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

Ole Jensen[a,*], Muhammad Rafehi[a], Mladen V. Tzvetkov[a,b], Jürgen Brockmöller[a]

[a] *Institute of Clinical Pharmacology, University Medical Center Göttingen, Georg-August University, D-37075 Göttingen, Germany*
[b] *Center of Drug Absorption and Transport (C_DAT), Department of Pharmacology, University Medical Center, Ernst-Moritz-Arndt-University, D-17487 Greifswald, Germany*

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$_2$-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

(R,R)-Fenoterol **(S,S)-Fenoterol** **(R,R)-Formoterol** **(S,S)-Formoterol**

(R)-Salbutamol **(S)-Salbutamol** **(R)-Pirbuterol** **(S)-Pirbuterol**

(R)-Orciprenaline **(S)-Orciprenaline** **(R)-Etilefrine** **(S)-Etilefrine**

(R)-Acebutolol **(S)-Acebutolol** **(R)-Atenolol** **(S)-Atenolol**
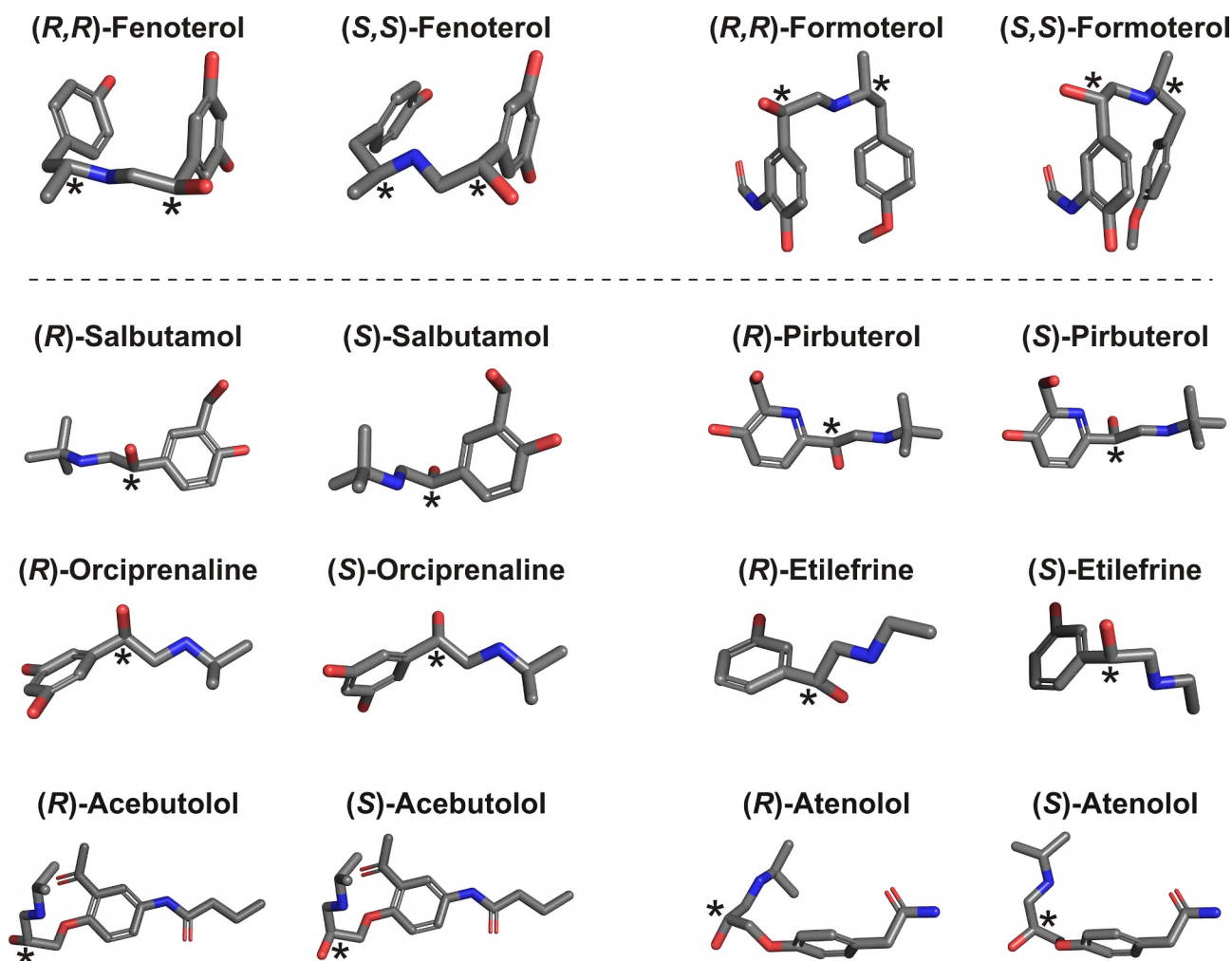
Fig. 1. Beta-adrenergic receptor agonists and antagonists investigated for stereoselective transport by OCTs and transporters of the MATE-family. These were selected for physicochemical properties ($pK_a > 8.0$ and $logD_{pH7.4} < 1$) that renders them likely transporter substrates. Chiral centres are 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$_1$-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_2. Cell lines overexpressing MATE1 and MATE2-K were incubated with 30 mM NH_4Cl 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[a]	Mobile phase[b]	Flow rate [μl × min^{-1}]	Retention time A [min]	Retention time B [min]
Fenoterol	Chiral-CBH	10 mM NH$_4$Ac, pH 5.8, 5% IPA	500	5.3 (R,R)	6.1 (S,S)
Formoterol	Chiral-CBH	10 mM NH$_4$Ac, 10% ACN	300	15.4 (R,R)	17.0 (S,S)
Salbutamol	Chirobiotic T	20 mM NH$_4$Ac, pH 4.5, 96% MeOH	1000	6.8 (R)	7.7 (S)
Pirbuterol	Chiral-CBH	10 mM NH$_4$Ac, pH 5.8, 5% IPA	300	3.3 (P1)	3.5 (P2)
Orciprenaline	Chirobiotic T	20 mM NH$_4$Ac, pH 4.5, 93% MeOH	500	9.2 (P1)	10.7 (P2)
Etilefrine	Chirobiotic T	20 mM NH$_4$Ac, pH 4.5, 93% MeOH	500	10.8 (P1)	11.6 (P2)
Acebutolol	Chiral-CBH	10 mM NH$_4$Ac, pH 5.8, 10% ACN	500	2.9 (R)	4.8 (S)
Atenolol	Chiral-CBH	10 mM NH$_4$Ac, pH 5.8, 5% IPA	300	3.8 (R)	5.1 (S)

[a] CBH, cellobiohydrolase
[b] ACN, acetonitrile; IPA, isopropanol; MeOH, methanol; NH$_4$Ac, ammonium acetate

Table 2
Chemical properties pK$_a$ and logD$_{pH7.4}$ and relevant data for mass-spectrometric detection of the substrates and analytical internal standards.

Drug	pK$_a$	logD$_{pH7.4}$	Q1 Mass[a] [Da]	Q3 Mass[b] [Da]	DP[c] [V]	CE[d] [V]	CXP[e] [V]
Substrates							
Fenoterol[1]	9.63	0.33	304.1 (304.1)	107.1 (135.2)	70 (70)	44 (24)	12 (12)
Formoterol[1]	9.81	0.04	345.2 (345.2)	149.1 (121.1)	70 (70)	28 (42)	15 (15)
Salbutamol[2]	9.40	−1.32	240.2 (240.2)	148.2 (222.2)	60 (60)	24 (24)	15 (15)
Pirbuterol[1]	9.59	−1.78	241.3 (241.3)	149.1 (167.2)	65 (65)	30 (24)	15 (15)
Orciprenaline[3]	9.70	−0.94	212.1 (212.1)	152.0 (107.0)	56 (56)	23 (39)	10 (8)
Etilefrine[4]	9.73	−1.07	182.1 (182.1)	164.0 (91.0)	51 (51)	17 (37)	10 (6)
Acebutolol[2]	9.65	−0.68	337.2 (337.2)	116.0 (98.1)	91 (91)	31 (29)	8 (8)
Atenolol[5]	9.67	−1.80	267.2 (267.2)	145.2 (74.0)	130 (1 3 0)	38 (35)	10 (14)
Internal standards							
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)

[a] Q1, first quadrupole (qualifiers below in parentheses);
[b] Q3, third quadrupole (qualifiers in parentheses);
[c] DP, declustering potential;
[d] CE, collision energy;
[e] CXP, collision cell exit potential;
[1] quantified with internal standard fenoterol-d6;
[2] quantified with internal standard desvenlafaxine;
[3] quantified with internal standard metoprolol;
[4] quantified with internal standard (S)-propranolol;
[5] 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 μm; Sigma-Aldrich, Darmstadt, Germany) with a corresponding 10 × 3 mm guard column or an Astec Chirobiotic T (15 cm × 2.1 mm, 5 μm; 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$_{max}$ were estimated by regression analysis using the Michaelis-Menten equation. Means and standard errors were calculated from individual K$_m$ and v$_{max}$ values of at least three independent experiments. The intrinsic clearance Cl$_{int}$ was calculated as the ratio of v$_{max}$ over K$_m$. The kinetic parameters v$_{max}$, K$_m$, and Cl$_{int}$ 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 (pk$_A$ > 8.0 and logD$_{pH7.4}$ < 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$_{max}$) 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$_{max}$ between the enantiomers of salbutamol, pirbuterol, orciprenaline (metaproterenol), etilefrine, acebutolol, and atenolol (Figs. 2, 3, Table 3). However, no notable differences
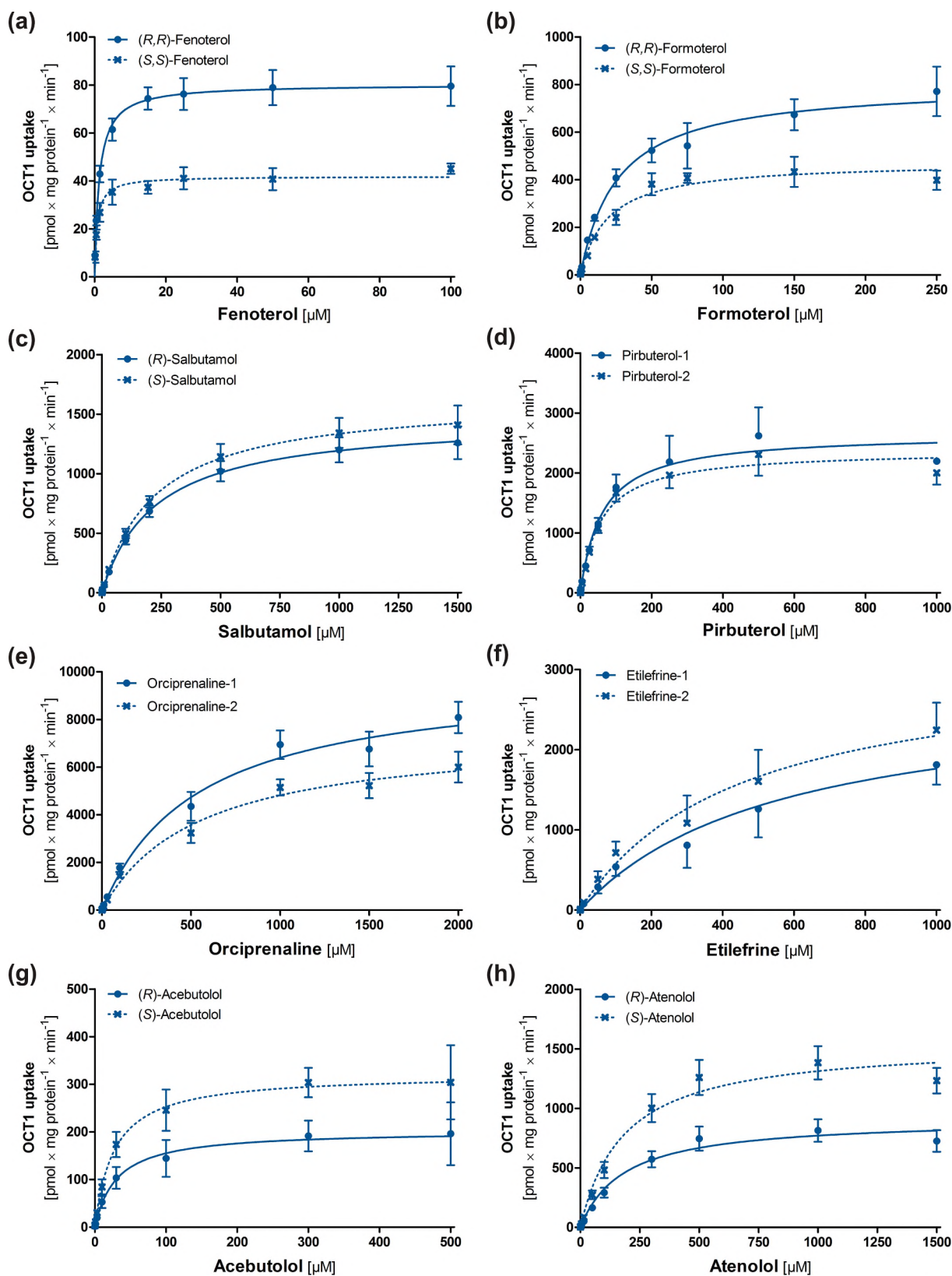
Fig. 2. Transport of (anti)adrenergic drug enantiomers by wild-type OCT1, determined using stably transfected HEK293 cells. Shown are the mean ± SEM of at least three independent experiments for each drug. Enantiomers that could not be identified were numbered according to the order of HPLC elution.

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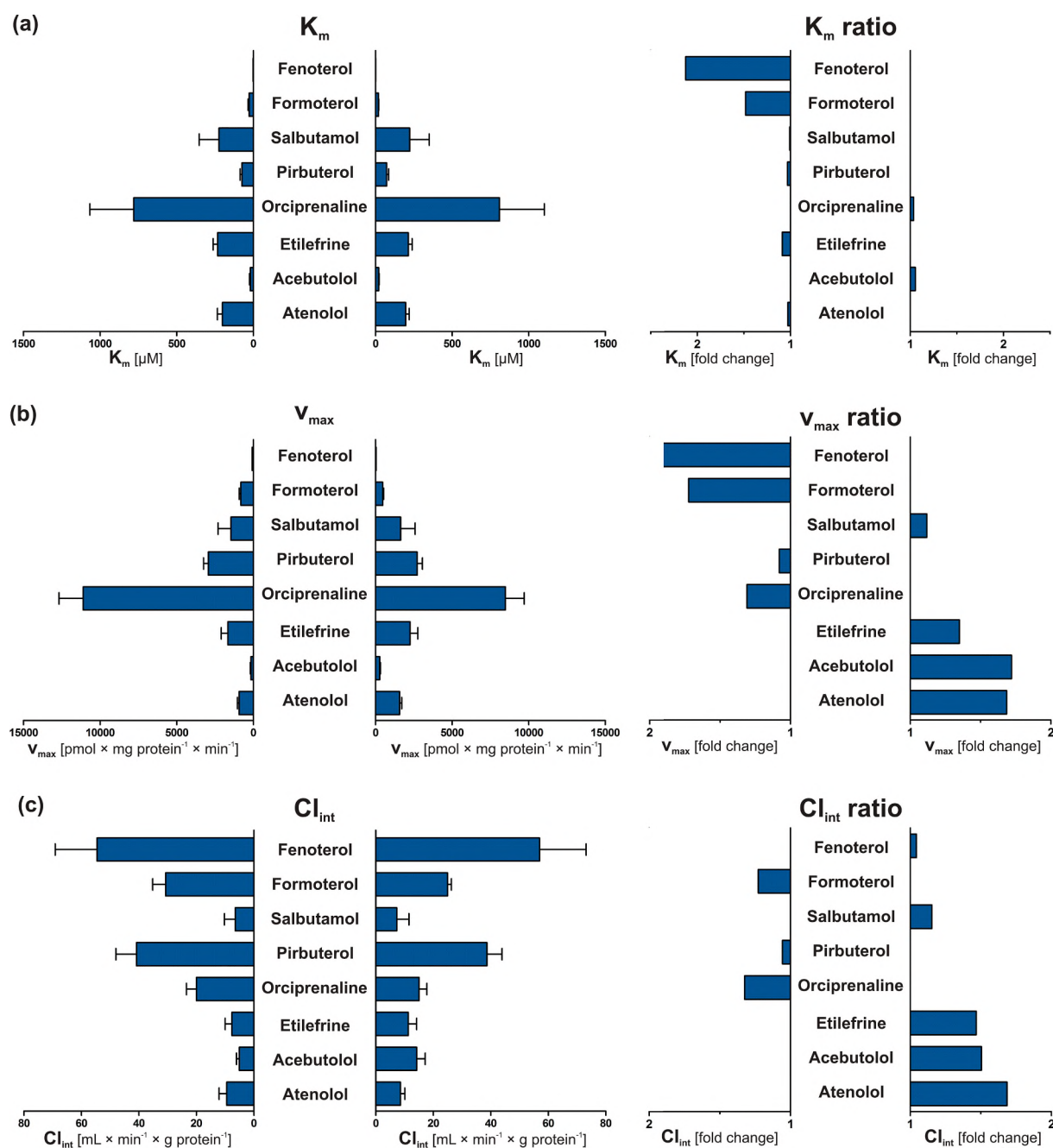
Fig. 3. Stereoselectivity in the OCT1-mediated transport of sympathomimetic and sympatholytic drugs. The ratios on the right were calculated as the quotients of the higher and the lower values.

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^{-1} × min^{-1}]	Cl_{int} (± SEM) [ml × min^{-1} × g protein^{-1}]	Stereoselectivity		
					K_m	v_{max}	Cl_{int}
OCT1	(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^{-1} × min^{-1}]	Cl_{int} (± SEM) [ml × min^{-1} × g protein^{-1}]	Stereoselectivity		
					K_m	v_{max}	Cl_{int}
OCT1*2	(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)			
OCT1*3	(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						
	(R)-Atenolol	no transport	no transport	no transport	–	–	–
	(S)-Atenolol						
OCT1*4	(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						
	(R)-Atenolol	no transport	no transport	no transport	–	–	–
	(S)-Atenolol						
OCT1*7	(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)			
	(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)			
OCT1*8	(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)			
	(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
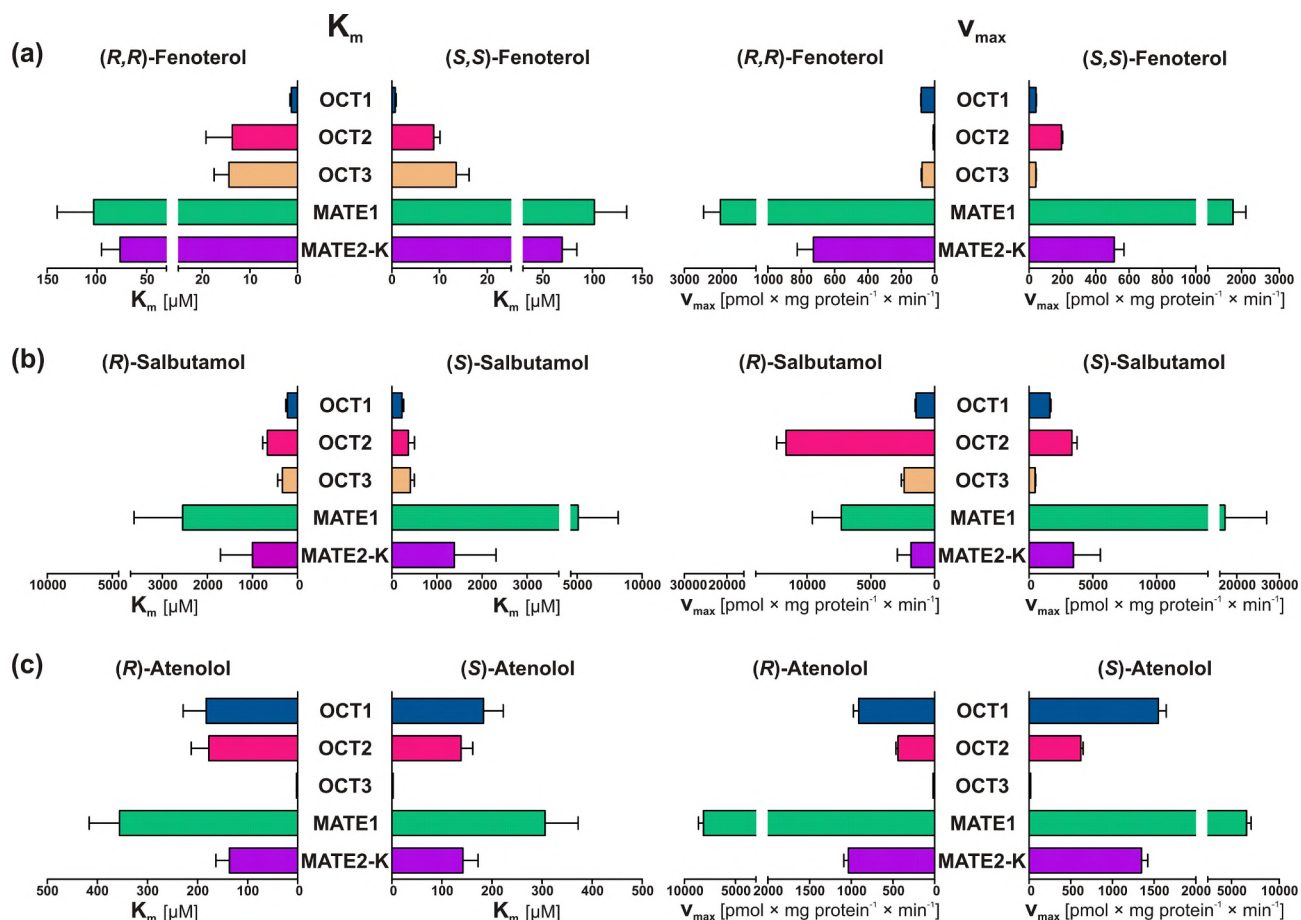

Fig. 4. Comparison of K_m (left) and v_{max} (right) between wild-type OCT1 and related cation transporters for (a) fenoterol, (b) salbutamol, and (c) atenolol. Formoterol transport kinetic parameters could not be determined with high precision and are given in Table 5 only.

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^{-1} × min^{-1}]	Cl_{int} (± SEM) [ml × min^{-1} × g protein^{-1}]	Stereoselectivity		
					K_m	v_{max}	Cl_{int}
OCT2	(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)			
OCT3	(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)	1.71-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						
MATE1	(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)	1.14-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)	1.49-fold for (S)	1.48-fold for (S,S)	1.04-fold for (R,R)
	(S,S)-Formoterol	343.8 (± 321.0)	447.3 (± 363.4)	2.5 (± 1.2)			
	(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)			
MATE2-K	(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)	1.28-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)	5.09-fold for (R,R)	2.30-fold for (R,R)	1.10-fold for (S,S)
	(S,S)-Formoterol	35.7 (± 12.5)	33.7 (± 6.1)	1.1 (± 0.6)			
	(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$_2$-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$_2$-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* sulfo-conjugation 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.

Acknowledgements

References

[1] R. Hendrickx, J.G. Johansson, C. Lohmann, R.M. Jenvert, A. Blomgren, L. Borjesson, L. Gustavsson, Identification of novel substrates and structure-activity relationship of cellular uptake mediated by human organic cation transporters 1 and 2, J. Med. Chem. 56 (18) (2013) 7232–7242.

[2] D.A. Handley, C.H. Senanayake, W. Dutczak, J.L. Benovic, T. Walle, R.B. Penn, H.S. Wilkinson, G.J. Tanoury, R.G. Andersson, F. Johansson, J. Morley, Biological actions of formoterol isomers, Pulm. Pharmacol. Ther. 15 (2) (2002) 135–145.

[3] J. Lötvall, M. Palmqvist, P. Arvidsson, A. Maloney, G.P. Ventresca, J. Ward, The therapeutic ratio of R-albuterol is comparable with that of RS-albuterol in asthmatic patients, J. Allergy Clin. Immunol. 108 (5) (2001) 726–731.

[4] T. Niwa, N. Murayama, H. Yamazaki, Stereoselectivity of human cytochrome p450 in metabolic and inhibitory activities, Curr. Drug Metab. 12 (6) (2011) 549–569.

[5] U.K. Walle, T. Walle, Stereoselective sulfation of terbutaline by the rat liver cytosol: evaluation of experimental approaches, Chirality 1 (2) (1989) 121–126.

[6] M.J. Dresser, M.K. Leabman, K.M. Giacomini, Transporters involved in the elimination of drugs in the kidney: organic anion transporters and organic cation transporters, J. Pharm. Sci. 90 (4) (2001) 397–421.

[7] T. Seitz, R. Stalmann, N. Dalila, J. Chen, S. Pojar, J.N. Dos Santos Pereira, R. Kratzner, J. Brockmöller, M.V. Tzvetkov, Global genetic analyses reveal strong inter-ethnic variability in the loss of activity of the organic cation transporter OCT1, Genome Med 7(1) (2015) 56.

[8] Q. Zhou, L.S. Yu, S. Zeng, Stereoselectivity of chiral drug transport: a focus on enantiomer-transporter interaction, Drug Metab. Rev. 46 (3) (2014) 283–290.

[9] A.R. Saadatmand, S. Tadjerpisheh, J. Brockmöller, M.V. Tzvetkov, The prototypic pharmacogenetic drug debrisoquine is a substrate of the genetically polymorphic organic cation transporter OCT1, Biochem. Pharmacol. 83 (10) (2012) 1427–1434.

[10] J. Chen, J. Brockmöller, T. Seitz, J. König, M.V. Tzvetkov, X. Chen, Tropane alkaloids as substrates and inhibitors of human organic cation transporters of the SLC22 (OCT) and the SLC47 (MATE) families, Biol. Chem. 398 (2) (2017) 237–249.

[11] H. Jiang, C. Randlett, H. Junga, X. Jiang, Q.C. Ji, Using supported liquid extraction together with cellobiohydrolase chiral stationary phases-based liquid chromatography with tandem mass spectrometry for enantioselective determination of acebutolol and its active metabolite diacetolol in spiked human plasma, J. Chromatogr. B 877 (3) (2009) 173–180.

[12] T. Fornstedt, A.M. Hesselgren, M. Johansson, Chiral assay of atenolol present in microdialysis and plasma samples of rats using chiral CBH as stationary phase, Chirality 9 (4) (1997) 329–334.

[13] M. Sanghvi, A. Ramamoorthy, J. Strait, I.W. Wainer, R. Moaddel, Development and validation of a sensitive LC-MS/MS method for the determination of fenoterol in human plasma and urine samples, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 933 (2013) 37–43.

[14] M. Zhang, J.P. Fawcett, J.M. Kennedy, J.P. Shaw, Stereoselective glucuronidation of formoterol by human liver microsomes, Br. J. Clin. Pharmacol. 49 (2) (2000) 152–157.

[15] A. Halabi, C. Ferrayoli, M. Palacio, V. Dabbene, S. Palacios, Validation of a chiral HPLC assay for (R)-salbutamol sulfate, J. Pharm. Biomed. Anal. 34 (1) (2004) 45–51.

[16] T. Keller, V. Gorboulev, T.D. Mueller, V. Dotsch, F. Bernhard, H. Koepsell, Rat organic cation transporter 1 contains three binding sites for substrate 1-methyl-4-phenylpyridinium per monomer, Mol. Pharmacol. 95 (2) (2019) 169–182.

[17] H. Koepsell, Multiple binding sites in organic cation transporters require sophisticated procedures to identify interactions of novel drugs, Biol. Chem. (2019) 195.

[18] D.C. Li, C.G. Nichols, M. Sala-Rabanal, Role of a hydrophobic pocket in polyamine interactions with the polyspecific organic cation transporter OCT3, J. Biol. Chem. 290 (46) (2015) 27633–27643.

[19] H.C. Liu, A. Goldenberg, Y. Chen, C. Lun, W. Wu, K.T. Bush, N. Balac, P. Rodriguez, R. Abagyan, S.K. Nigam, Molecular properties of drugs interacting with SLC22 transporters OAT1, OAT3, OCT1, and OCT2: A machine-learning approach, J. Pharmacol. Exp. Ther. 359 (1) (2016) 215–229.

[20] B. Waldeck, Enantiomers of bronchodilating beta2-adrenoceptor agonists: is there a cause for concern? J. Allergy Clin. Immunol. 103 (5 Pt 1) (1999) 742–748.

[21] R. Mehvar, D.R. Brocks, Stereospecific pharmacokinetics and pharmacodynamics of beta-adrenergic blockers in humans, J. Pharm. Pharm. Sci. 4 (2) (2001) 185–200.

[22] A.A. Wilson, J. Wang, P. Koch, T. Walle, Stereoselective sulphate conjugation of fenoterol by human phenolsulphotransferases, Xenobiotica 27 (11) (1997) 1147–1154.

[23] M. Hostrup, C.K. Narkowicz, S. Habib, D.S. Nichols, G.A. Jacobson, Beta2-adrenergic ligand racemic formoterol exhibits enantioselective disposition in blood and skeletal muscle of humans, and elicits myocellular PKA signaling at therapeutic inhaled doses, Drug Test. Anal. 11 (7) (2019) 1048–1056.

[24] J.B. Lecaillon, G. Kaiser, M. Palmisano, J. Morgan, G. Della Cioppa, Pharmacokinetics and tolerability of formoterol in healthy volunteers after a single high dose of Foradil dry powder inhalation via Aerolizer, Eur. J. Clin. Pharmacol. 55 (2) (1999) 131–138.

[25] G.A. Jacobson, M. Hostrup, C.K. Narkowicz, D.S. Nichols, E.H. Walters, Enantioselective disposition of (R, R)-formoterol, (S, S)-formoterol and their respective glucuronides in urine following single inhaled dosing and application to doping control, Drug Test. Anal. 11 (7) (2019) 950–956.

[26] M. Zhang, J.P. Fawcett, J.P. Shaw, Stereoselective urinary excretion of formoterol and its glucuronide conjugate in human, Br. J. Clin. Pharmacol. 54 (3) (2002) 246–250.

[27] M. Vakily, R. Mehvar, D. Brocks, Stereoselective pharmacokinetics and pharmacodynamics of anti-asthma agents, Ann. Pharmacother. 36 (4) (2002) 693–701.

[28] B.J. Lipworth, D.J. Clark, P. Koch, C. Arbeeny, Pharmacokinetics and extrapulmonary beta 2 adrenoceptor activity of nebulised racemic salbutamol and its R and S isomers in healthy volunteers, Thorax 52 (10) (1997) 849–852.

[29] G.A. Jacobson, F.V. Chong, R. Wood-Baker, (R, S)-Salbutamol plasma concentrations in severe asthma, J. Clin. Pharm. Ther. 28 (3) (2003) 235–238.

[30] D.W. Boulton, J.P. Fawcett, Enantioselective disposition of salbutamol in man following oral and intravenous administration, Br. J. Clin. Pharmacol. 41 (1) (1996) 35–40.

[31] T. Zhou, J. Zeng, S. Liu, T. Zhao, J. Wu, W. Lai, M. He, B. Xu, S. Qu, L. Xu, W. Tan, Study on the determination and chiral inversion of R-salbutamol in human plasma and urine by liquid chromatography-tandem mass spectrometry, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 1002 (2015) 218–227.

[32] K. Gumbhir-Shah, D.J. Kellerman, S. DeGraw, P. Koch, W.J. Jusko, Pharmacokinetic and pharmacodynamic characteristics and safety of inhaled albuterol enantiomers in healthy volunteers, J. Clin. Pharmacol. 38 (12) (1998) 1096–1106.

[33] D.W. Boulton, J.P. Fawcett, The pharmacokinetics of levosalbutamol: what are the clinical implications? Clin. Pharmacokinet. 40 (1) (2001) 23–40.

[34] U.K. Walle, G.R. Pesola, T. Walle, Stereoselective sulphate conjugation of salbutamol in humans: comparison of hepatic, intestinal and platelet activity, Br. J. Clin. Pharmacol. 35 (4) (1993) 413–418.

[35] T. Nakpheng, S. Songkarak, T. Suwandecha, R. Sritharadol, C. Chunhachaichana, T. Srichana, Evidences for salbutamol metabolism by respiratory and liver cell lines, Drug Metab. Pharmacokinet. 32 (2) (2017) 127–134.

[36] J.K. Ward, J. Dow, N. Dallow, P. Eynott, S. Milleri, G.P. Ventresca, Enantiomeric disposition of inhaled, intravenous and oral racemic-salbutamol in man–no evidence of enantioselective lung metabolism, Br. J. Clin. Pharmacol. 49 (1) (2000) 15–22.

[37] T. Walle, E.A. Eaton, U.K. Walle, G.R. Pesola, Stereoselective metabolism of RS-albuterol in humans, Clin. Rev. Allergy Immunol. 14 (1) (1996) 101–113.

[38] G.A. Jacobson, K.C. Yee, R. Wood-Baker, E.H. Walters, SULT 1A3 single-nucleotide polymorphism and the single dose pharmacokinetics of inhaled salbutamol enantiomers: are some athletes at risk of higher urine levels? Drug Test. Anal. 7 (2) (2015) 109–113.

[39] D.W. Boulton, J.P. Fawcett, Enantioselective disposition of albuterol in humans, Clin. Rev. Allergy Immunol. 14 (1) (1996) 115–138.

[40] M. Piquette-Miller, R.T. Foster, C.T. Kappagoda, F. Jamali, Pharmacokinetics of acebutolol enantiomers in humans, J. Pharm. Sci. 80 (4) (1991) 313–316.

[41] M. Piquette-Miller, R.T. Foster, C.T. Kappagoda, F. Jamali, Effect of aging on the pharmacokinetics of acebutolol enantiomers, J. Clin. Pharmacol. 32 (2) (1992) 148–156.

[42] M.G. Sankey, A. Gulaid, C.M. Kaye, Preliminary study of the disposition in man of acebutolol and its metabolite, diacetolol, using a new stereoselective hplc method, J. Pharm. Pharmacol. 36 (4) (1984) 276–277.

[43] R. Mehvar, M.E. Gross, R.N. Kreamer, Pharmacokinetics of atenolol enantiomers in humans and rats, J. Pharm. Sci. 79 (10) (1990) 881–885.

[44] K. Stoschitzky, G. Egginger, G. Zernig, W. Klein, W. Lindner, Stereoselective features of (R)- and (S)-atenolol: clinical pharmacological, pharmacokinetic, and radioligand binding studies, Chirality 5 (1) (1993) 15–19.

[45] R.A. Boyd, S.K. Chin, O. Don-Pedro, R.L. Williams, K.M. Giacomini, The pharmacokinetics of the enantiomers of atenolol, Clin. Pharmacol. Ther. 45 (4) (1989) 403–410.

[46] G. Egginger, W. Lindner, S. Kahr, K. Stoschitzky, Stereoselective HPLC bioanalysis of atenolol enantiomers in plasma: application to a comparative human pharmacokinetic study, Chirality 5 (7) (1993) 505–512.

[47] K. Stoschitzky, W. Lindner, W. Klein, Stereoselective release of (S)-atenolol from adrenergic nerve endings at exercise, Lancet 340 (8821) (1992) 696–697.

[48] S. Xie, S. Zeng, Stereoselective glucuronidation of propafenone and its analogues by human recombinant UGT1A9, Chem Pharm Bull (Tokyo) 58 (6) (2010) 879–883.