higher for the (S)-enantiomer following oral administration of racemic acebutolol, which corresponded to a 1.2-fold higher oral clearance of (R)-acebutolol. This may be explained by stereoselectivity in first-pass metabolism and renal excretion of the main metabolite diacetolol. For diacetolol,  $C_{max}$  and renal clearance was greater for the (R)-enantiomer; no significant difference was seen with respect to plasma AUC [40]. The (S):(R) ratio for urinary excretion correlated with age in a subsequent study involving elderly participants, possibly as a result of altered stereoselectivity in tubular secretion [41]. A different study preceding the above two found no stereoselectivity in the disposition of acebutolol and diacetolol following single and repeated oral administration [42]. Plasma protein binding of acebutolol appears not to be stereoselective and the majority of the drug (greater than 85%) is found in the

unbound fraction [21]. Our data showed a 1.5-fold higher intrinsic clearance of (S)-acebutolol for OCT1.

With respect to atenolol, it was found in several independent studies that the plasma AUCs were slightly (1.1-fold) higher for (R)-atenolol following a single oral dose of the racemate [43–46]. In the study conducted by Mehvar et al., a slightly (1.1-fold) but statistically significantly higher renal clearance of the (S)-enantiomer was proposed as possible underlying reason; however, no significant difference in the renal clearance was seen in the study by Boyd et al. [43,45]. Intriguingly, exercise appeared to alter the stereoselectivity of atenolol pharmacokinetics, as the (R):(S) ratio of the mean plasma concentrations changed from 1.1 at rest to 0.7 following exercise [47]. Given its more hydrophilic structure, atenolol is almost exclusively eliminated unchanged in urine, a process that appears to be modestly stereoselective towards the (R)-enantiomer [21,43,45]. A possible interaction between the enantiomers in a racemic mixture, as seen for salbutamol, apparently does not occur with atenolol [44,46]. A high degree of stereoselectivity was observed in the glucuronidation of atenolol, as 3.1-fold more of the (S)-glucuronide conjugate was formed after incubation of the racemate with UGT1A9 [48]. These clinical observations may partially be explained by the higher intrinsic clearance of the (S)-enantiomer via OCT1 (including variants) and OCT2, but the almost 2-fold difference in cell uptake via OCT2 is apparently not fully reflected by the pharmacokinetic data.

Altogether, our *in vitro* data on stereospecificity in organic cation transporter-mediated drug transport is in accordance with, and may partially account for, stereospecificity in clinical pharmacokinetics, especially with respect to formoterol, salbutamol, and atenolol. It is notable that, for many racemic drugs, the pharmacokinetics were not analysed with regard to stereospecificity, despite receptor interactions being stereoselective in most cases. Here we showed that membrane transport of several beta-(anti)adrenergic drugs can be stereospecific, but it appears that stereoselectivity is more pronounced in biotransformation of some of the studied drugs. From a molecular perspective, the observed stereoselectivity in membrane transport may indicate a relatively tight interaction between solute carriers and some substrates. This could be explored in more detail using computational molecular modelling as a follow-up to this study.

#### CRediT authorship contribution statement

**Ole Jensen:** Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Muhammad Rafehi:** Writing - original draft, Writing - review & editing. **Mladen V. Tzvetkov:** Conceptualization, Resources. **Jürgen Brockmöller:** Conceptualization, Resources, Writing - original draft, Writing - review & editing, Project administration, Supervision.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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