sheep revealed that a saturable mechanism is responsible for the uptake of T_4 through the choroid plexus from blood side to the cerebrospinal fluid (28). Apparent Km value with respect to the concentration in the injectate was 11 μM. However, taking into consideration the dilution factor after the injection, the corrected Km value was to be 1.6 μM, comparable with that determined in this study for the uptake by the cerebral cortex across the BBB. mOatp14 may play a role in the uptake of T_4 at the basolateral side of the choroid plexus epithelial cells.

The present study highlights the importance of membrane transporters, especially Oatp14, for the brain uptake of T_4 across the BBB. In addition to the BBB and blood-cerebrospinal fluid barrier, transporters will play an important role in the disposition of thyroid hormones in the central nervous system. Carrier-mediated uptake of T_4 and T_3 has been reported in primary cultured neuronal cells (29). Although the transporter uptake by brain parenchymal cells has not been identified, there are some likely candidate transporters. OATP-E, another Oatp/OATP isoform, is known to accept thyroid hormones as substrates (30) and is expressed in the brain (30). In addition to Oatp/OATP isoforms, MCT8 has been identified as a thyroid hormone transporter (17). MCT8 is expressed weakly in the brain but most abundantly in the liver (17). Recently Dumitrescu *et al.* (31) reported that mutations in the MCT8 gene are associated with thyroid hormonal and neurological abnormalities in humans, although whether these are due to the functional loss of MCT8 in the central nervous system remains to be elucidated. Further studies are necessary to determine the roles of transporters in regulating the disposition of thyroid hormones in the central nervous system.

Thyroid hormones are activated/inactivated by deiodinases in the brain. Of three subtypes, types 2 and 3 deiodinases are expressed in the brain. Type 2 deiodinase is responsible for the conversion T_4 to T_3, whereas type 3 deiodinase inactivates thyroid hormone by converting T_4 to rT_3, and T_3 to T_2 (32, 33). In the brain, type 2 deiodinase has been shown to be predominantly expressed in the nonneuronal cells and glial cells, such as tanycytes and astrocytes (32), whereas type 3 deiodinase is expressed in the neurons (33). Therefore, it can be hypothesized that T_4 is transported into the brain via the BBB by specific transport systems followed by activation to T_3 mainly in nonneuronal and glial cells. Thereafter, T_3, produced by nonneuronal and glial cells, is taken up by neuronal cells to exert its action via the nuclear receptors followed by inactivation by type 3 deiodinase. Membrane transport processes in nonneuronal and glial cells and neurons will play an important role in regulating the effect of thyroid hormones in the central nervous system together with deiodinases.

In conclusion, taurocholate-sensitive transporters play a predominant role in the uptake of T_4 across the BBB. It is suggested that mOatp14 accounts for E-sul inhibitable fraction, at least partly, and the E-sul-insensitive fraction is accounted for by unknown transporters. In addition to the BBB, Oatp14 may also play a role in the uptake of T_4 in the choroid plexus.

Acknowledgments

We are grateful to Dr. Toshimasa Ohnishi and Dr. Young-Joo Lee for their kind instruction to perform *in situ* brain perfusion in mice.

Received January 19, 2004. Accepted May 19, 2004.

Address all correspondence and requests for reprints to: Yuichi Sugiyama, Ph.D., Department of Molecular Pharmacokinetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. E-mail: sugiyama@mol.f.u-tokyo.ac.jp.

This study was performed through the Advanced and Innovational Research program in Life Sciences from the Ministry of Education, Culture, Sports, Science and Technology, the Japanese Government.

References

1. **Bernal J** 2002 Action of thyroid hormone in brain. J Endocrinol Invest 25: 268–288
2. **Sarkar PK** 2002 In quest of thyroid hormone function in mature mammalian brain. Indian J Exp Biol 40:865–873
3. **Alvarez-Dolado M, Iglesias T, Rodriguez-Pena A, Bernal J, Munoz A** 1994 Expression of neurotrophins and the trk family of neurotrophin receptors in normal and hypothyroid rat brain. Brain Res Mol Brain Res 27:249–257
4. **Ekins R** 1992 The free hormone hypothesis and measurement of free hormones. Clin Chem 38:1289–1293
5. **Dratman MB, Crutchfield FL, Schoenhoff MB** 1991 Transport of iodothyronines from bloodstream to brain: contributions by blood:brain and choroid plexus:cerebrospinal fluid barriers. Brain Res 554:229–236
6. **Cheng LY, Outterbridge LV, Covatta ND, Martens DA, Gordon JT, Dratman MB** 1994 Film autoradiography identifies unique features of [125I]3,3′5′-(reverse) triiodothyronine transport from blood to brain. J Neurophysiol 72: 380–391
7. **Pardridge WM** 1979 Carrier-mediated transport of thyroid hormones through the rat blood-brain barrier: primary role of albumin-bound hormone. Endocrinology 105:605–612
8. **Hagen GA, Solberg Jr LA** 1974 Brain and cerebrospinal fluid permeability to intravenous thyroid hormones. Endocrinology 95:1398–1410
9. **Banks WA, Kastin AJ, Michals EA** 1985 Transport of thyroxine across the blood-brain barrier is directed primarily from brain to blood in the mouse. Life Sci 37:2407–2414
10. **Li JY, Boado RJ, Pardridge WM** 2001 Blood-brain barrier genomics. J Cereb Blood Flow Metab 21:61–68
11. **Sugiyama D, Kusuhara H, Taniguchi H, Ishikawa S, Nozaki Y, Aburatani H, Sugiyama Y** 2003 Functional characterization of rat brain-specific organic anion transporter (Oatp14) at the blood-brain barrier: high affinity transporter for thyroxine. J Biol Chem 278:43489–43495
12. **Pizzagalli F, Hagenbuch B, Stieger B, Klenk U, Folkers G, Meier PJ** 2002 Identification of a novel human organic anion transporting polypeptide as a high affinity thyroxine transporter. Mol Endocrinol 16:2283–2296
13. **Abe T, Kakyo M, Sakagami H, Tokui T, Nishio T, Tanemoto M, Nomura H, Hebert SC, Matsuno S, Kondo H, Yawo H** 1998 Molecular characterization and tissue distribution of a new organic anion transporter subtype (oatp3) that transports thyroid hormones and taurocholate and comparison with oatp2. J Biol Chem 273:22395–22401
14. **Reichel C, Gao B, Van Montfoort J, Cattori V, Rahner C, Hagenbuch B, Stieger B, Kamisako T, Meier PJ** 1999 Localization and function of the organic anion-transporting polypeptide Oatp2 in rat liver. Gastroenterology 117:688–695
15. **Friesema EC, Docter R, Moerings EP, Verrey F, Krenning EP, Hennemann G, Visser TJ** 2001 Thyroid hormone transport by the heterodimeric human system L amino acid transporter. Endocrinology 142:4339–4348
16. **Matsuo H, Tsukada S, Nakata T, Chairoungdua A, Kim DK, Cha SH, Inatomi J, Yorifuji H, Fukuda J, Endou H, Kanai Y** 2000 Expression of a system L neutral amino acid transporter at the blood-brain barrier. Neuroreport 11: 3507–3511
17. **Friesema EC, Ganguly S, Abdalla A, Manning Fox JE, Halestrap AP, Visser TJ** 2003 Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter. J Biol Chem 278:40128–40135
18. **Hearn MT, Hancock WS, Bishop CA** 1978 High-pressure liquid chromatography of amino acids, peptides and proteins. V. Separation of thyroidal iodoamino acids by hydrophilic ion-paired reversed-phase high-performance liquid chromatography. J Chromatogr 157:337–344
19. **Dagenais C, Rousselle C, Pollack GM, Scherrmann JM** 2000 Development of an *in situ* mouse brain perfusion model and its application to mdr1a P-glycoprotein-deficient mice. J Cereb Blood Flow Metab 20:381–386
20. **Yamaoka K, Tanigawara Y, Nakagawa T, Uno T** 1981 A pharmacokinetic analysis program (multi) for microcomputer. J Pharmacobiodyn 4:879–885
21. **Ball HJ, McParland B, Driussi C, Hunt NH** 2002 Isolating vessels from the mouse brain for gene expression analysis using laser capture microdissection. Brain Res Brain Res Protoc 9:206–213

22. Dallaire L, Tremblay L, Beliveau R 1991 Purification and characterization of metabolically active capillaries of the blood-brain barrier. Biochem J 276(Pt 3):745–752
23. Sugiyama D, Kusuhara H, Shitara Y, Abe T, Sugiyama Y 2002 Effect of 17 β-estradiol-D-17 β-glucuronide on the rat organic anion transporting polypeptide 2-mediated transport differs depending on substrates. Drug Metab Dispos 30:220–223
24. Hulbert AJ 2000 Thyroid hormones and their effects: a new perspective. Biol Rev Camb Philos Soc 75:519–631
25. Palha JA, Episkopou V, Maeda S, Shimada K, Gottesman ME, Saraiva MJ 1994 Thyroid hormone metabolism in a transthyretin-null mouse strain. J Biol Chem 269:33135–33139
26. Abe T, Suzuki T, Unno M, Tokui T, Ito S 2002 Thyroid hormone transporters: recent advances. Trends Endocrinol Metab 13:215–220
27. Hennemann G, Docter R, Friesema EC, de Jong M, Krenning EP, Visser TJ 2001 Plasma membrane transport of thyroid hormones and its role in thyroid hormone metabolism and bioavailability. Endocr Rev 22:451–476
28. Zheng W, Deane R, Redzic Z, Preston JE, Segal MB 2003 Transport of L-[125I]thyroxine by *in situ* perfused ovine choroid plexus: inhibition by lead exposure. J Toxicol Environ Health A 66:435–451
29. Chantoux F, Blondeau JP, Francon J 1995 Characterization of the thyroid hormone transport system of cerebrocortical rat neurons in primary culture. J Neurochem 65:2549–2554
30. Fujiwara K, Adachi H, Nishio T, Unno M, Tokui T, Okabe M, Onogawa T, Suzuki T, Asano N, Tanemoto M, Seki M, Shiiba K, Suzuki M, Kondo Y, Nunoki K, Shimosegawa T, Iinuma K, Ito S, Matsuno S, Abe T 2001 Identification of thyroid hormone transporters in humans: different molecules are involved in a tissue-specific manner. Endocrinology 142:2005–2012
31. Dumitrescu AM, Liao XH, Best TB, Brockmann K, Refetoff S 2004 A novel syndrome combining thyroid and neurological abnormalities is associated with mutations in a monocarboxylate transporter gene. Am J Hum Genet 74:168–175
32. Guadano-Ferraz A, Obregon MJ, St. Germain DL, Bernal J 1997 The type 2 iodothyronine deiodinase is expressed primarily in glial cells in the neonatal rat brain. Proc Natl Acad Sci USA 94:10391–10396
33. Tu HM, Legradi G, Bartha T, Salvatore D, Lechan RM, Larsen PR 1999 Regional expression of the type 3 iodothyronine deiodinase messenger ribonucleic acid in the rat central nervous system and its regulation by thyroid hormone. Endocrinology 140:784–790

Endocrinology is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

Available online at www.sciencedirect.com

SCIENCE @ DIRECT

Advanced Drug Delivery Reviews 56 (2004) 1741–1763

Advanced DRUG DELIVERY Reviews

www.elsevier.com/locate/addr

Efflux transport systems for organic anions and cations at the blood–CSF barrier

Hiroyuki Kusuhara, Yuichi Sugiyama*

Graduate School of Pharmaceutical Sciences, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

Received 10 August 2003; accepted 4 July 2004
Available online 25 August 2004

Abstract

The choroid plexus (CP), located in the lateral, third and fourth ventricles, is the site of elimination of xenobiotics and endogenous waste from the cerebrospinal fluid (CSF) together with convective flow associated with CSF turnover. Active efflux transport systems, as well as metabolic enzymes in the choroid plexus epithelial cells (CPE), which form a tight monolayer, play a protective role by facilitating the elimination of xenobiotics including drugs and endogenous waste from the CSF to prevent their accumulation in the central nervous system. Except in the case of lipophilic cationic and neutral compounds, uptake and efflux transporters carry out the vectorial transport across the cell monolayer to transfer their common substrates efficiently from the CSF to the blood side. Many published studies have given us some insights into the uptake mechanisms for organic compounds at the brush border side of the CP. Organic anion transporters, such as Oatp3 and Oat3, play a major role in the uptake of amphipathic and hydrophilic organic anions, respectively, at the brush border surface of the CPE, while the organic cation transporters, Oct2 and/or Oct3, have been suggested to be involved in the uptake of hydrophilic organic cations. In contrast, the molecular characteristics of basolateral transporters have not been fully elucidated. Mrp1 is involved in the excretion of etoposide at the basolateral membrane of the CPE, but its contribution to the excretion of organic anions, especially amphipathic conjugated metabolites, remains controversial. The present manuscript summarizes the efflux transport mechanisms at the choroid plexus and focuses on the molecular characteristics of these transporters.
© 2004 Published by Elsevier B.V.

Keywords: Efflux transport; Organic anion; Organic cation; OCT; OAT; OATP; MRP

Abbreviations: ABC, ATP-binding cassette; BBB, blood–brain barrier; BCSFB, blood–cerebrospinal fluid barrier; BLM, basolateral membrane; BBM, brush border membrane; CP, choroid plexus; CPE, choroid plexus epithelial cells; OAT, organic anion transporter; OCT, organic cation transporter; OATP, organic anion-transporting polypeptide; PEPT, peptide transporter; MRP, multidrug resistance-associated protein; P-gp, P-glycoprotein; i.c.v., intracerebroventricular; E217βG, 17β-estradiol-D-17β glucuronide; FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; NMN, *N*-methylnicotineamide; PAH, *p*-aminohippurate; PGE2, prostaglandin E2; TEA, tetaraethylammonium.

* Corresponding author. Tel.: +81 3 5841 4774; fax: +81 3 5841 4766.
E-mail address: sugiyama@mol.f.u-tokyo.ac.jp (Y. Sugiyama).

0169-409X/$ - see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.addr.2004.07.007

Contents

1. Introduction 1742
2. Pharmacokinetic quantification of efflux transport from the CSF 1744
3. Molecular characteristics of drug transporters 1744
   3.1. Organic anion-transporting polypeptide (Oatp/OATP; SLCO) 1744
   3.2. Organic ion transporter (SLC22A) 1746
      3.2.1. Organic anion transporter (Oat/OAT; SLC22A) 1746
      3.2.2. Organic cation transporter (Oct/OCT; SLC22A) 1747
      3.2.3. OCTN (SLC22A) 1748
   3.3. Peptide transporter (PEPT; SLC15A) 1748
   3.4. ABC transporters 1748
      3.4.1. Multidrug resistance-associated protein (MRP; ABCC) 1748
      3.4.2. P-glycoprotein (ABCB1) 1749
4. Efflux transport mechanisms for organic anions in the choroid plexus 1749
   4.1. Amphipathic organic anions 1749
   4.2. Hydrophilic organic anions 1752
5. Efflux transport mechanism for organic cations in the choroid plexus 1754
   5.1. Cimetidine 1755
   5.2. Tetraethylammonium and choline 1755
6. Discussion and future aspects 1757
References 1757

1. Introduction

The choroid plexus (CP) is a leaf-like, highly vascularized organ that protrudes into the ventricles. It secretes the cerebrospinal fluid (CSF) which fills the ventricular system and the subarachnoideal space, and circulates around the brain and spinal cord before it is reabsorbed into the blood circulation primarily by the arachnoid villi [1,2]. The CSF maintains the working environment of the brain by providing buoyancy to protect the brain and by acting as a buffer reservoir or as a source of necessary osmolytes. The CP consists of fenestrated capillaries surrounded by a tight monolayer of epithelial cells. The choroid plexus epithelial cells (CPE) are polarized to form brush border (BBM) and basolateral (BLM) membranes facing towards the CSF and plasma, respectively. Due to fenestrated capillaries in the CP, compounds in the blood have free access to the BLM of the CPE; however, tightly sealed cell junctions between the epithelial cells prevent free exchange of compounds between the blood and CSF, and provide a barrier function between the CSF and the blood circulation (blood–CSF barrier). In addition, the CPE has detoxification systems, including metabolic enzymes and efflux transport systems, to facilitate the elimination of xenobiotics and endogenous wastes from the CSF to the circulating blood. This, together with the blood–brain brain barrier formed by brain capillary endothelial cells, prevents their accumulation in the central nervous system [3–8]. Drugs acting in the central nervous system have to overcome these

Notes to Table 1:
Tissue distribution: k, kidney; li, liver; lu, lung; b, brain; si, small intestine; t, testis; r, retina; pl, placenta; m, muscle; bl, bladder; e, eye; cp, choroid plexus; h, heart; sp, spleen; tg, thyroid gland; ag, adrenal gland; bc, brain capillary; Membrane localization: AM, abluminal membrane; LM, luminal membrane; SM, sinusoidal membrane; CM, canalicular membrane; BLM, basolateral membrane; BBM, brush border membrane; OA, organic anion.

[a] Controversial.

[b] Localization of the chimeric protein (rOct2-GFP).

Table 1
Drug transporters potentially involved in the efflux transport across the CP

Name	Species	Locus ID	Gene symbol	Tissue distribution	Membrane localization	Transport mechanism
Facilitate, secondary/tertiary active transporter organic anion-transporting polypeptide (Oatp/OATP) family						
Oatp2	rat	170698	*Slco1a4*/Oatp1a4	li, b, r, cp	SM(li), LM/AM(bc), BLM(cp)	ND
Oatp3	rat	80900	*Slco1a5*/Oatp1a5	k, r, b, lu, si, cp[a]	BBM	ND
OATP A	human	6579	*SLCO1A2*/OATP1A2	b, low: k, li, lu, t	LM/AM(bc)	ND
Oatp9/moat1	rat	140860	*Slco2b1*/Oatp2b1	ubiquitously		ND
OATP B	human	11309	*SLCO2B1*/OATP2B1	ubiquitously	SM(li), BBM(si)	ND
Oatp12	rat	171144	*Slco4a1*/Oatp4a1	ubiquitously	ND	ND
OATP E	human	28231	*SLO4A1*/OATP4A1	ubiquitously	ND	ND
Oatp14/BSAT1	rat	84511	*Slco1c1*/Oatp1c1	b, cp	LM/AM(bc)	ND
OATP-F	human	53919	*SLCO1C1*/OATP1C1	b, t	ND	ND
Organic anion transporter (Oat/OAT) family						
Oat1	rat	29509	*Slc22a6*	k, cp	BLM(k)	OA/dicarboxylate antiport
OAT1	human	9356	*SLC22A6*	k	BLM	ND
Oat2/NLT	rat	89776	*Slc22a7*	li, k (female), cp	SM(li), BBM(k)	ND
OAT2	human	10864	*SLC22A7*	li, k	BLM(k)	ND
Oat3/Roct	mouse	19879	*Slc22a8*	k	ND	ND
Oat3	rat	83500	*Slc22a8*	li(male), k, b, e, cp	BLM(k), AM(bc), BBM(cp)	OA/dicarboxylate antiport
OAT3	human	9376	*SLC22A8*	k	BLM	OA/dicarboxylate antiport
Organic cation transporter (Oct/OCT) family						
Oct2	rat	19503	*Slc22a2*	k, b, cp	BLM(k), BBM(cp)[b]	facilitative
OCT2	human	6582	*SLC22A2*	k (distal tubul)	BLM	facilitative
Oct3	rat	29504	*Slc22a3*	ubiquitously	ND	facilitative
OCT3	human	6581	*SLC22A3*	ubiquitously	ND	facilitative
Octn/OCTN family						
Octn1	rat	19503	*Slc22a2*	li, si, b, k, h, pl	ND	
OCTN1	human	6582	*SLC22A2*	ubiquitously	ND	H^+ antiport
Octn2/CT1	rat	29504	*Slc22a3*	ubiquitously	ND	
OCTN2	human	6581	*SLC22A3*	ubiquitously	ND	Na^+ symport
Peptide transporter						
PEPT2	rat	60577	*Slc15a2*	k, b, lu, sp	BBM	H^+ symport
PEPT2	human	6565	*SLC15A2*	k	ND	H^+ symport
ABC transporter P-glycoprotein						
Mdr1a	mouse	18671	*Abcb1a*	si, h, b, li, k, lu, t	CM(li), BBM(si, k), LM(bc)	primary active
Mdr1b	mouse	18669	*Abcb1b*	pl (during pregnancy), ag, k, h	CM(li), BBM(k)	primary active
MDR1	human	5243	*ABCB1*	b, li, k, si	CM(li), BBM(si), LM(bc)	primary active
Multidrug resistance-associated protein (Mrp/MRP) family						
Mrp1	mouse	17250	*Abcc1*			primary active
Mrp1	rat	24565	*Abcc1*	b, cp	BLM(cp)	primary active
MRP1	human	4363	*ABCC1*	lu, sp, tg, t, bl, ag	ND	primary active
Mrp4	mouse	239273	*Abcc4*	ND	ND	primary active
MRP4	human	10257	*ABCC4*	ubiquitously	BBM(k)	primary active
Mrp5	mouse	27416	*Abcc5*	ND	ND	primary active
MRP5	human	10057	*ABCC5*	ubiquitously	ND	primary active

barriers to achieve clinically significant concentrations in the central nervous system.

The efflux transport of organic compounds across the cell monolayer is characterized by vectorial transport, which plays a major role in the hepatobiliary transport and urinary secretion of organic anions and hydrophilic organic cations. Recently, a number of transporters have been cloned and their functional characterization has been carried out [6,9–15]. This has allowed the elucidation of the molecular characteristics of the efflux transport systems expressed at the CP as summarized in Table 1. The primary purpose of the present manuscript is to illustrate the efflux transport systems for organic anions and cations in the CP.

2. Pharmacokinetic quantification of efflux transport from the CSF

A sequential determination of the CSF concentration after intracerebroventricular (i.c.v.) administration (C_{CSF}) allows us to determine the elimination rate constant (k_e) as described by the following differential equation,

$$\frac{dC_{CSF}}{dt} = -k_e C_{CSF} = -CL_{CSF}/V_{d,CSF} C_{CSF} \quad (1)$$

where CL_{CSF} represents the elimination clearance from the CSF. The time profile of the drug concentration in the CSF is affected not only by the elimination clearance, but also by the distribution volume in the ventricles ($V_{d,CSF}$). The CL_{CSF} is experimentally obtained from the elimination rate constant (k_e) and distribution volume ($V_{d,CSF}$). $V_{d,CSF}$ can be calculated using the amount of drug injected into the ventricles and the initial CSF concentration extrapolated to time 0 assuming rapid equilibrium of distribution in the ventricles.

The CL_{CSF} represents the sum of the elimination clearance of three different elimination routes from the CSF, i.e. (1) bulk flow rate, (2) active efflux through the CP and (3) diffusion into the brain parenchyma through the ependyma surface followed by efflux across the brain capillaries and/or metabolism. Suzuki et al. [17] introduced a spatially distributed model, which was initially developed by Collins and Dedrick [16], to handle each elimination process quantitatively. Based on this model, the CL_{CSF} can be expressed by the following equation,

$$CL_{CSF} = Q_{CSF} + \sqrt{A_r^2 D_t PS_{eff} V_{br}} + PS_{eff,cp} \quad (2)$$

where Q_{CSF} is the bulk flow rate, A_r is the ependymal surface area, D_t is the apparent diffusion constant for the ligand in the brain extracellular fluid, V_{br} is the volume of distribution in the brain, defined as the brain concentration divided by the concentration in the brain extracellular fluid, and PS_{eff} and $PS_{eff,CP}$ are the PS products for the efflux of ligand across the brain capillaries and CP, respectively. The second and third terms represent the elimination via the brain parenchyma and the CP, respectively. The efflux clearance across the brain capillaries (PS_{eff}) can be evaluated in separate experiments, such as in vivo microdialysis and the brain efflux index method [18,19]. The details of these experimental methods are given in Ref. [19]. Ogawa et al. [20] applied this model to analyze the elimination of a β-lactam antibiotic, benzylpenicillin, from the CSF. According to this analysis, the efflux transport through the CP accounted for the major part of the total elimination clearance (64%), while the remainder is accounted for by the CSF convective flow (12%) and elimination via the brain parenchyma followed by the efflux across the brain capillaries (24%).

3. Molecular characteristics of drug transporters

In this section, the molecular characteristics of the uptake transporters, such as Oatp/OATP, Oat/OAT, Oct/OCT and PEPT are described, as well as the ABC transporters, such as Mrp/MRP and P-glycoprotein. The prefixes m, r and h represent different species, i.e. mice, rats and humans, in the following text.

3.1. Organic anion-transporting polypeptide (Oatp/OATP; SLCO)

The organic anion-transporting polypeptides (referred to as Oatp in rodents and OATP in humans) belong to the growing gene family of organic anion/prostaglandin transporters that mediate sodium-independent transport of numerous endogenous and xenobiotic amphipathic compounds. They are classified

within the gene superfamily of solute carriers (SLC) as the *SLCO* gene family (Human Gene Nomenclature Committee DataBase). Fourteen members of the *Oatp/OATP* family have been identified in rodents and humans [12]. Review articles of the Oatp/OATP family are available in Refs. [12,21,22].

In the CP, rOatp1 (*Slco1a1*, Oatp1a1) was initially identified along the brush border membrane (BBM) of the CPE [23]. However, recent publications have revealed that rOatp3 (*Slco1a5*, Oatp1a5), and not rOatp1, is the most abundant isoform in the CP [24–26] where it is localized to the BBM [25]. Due to the high degree of homology between rOatp1 and rOatp3, this discrepancy is presumably due to the cross-reaction of probes. Among rat isoforms, rOatp2 (*Slco1a4*, Oatp1a4) has been identified at the BLM of the CPE [27]. In addition, the expression of rOatp9 (*Slco2b1*, Oatp2b1), rOatp12 (*Slco4a1*, Oatp4a1) and rOatp14 (*Slco1c1*, Oatp1c1) has been reported, but the expression of Oatp4 (*Slco1b2*, Oatp1b2), Oatp5 (*Slco1a6*, Oatp1a6) and Oat-K1/Oat-K2 (*Slco1a3*, Oatp1a3) was below the detection limit [26,28]. According to the quantification by Choudhuri [26], the mRNA expression of rOatp9, rOatp12 and rOatp2 in the rat CP is much lower than that of rOatp3.

rOatp3 was isolated from the rat retina cDNA library using homology cloning [29]. The cDNA encodes a 670-amino-acid protein of approximately 80 kDa with 12 putative transmembrane domains. The tissue distribution of rOatp3 is still under discussion. Northern blot analysis using the 3′ non-coding region as a probe revealed its expression in the kidney [29]. However, subsequent analyses using the RNase protection assay revealed its expression in the brain, small intestine, lung and retina [30], but not in the kidney and liver. Li et al. [31] quantified the Oatp3 mRNA expression using the branched DNA signal amplification method. An abundant Oatp3 expression was found in the lung, cerebellum and female cerebral cortex and, to a lesser extent, in the intestine. Functional expression studies of rOatp3 revealed its broad substrate specificity including amphipathic organic anions, such as bile acids, E217βG, estrone sulfate, dehydroepiandrosterone sulfate, and thyroid hormones [24,25,29,32]. In *Xenopus laevis* oocytes, the K_m values of estrone sulfate, dehydroepiandrosterone sulfate and estrone sulfate for rOatp3 were greater than those for rOatp1 and rOatp2 [32], whereas they were similar to those for rOatp1 when they are expressed in LLC-PK1 cells [25].

rOatp2 was isolated from the rat brain and retina cDNA library using homology cloning [29,33]. The cDNA encodes a 661-amino-acid protein with an apparent molecular mass of 92 kDa [34]. Its substrate specificity is similar to rOatp1 and rOatp3 [32], but cardiac glycosides, such as ouabain and digoxin, show a higher affinity for rOatp2 [32,33]. In addition, rOatp2 accepts bulky organic cations, such as *N*-(4,4-azo-n-pentyl)-21-deoxyajmalinium, *N*-methyl-quinidine, *N*-methyl-quinine and rocuronium, as well as anionic peptides, such as BQ-123, [D-Pen2,D-Pen5]enkephalin and deltorphin II [35,36]. Bi-directional transport by rOatp2 has been shown by Li et al. [37]. They demonstrated that taurocholate uptake by rOatp2-expressed oocytes was increased in the presence of an outward concentration gradient of taurocholate and glutathione conjugates. Thus, rOatp2 at the BLM of the CP may be involved in the excretion of organic anions, as well as uptake from the circulating blood.

OATP-A (*SLCO1A2*, OATP1A2) is the human isoform which exhibits the highest homology with rOatp2 and rOatp3 [12,38]. Its cDNA is abundantly expressed in the brain and, to a lesser extent, in the lung, liver, kidney and testis [39,40]. It is ubiquitously distributed in the brain, although its expression in the CP remains unknown [41]. Functional expression studies of OATP-A show its broad substrate specificity, including ouabain (cardiac glycoside), type II organic cations, such as *N*-(4,4-azo-n-pentyl)-21-ajmalinium, *N*-methylquinine and *N*-methylquinidine, as well as amphipathic organic anions [35,38,40].

rOatp9 is a rodent ortholog of hOATP-B (*SLCO2B1*, OATP2B1). rOatp9 and hOATP-B are ubiquitously expressed in the body [42–44]. Northern blot and in situ hybridization analyses of rat brain further indicated that Oatp9 mRNA is widely distributed in the neuronal cells of the central nervous system, especially in the hippocampus and cerebellum, but the expression in the CP was below the detection limit in this analysis [42]. hOATP-B has been shown to be localized to the sinusoidal membrane in the liver and the BBM of intestinal epithelial cells [44,45]. The substrates of rOatp9 include

prostaglandins (PGE_2, PGD_2 and PGE_1), leukotriene C_4 and thromboxan B_2, while the substrates of hOATP-B include bromosulfophtalein, estrone sulfate, dehydroepiandrosterone sulfate and pravastatin [43–45].

rOatp12 is a rodent ortholog of hOATP-E (*SLCO4A1*, OATP4A1). The tissue distribution of hOATP-E is ubiquitous [46]. Triiodothyronine (T3) is the only substrate of rOatp12 which has been reported [46], while the substrates of hOATP-E include T3, thyroxine (T4), reverse T3 and E217βG [43,46].

rOatp14 was originally referred to as BBB-specific anion transporter 1 (BSAT1), which was isolated using gene microarray techniques by comparing the gene-expression profile of cDNA from the brain capillaries with that from the liver and kidney [47]. rOatp14 cDNA consists of 2148 base pairs that encode a 716-amino-acid residue protein with 12 putative membrane-spanning domains. The substrates of rOatp14 include organic anions, such as E217βG, cerivastatin and troglitazone sulfate, as well as T4 and reverse T3 [28]. T4 shows the highest transport activity and affinity for rOatp14 among the known substrates [28]. Western blot analysis detected a band in the CP in addition to the brain capillary enriched fraction [28]. OATP-F (*SLCO1C1*, OATP1C1), the human ortholog of rOatp14, accepts T4 and reverse T3 specifically as substrates, and the transport activity of organic anions by OATP-F is low [48].

3.2. Organic ion transporter (SLC22A)

The organic ion transporter family is a superfamily which consists of Oat/OAT, Oct/OCT, Octn/OCTN, CT2 and URAT1. CT2 and URAT1 are transporters for carnitine and urate, respectively [49,50]. This section focuses on the molecular characteristics of Oat/OAT, Oct/OCT and Octn/OCTN.

3.2.1. Organic anion transporter (Oat/OAT; SLC22A)

Four *OAT* genes (OAT1–OAT4 in human, and Oat1–Oat3 and Oat5 in rodents) have been identified. Review articles of the Oat/OAT family are available in Refs. [9,11,13,14,51–53]. RT-PCR analysis revealed the expression of three rat Oat mRNAs in the CP [54] and quantification by Choudhuri et al. [26] revealed that rOat3 (*Slc22a8*) is the most abundant isoform expressed in the CP followed by rOat1 (*Slc22a6*) and rOat2 (*Slc22a7*). Western blot analysis revealed the protein expression of rOat3 in the CP, but no rOat1 protein was detected in the CP [55]. In human CP, immunohistochemical staining showed both hOAT1 (*SLC22A6*) and hOAT3 (*SLC22A8*) are expressed although their membrane localization remains to be elucidated [56].

The partial sequence of rOat3 was cloned from rat brain cDNA using degenerative primers designed for the conserved region among rOat1, rOat2 and rOct1. Its full-length cDNA was cloned from rat kidney cDNA library using the partial sequence as a probe [57]. The cDNA encodes 551 amino acids with 12 putative transmembrane domains. Northern blot analysis revealed its abundant expression in the liver, kidney, and, to lesser extent, in the brain and eye [57], whereas hOAT3 is predominantly expressed in the kidney [58]. Immunofluorescence studies show that the membrane localization of rOat3 is the BBM of the CP and the BLM of the renal proximal tubules [55,59]. Functional expression in *X. laevis* oocytes and mammalian cells has revealed that rOat3 has a broad substrate specificity including amphipathic organic anions, such as E217βG, estrone sulfate and dehydroepiandrosterone sulfate; hydrophilic organic anions, such as PAH and benzylpenicillin; and the organic cation, cimetidine [55,57,59–63].

Estrone sulfate uptake and efflux via rOAT3 are not *trans*-stimulated by ochratoxin A, PAH or estrone sulfate in rOat3-cRNA injected *Xenopus* oocytes[57]. In contrast, Sweet et al. [64] demonstrated that estrone sulfate and PAH uptake by rOat3-expressing oocytes was stimulated by an outward concentration gradient of glutarate formed by co-expression of the sodium-dicarboxylate cotransporter (NaDC-1) in *X. laevis* oocytes. The efflux of glutarate from inside the cells was greater in hOAT3-expressing oocytes than in control oocytes and was stimulated by extracellular OAT3 substrates, such as α-ketoglutarate, glutarate, PAH, cimetidine and urate. Estrone sulfate inhibited the efflux [65]. Thus, it is likely that rOat3/hOAT3 is an exchanger and an outward concentration gradient of dicarboxylates, such as α-ketoglutarate, formed by the sodium-dependent dicarboxylate co-transporter and the TCA cycle, may drive rOat3-mediated transport.

The Oat3 knockout mouse was established by Sweet et al. [54] and this gives more direct insight

into its role in the kidney and CP. This mouse strain is healthy and exhibits no significant physiological abnormalities compared with the corresponding wild-type mouse. Most of the uptake of amphipathic organic anions, such as taurocholate and estrone sulfate, is markedly reduced in kidney slices from the mOat3 knockout mouse compared with that from the wild-type mouse [54]. In addition, the accumulation of fluorescein is markedly reduced in the isolated CP from the mOat3 knockout mouse [54].

rOat1 has been isolated as a "classical" organic anion transporter at the BLM of the renal proximal tubules by expression cloning using *X. laevis* oocytes and it accepts PAH as a typical substrate [66,67]. rOat1 is predominantly expressed in the kidney and shows broad substrate specificity to organic anions, such as PAH, β-lactam antibiotics, nucleoside analogs and nonsteroidal anti-inflammatory drugs [11,13,51,66,67]. rOat1-mediated transport has been characterized by *trans*-stimulation. An outward concentration gradient of substrates, including dicarboxylates preloaded by preincubation or via sodium-dicarboxylate cotransporter, stimulates rOat1-mediated transport, suggesting that rOat1 functions as an exchanger [66,67]. rOat2 was found by a database-search and registered as a novel-liver specific transporter (NLT) [68,69]. Functional expression in *X. laevis* oocytes revealed that NLT transports α-ketoglutarate, PGE_2, PAH, methotrexate and acetylsalicylate [69]. When expressed in LLC-PK1 cells as a host, it transports indomethacin, PGE2, and nucleoside analogs, such as azidodeoxythymidine and dideoxycytidine, although specific uptake of methotrexate and PAH was not detected [70].

3.2.2. Organic cation transporter (Oct/OCT: SLC22A)

Organic cation transporters (Oct/OCT) are multispecific facilitative transporters of hydrophilic and small organic cations. Review articles of the Oct/OCT family are available in Refs. [10,11,13,21]. Three isoforms (Oct1/OCT1–Oct3/OCT3) have been isolated from rodents and in human. The Oct isoform expressed in the CP is controversial. RT-PCR analysis using cDNA from rat CP as a template revealed expression of rOct2 (*Slc22a2*) and rOct3 (*Slc22a3*) mRNA, but not rOct1 (*Slc22a1*) mRNA, in the CP [71]. However, mRNA quantification by Choudhuri et al. [26] revealed the low-level expression of rOct1 and rOct3 in the rat CP, and the expression level of Oct2 mRNA was below the detection limit. rOct1 was initially cloned from the rabbit by expression cloning using *X. laevis* oocytes [72]. Subsequently other isofoms, rOct2 and rOct3, have been cloned from kidney and placenta cDNA libraries using homology screening [73,74]. rOct1 is expressed abundantly in the kidney, liver and, to a lesser extent, in the intestine [72], while hOCT1 is abundantly expressed in the liver [75,76]. rOct2/hOCT2 is expressed predominantly in the kidney [73,75]. rOct3 has a ubiquitous expression profile and is relatively abundant in the placenta [74].

The Oct1-mediated uptake is characterized by its membrane voltage dependence [72]. For example, an intracellular negative membrane voltage facilitates rOct1-mediated transport of hydrophilic organic cations and substitution of Na^+ for K^+ reduces the transport activity, due to depolarization of the membrane voltage [72]. Oct/OCT-mediated uptake is reduced under conditions that cause depolarization of the membrane voltage, such as substitution of K^+ for Na^+, Ba^{2+} and ouabain treatment, as well as an extracellular acidic pH [10,11,13,21]. It has been shown that members of the Oct/OCT family have a similar substrate specificity, i.e. small and hydrophilic organic cations, such as tetaraethylammonium (TEA) and *N*-methylnicotineamide (NMN), although rOct3 exhibits a relatively lower affinity for TEA compared with rOct1 and rOct2 [10,11,13,21,74,77,78]. The membrane localization of rOct1, rOct2 and rOct3 in the CP remains unknown. Sweet et al. [71] produced a chimeric protein of rOct2-GFP and transfected it to rat isolated CP. The fluorescence associated with the chimeric protein was localized at the BBM of the CP, suggesting the apical localization of endogenous rOct2 in the CP, if it is expressed in the CP.

Oct1, Oct2 and Oct3, as well as Oct1/Oct2 double knockout mice have been developed [79–81]. These mouse strains are healthy and exhibit no significant physiological abnormalities. No functional characterization of the organic cation transport in the CP has been carried out using these animals. These mice will allow us to investigate the role of Oct isoforms in the transport of organic cation in the CP.

3.2.3. OCTN (SLC22A)

Three isoforms (Octn1–Octn3) have been isolated in rodents, while two isoforms (OCTN1 and OCTN2) have been isolated in humans. Review articles of the Octn/OCTN family are available in Refs. [14,51,53]. Choudhuri et al. [26] demonstrated abundant expression of rOctn2 (*Slc22a5*) in the CP and, to a lesser extent, rOctn1 (*Slc22a4*), while the expression of rOctn3 remains to be quantified. Both hOCTN1 (*SLC22A4*) and hOCTN2 (*SLC22A5*) show ubiquitous tissue distribution [82,83] Octn2/OCTN2 has been characterized as a sodium-dependent carnitine transporter [83,84], although it also transports TEA [85]. Octn2 is hereditarily deficient in the juvenile visceral steatosis (*jvs*) mouse [86], a model animal of primary systemic carnitine deficiency (OMIM 212140). A single nucleotide mutation (TG) causes substitution of Leu by Arg at codon 352 leading to a loss of transport function [86]. Lack of Octn2 causes marked reduction in the renal reabsorption of carnitine and the tissue distribution in the heart, liver and brain capillaries [87–89]. Furthermore, pharmacokinetic studies in jvs mouse suggested an involvement of Octn2 in the tubular secretion of TEA [90]. The renal clearance of TEA was significantly decreased in the jvs mouse compared with its corresponding wild-type mice, while that of an organic anion, cefazolin [90]. Mutations, causing functional loss, have been found in patients suffering from primary systemic carnitine deficiency [86].

OCTN1 has been considered to be a proton/organic cation exchanger since an acidic pH stimulates the efflux of TEA in OCTN1-expressing HEK293 cells [82]. The substrates of OCTN1 include organic cations, such as pyrilamine, quinidine and verapamil, as well as carnitine. In contrast to the transport of cationic compounds by hOCTN1, hOCTN1-mediated carnitine uptake shows sodium dependency [82]. The transport activity of carnitine by mOctn1 is much lower than that by mOctn2 [85].

3.3. Peptide transporter (PEPT; SLC15A)

Peptide transporters are classified within the gene superfamily of solute carriers (SLC) as the *SLC15A* gene family. In rat CP, PEPT2 (*Slc15a2*), but not PEPT1 (*Slc15a1*), has been identified at the BBM of CPE [91]. The details of this transporter are summarized in another review of this issue. Briefly, PEPT2 accepts di- and tripeptides, as well as peptide-mimetic drugs, such as β-lactam antibiotics containing an α-amino group, and angiotensin-converting enzyme inhibitors are also substrates of PEPT2 [92–96]. The functional role of PEPT2 in the CP has been shown using the Pept2 knockout mouse [97]. The cellular accumulation of glycylsarcosine and cefadroxil, typical ligands of PEPT2, by the isolated CP is considerably reduced in Pept2 knockout mice [97,98].

3.4. ABC transporters

ABC transporters are characterized by the cytoplasmically located ATP-binding cassette acting as a catalytic domain for ATP hydrolysis and by the unidirectional efflux to the outside of the cells. This section focuses on the molecular characterization of multidrug resistance-associated proteins (MRPs) and P-glycoprotein (P-gp).

3.4.1. Multidrug resistance-associated protein (MRP; ABCC)

MRPs have been classified within the gene superfamily of ABCC and eight different ABCC proteins (MRP1–8) have been cloned [99–102]. Review articles of the Mrp/MRP family are available in Refs. [103–106].

As far as the Mrp/MRP members are concerned, RT-PCR and Western blot analyses have revealed the expression of rMrp1 (*Abcc1*) in the CP where it is localized on the basolateral membrane of the CPE [24,107,108]. MRP1 has been found to be present as a protein overexpressed in the doxorubicin-selected lung cancer cell line H69AR, which confers multidrug resistance to tumor cells [109]. The mRNA of human MRP1 (*ABCC1*) encodes 1531 amino acids with an apparent molecular weight of 190 kDa and 17 transmembrane domains with two cytoplasmically located ATP-binding cassettes [109]. MPR1 has a broad substrate specificity including amphipathic glucuronide and glutathione conjugates [110,111]. In the presence of glutathione, hMRP1 can accept vincristine and estrone sulfate as substrates [106,112].

Taking the unidirectional transport as being directed from inside the cells to the outside, Mrp1 is a candidate transporter for basolateral excretion of its substrates, including amphipathic organic anions in

the CP. In accordance with this hypothesis, the CSF concentration of etoposide is significantly increased in triple knockout mice (Mrp1/Mdr1a/Mdr1b knockout mouse) compared with that in double knockout mice (Mdr1a/Mdr1b knockout mouse), although there is no difference in the plasma and brain concentrations of etoposide between double and triple knockout mice [113]. However, Lee et al. [114] reported only a minimal difference in the CSF concentration of E217βG and dinitrophenyl-*S*-glutathione after i.c.v. administration between wild-type and mMrp1 knockout mice, suggesting that Mrp1 may not play a major role in the efflux of organic anions across the BLM of the CP.

Quantification of Mrp mRNAs (mMrp1–mMrp6) has revealed abundant expression of mMrp4 (*Abcc4*) and mMrp5 (*Abcc5*) in the mouse CP [26,114]. hMRP4 (*ABCC4*) and hMRP5 (*ABCC5*) mRNAs encode 1325 and 1437 amino acid proteins, respectively, with 12 putative transmembrane domains. The apparent molecular weight of hMRP4/rMrp4 is approximately 170 kDa in the BBM from the kidney [115]. The substrates of hMRP4 include nucleoside analogs and cyclic nucleotides, as well as amphipathic organic anions, such as E217βG, dehydroepiandrosterone sulfate, methotrexate and prostaglandins [115–120], whereas cAMP, cGMP, 6-mercaptopurine and adefovir are known substrates of hMRP5 [121,122]. Further studies are necessary to reveal their functional role in the CP.

3.4.2. P-glycoprotein (ABCB1)

P-glycoprotein (P-gp: *ABCB1*) was originally found as an overexpressed protein at the plasma membrane of multidrug resistant tumor cells. The mRNA of human MDR1 encodes 1280 amino acids with an apparent molecular weight of 140–170 kDa and 12 transmembrane domains with two cytoplasmically located ATP-binding cassettes. In rodents, two isoforms, namely, Mdr1a (*Abcb4*) and Mdr1b (*Abcb1*), correspond to the human ortholog (MDR1). Mdr1a plays a central role in efflux transport at the brain capillary and small intestine [123,124]. P-gp has a broad substrate specificity including hydrophobic neutral or cationic compounds, fexofenadine and E217βG [125–128]. The importance of P-gp is well recognized in the brain capillaries [6,7,103,124,129–131]. Rao et al. [108] demonstrated its expression in the CP by Western blotting. However, the signals detected by anti-P-gp antibody (C219) shows punctate or granular staining patterns at the subapical region of the primary cultured CPE, suggesting that it is confined to the vesicular compartment [108]. Whether P-gp plays a role as one of the detoxification mechanisms in the CP remains to be elucidated.

4. Efflux transport mechanisms for organic anions in the choroid plexus

The uptake mechanisms for organic anions at the brush border surface of the CP can be subdivided into two groups in terms of substrate specificity: One for amphipathic organic anions, such as taurocholate, E217βG and estrone sulfate, and the other for hydrophilic and small organic anions, such as PAH and benzylpenicillin (Fig. 1). These two systems have similar characteristics to the hepatobiliary and urinary transport systems for organic anions, respectively, and are primarily accounted for by the Oatp/OATP and Oat/OAT families.

4.1. Amphipathic organic anions

E217βG shows rapid elimination from the CSF compared with the CSF turnover rate (represented by the elimination of inulin). The CSF elimination clearance of E217βG is 12-fold greater than that of inulin (76 and 5.9 μl/min/rat, respectively) (Fig. 2). Similarly, estrone sulfate is rapidly eliminated from the CSF after i.c.v. administration [132]. According to the initial characterization by Nishino et al. [107], a saturable component accounts for part of the total uptake of E217βG and taurocholate by the isolated CP (~40% of the total uptake). The uptake is inhibited by probenecid and FCCP treatment causing a reduction in the intracellular ATP level, which reduces the accumulation of E217βG by the isolated rat CP, suggesting that the uptake process requires energy [107]. A second report by Kusuhara et al. [25] showed that a saturable component accounts for a major part of the total uptake of E217βG by the isolated rat CP with K_m values (55 μM) 16-fold greater than those previously reported (3.4 μM). The transport activity of TCA by the isolated rat CP was similar to that of E217βG (Fig. 3) and is saturable with a K_m value

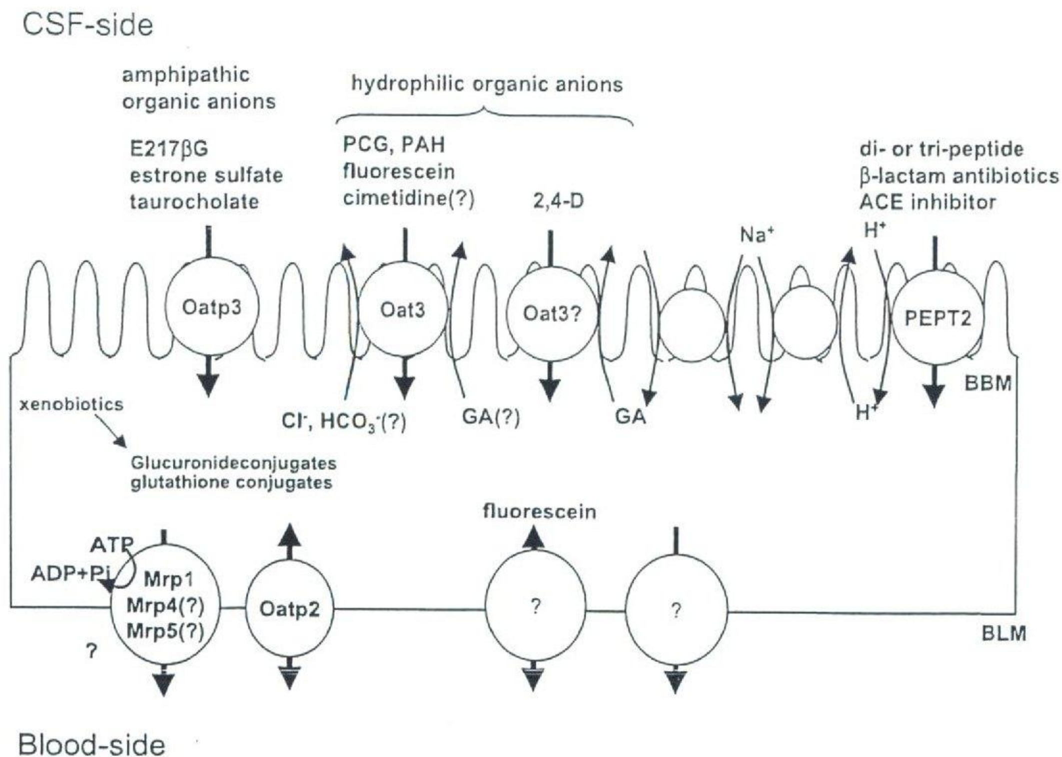

Fig. 1. Schematic diagram of the efflux transport systems for organic anions in the CP. The uptake of amphipathic organic anions, such as E217βG, taurocholate and estrone sulfate, at the BBM is mediated by organic anion-transporting polypeptide 3 (Oatp3). That of hydrophilic organic anions, such as benzylpenicillin (PCG), fluorescein and PAH, is mediated by organic anion transporter 3 (Oat3). The uptake mechanism for 2,4-dichlorophenoxyacetate (2,4-D) is unknown, but is mediated by the exchange of intracellular dicarboxylate (GA; glutarate). Peptide transporter 2 (PEPT2) may be involved in the uptake of β-lactam antibiotics and angiotensin-converting enzyme (ACE) inhibitors with an α-amino group, as well as di- and tripeptides. The basolateral excretion mechanisms for organic anions have not been fully elucidated. Mrp1 has been a candidate transporter, but its contribution to the efflux of amphipathic-conjugated metabolites remains controversial. Oatp2 and other ABC transporters, such as Mrp4 and Mrp5, are alternative candidate transporters. In addition, a membrane voltage-dependent efflux transport system has been suggested to be located on the basolateral membrane.

(116 μM), comparable with its K_i value for E217βG uptake by the isolated CP (124 μM) [25]. This suggests that they share the same uptake mechanism at the BBM. The K_m value of E217βG determined in the isolated CP is very close to that determined in oocytes expressing rOatp3 [32], but greater than that determined in rOatp3-expressed LLC-PK1 cells [25]. The spectrum of inhibitors, e.g. corticosterone, indomethacin, probenecid, diclofenac, estrone sulfate and quinine, for the uptake of E217βG by LLC-PK1-expressing rOatp3 and isolated CP is similar, although the absolute values of their K_is are not identical [25]. Since the effect of benzylpenicillin is minimal even at a concentration sufficient to saturate its own uptake by the isolated CP, the uptake of these two organic anions is mediated by different mechanisms at the BBM of the CP [25,107]. Although E217βG is a substrate of rOat3 [61], the minimal inhibition by benzylpenicillin indicates the minor contribution of rOat3 to the uptake of E217βG by the isolated rat CP. This is reasonable taking into consideration the transport activities of benzylpenicillin and E217βG in the CP and LLC-PK1 cells expressing rOat3. Their transport activities are similar in LLC-PK1 cells expressing rOat3 [55,61], whereas the uptake of E217βG by the isolated CP is fivefold greater than that of benzylpenicillin (Fig. 3).

Kitazawa et al. [132] have shown significant accumulation of estrone sulfate by the isolated rat CP, which is saturable with a K_m value of 18 μM. As observed for the uptake of E217βG, reduction of the intracellular ATP level during treatment of the CP with 2,4-dinitrophenol (metabolic inhibitor) and rotenone (inhibitor of mitochondrial respiration) markedly reduces the uptake of estrone sulfate. Amphipathic

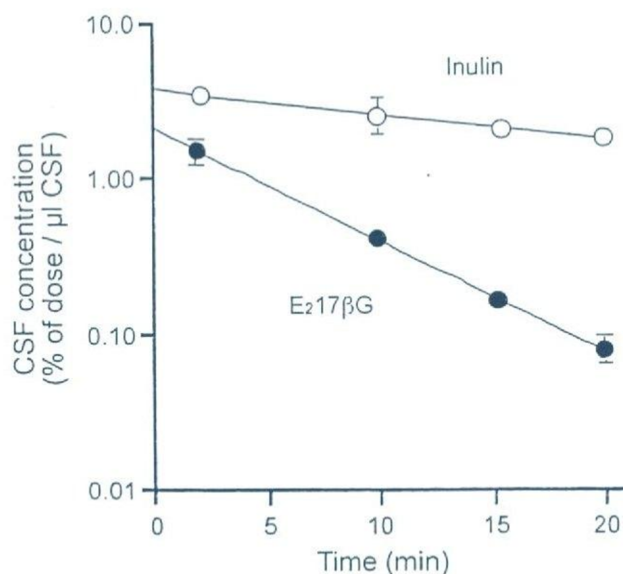

Fig. 2. The CSF concentration–time profiles of E217βG after intracerebroventricular administration in rats. Rats were given [^{3}H]E217βG (●; 0.38 µCi/rat) and [^{14}C]inulin (○; 0.02 µCi/rat) by i.c.v. administration and the cisternal CSF concentration of each compound was determined. Each point and vertical bar represents the mean ± S.E. of three independent experiments. The solid line is the regression line. Taken from Nishino et al. [107].

organic anions, such as dehydroepiandrosterone sulfate, taurocholate and cholate inhibit the uptake significantly, whereas the effect of PAH and digoxin is minimal. Taking into consideration the localization and substrate specificity, rOatp3 is a candidate for the uptake of amphipathic organic anions.

To carry out efficient elimination from the CSF, the transporter will be involved in excretion across the BLM of the CPE. Strazielle and Ghersi-Egea [4] demonstrated asymmetrical efflux transport of 1-naphthol-17β-glucuronide in primary cultured rat CPE. Incubation of 1-naphthol with cultured cells produces the glucuronide conjugate intracellularly and the cumulative amount of intracellularly formed 1-naphthol-17β-glucuronide excreted into the basal side was 2.6-fold greater than that into the apical side. As described previously in Section 3.4.1, Mrp1 is the candidate transporter for the basolateral excretion of amphipathic organic anions. However, Lee et al. [114] showed comparable CSF concentrations of E217βG and 2,4-dinitrophenyl-glutathione after i.c.v. administration to the Mrp1 knockout mouse

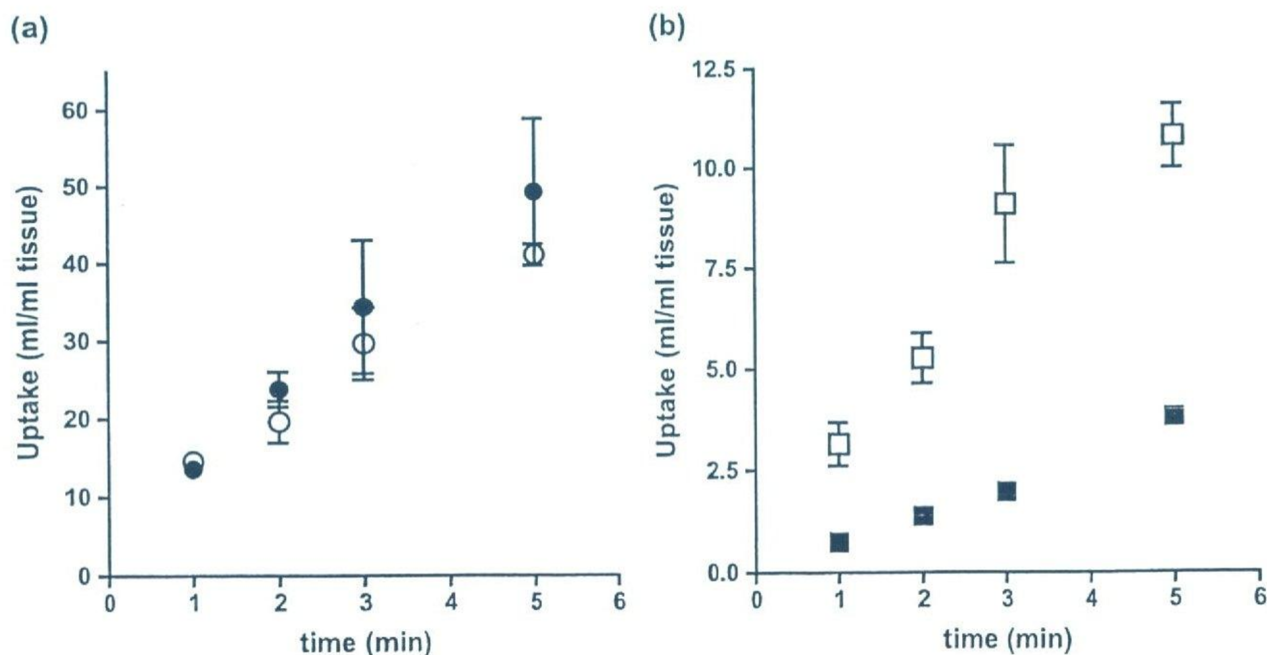

Fig. 3. Time profiles of the uptake of organic anions by the isolated rat CP. The CP was isolated from the lateral ventricles and the uptake of amphipathic and hydrophilic organic anions (panels a and b, respectively) by the isolated CP was determined by a centrifugal filtration method. The tissue-to-medium concentration ratio was calculated using [^{14}C]urea (panel a) or [^{3}H]water (panel b) as a cell water space marker and a correction was made for the adherent water space. (a) [^{3}H]E217βG (●; 0.01 µM). [^{3}H]taurocholate (○; 0.15 µM). (b) [^{14}C]benzylpenicillin (□; 0.2 µM) and [^{14}C]PAH (■; 0.2 µM). Taken from Kusuhara et al. [25] (panel a) and Nagata et al. [55] (panel b).

and the corresponding wild-type mouse. Another efflux mechanism may play a major role in the excretion of amphipathic organic anions across the BLM of the CP. According to the mRNA quantification, Mrp4 and Mrp5 may be alternative candidates [26,114]. Further studies are required to reveal their membrane localization in the CP and contribution to efflux transport via the CP.

4.2. Hydrophilic organic anions

In vivo kinetic analyses revealed the presence of a saturable elimination mechanism for benzylpenicillin in the ventricles [20,133]. Ogawa et al. [20] compared the kinetic parameters obtained in vivo using i.c.v. administration and in vitro using isolated rat CP. They found that the in vitro kinetic parameters (K_m 58 μM and V_{max} 504 pmol/min/rat) were comparable with those in vivo (K_m 43 μM and V_{max} 620 pmol/min/rat), indicating that the saturable efflux of benzylpenicillin in the ventricles is due to the uptake mechanism at the BBM of the CP [20]. This is also supported by a clear 1:1 correlation among the K_i values of probenecid and various β-lactam antibiotics for the efflux of benzylpenicillin from the CSF and for the uptake of benzylpenicillin by the isolated CP [20]. Hakvoort et al. [134] demonstrated that benzylpenicillin is accumulated on the basal side of the porcine CPE cultured on a porous membrane (Table 2). Similarly, fluorescein, phenol red, and riboflavin are accumulated in the basal compartment and this process is competitively inhibited by benzylpenicillin (Table 2) [134]. Mutual inhibition analyses have suggested multispecificity of the transporter for benzylpenicillin uptake at the BBM of the CP, which includes cefodizime (β-lactam antibiotic) [135], fleroxacin (quinolone antibiotic) [136] and PAH [55]. In addition to these compounds, a variety of β-lactam antibiotics have an inhibitory effect with K_i values ranging from 10 μM to 5.9 mM depending on their chemical structures [135,137]. Thus, it is likely that the transporter responsible for the benzylpenicillin uptake also plays a major role in regulating the CSF concentration of β-lactam antibiotics together with PEPT2 [98,138,139].

Table 2
Kinetic data of the investigated active transport systems in the primary cultured porcine choroid epithelial cells

Substrate	Transport direction	K_m [μM]	V_{max} [nmol/cm^2 h]
Ascorbic acid	b⇒a	67±12	3.91±0.29
myo-Inosipol	b⇒a	117±9	1.65±0.05
Penicillin G	a⇒b	107±8	1.82±0.05
Fluorescein	a⇒b	22±1.5	1.92±0.05
+400 μM Penicillin G		108±21	1.32±0.18
Phenol red	a⇒b	68±2.9	3.01±0.06
+400 μM Penicillin G		326±18	3.50±0.12
+20 μM Penicillin		340±23	2.52±0.12
Riboflavin	a⇒b	78±4	1.84±0.05
+400 μM Penicillin G		217±42	2.16±0.28

a: apical; b: basolateral.
Taken from Hakvoort et al. [134].

Suzuki et al. [137,140] investigated the driving force for the uptake of benzylpenicillin by the isolated CP. The uptake was markedly reduced by metabolic inhibitors, such as 2,4-dinitrophenol and KCN, suggesting the involvement of active transport [137]. Substitution of mannitol, Tris or *N*-methyl-D-glucamine, but not choline, for Na^+ slightly reduced the uptake to 70–80% of the control value, but ouabain, a Na^+K^+ ATPase inhibitor, did not affect the uptake [137]. Thus, benzylpenicillin uptake does not require an inward Na^+ concentration gradient. Whether benzylpenicillin uptake coupled to the export of intracellular anions was investigated using ATP-depleted CP, which was produced by incubating the isolated CP with 25 μM rotenone for 20 min, the intracellular ATP content was reduced from 27 to 0.97 nmol/mg protein [140]. The ATP-depleted choroid plexus has been incubated in the presence of anions, such as Cl^-, HCO_3^-, SCN^- and SO_4^{2-}, for their intracellular accumulation and subsequently transferred to the buffer without the anion to produce an outward-directed concentration gradient [140]. The uptake of benzylpenicillin was greater in the CP preloaded with Cl^- than in the CP preloaded with gluconate and the peak uptake in the presence of the Cl^- gradient was greater than that under equilibrium conditions [140]. Apart from Cl^-, the uptake was stimulated by the outwardly directed gradient of HCO_3^- and, to a lesser extent, by SCN^-. These anions are candidates for the driving force behind benzylpenicillin uptake into the choroid plexus.

As described previously, RT-PCR has detected the expression of rOat1, rOat2 and rOat3 mRNA in rat CP [54]. Especially, rOat3 has been shown to be expressed at the brush border surface of the rat CP.

Nagata et al. [55] investigated the uptake of PAH and benzylpenicillin as probe compounds for rOat1 and rOat3, respectively, by the isolated rat CP and compared their kinetic parameters (Fig. 4). Significant accumulation of PAH was observed in the isolated rat CP, but the transport activity was four-fold less than that of benzylpenicillin (Fig. 3) [55]. The K_m value of PAH was very close to the K_i value for benzylpenicillin uptake and vice versa. The inhibitors including cimetidine and E217βG, which do not affect rOat1-mediated transport, showed similar K_i values for PAH and benzylpenicillin uptake by isolated CP (Fig. 4). These results suggest that they share the same uptake mechanism at the brush border surface of the CPE. Furthermore, the K_i values for the inhibitors of benzylpenicillin uptake by isolated CP were comparable with those for rOat3 (Fig. 4). These kinetic results suggest that rOat3 plays a major role in the uptake of PAH and benzylpenicillin at the BBM of the CP. In addition, a marked reduction in the cellular accumulation of fluorescein in the isolated CP from the mOat3 knockout mouse [54] supports the idea that rOat3 is the likeliest candidate for the uptake of hydrophilic organic anions in the CP. Involvement of rOat3 has also been suggested in the uptake of a nucleoside analog, zidovudine [141]. The apical-to-basal transport across the primary cultured CPE, corresponding to the efflux from the CSF, of zidovudine, didanosine and lamivudine is greater than that in the opposite direction. The apical-to-basal transport of ziduvudine is saturable, and inhibited by 2,4-dichlorophenoxyacetate and benzylpenicillin [141].

The driving force for rOat3-mediated transport is considered to be an outward concentration gradient of dicarboxylate as described in Section 3.2.1. Similar transport properties were observed for the uptake of 2,4-dichlorophenoxylacetate (2,4-D) by the brush border surface of the CP. Pritchard et al. [142] clearly showed that extracellular or -vesicular glutarate stimulated the uptake of 2,4-D by the isolated CP and BBMV in the presence of an inward Na^+ concentration gradient (Fig. 5). Intravesicular glutarate, preloaded into the vesicles prior to the uptake experiment, could also stimulate the uptake of 2,4-D, suggesting the involvement of an exchanger for 2,4-D

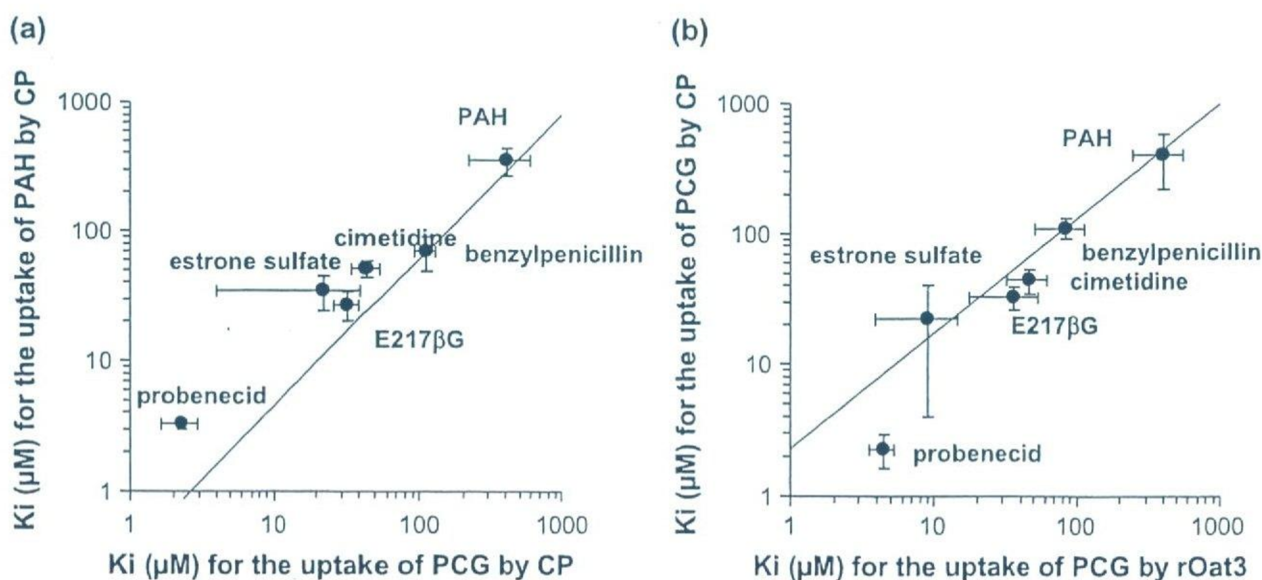

Fig. 4. Relationship between the K_m and K_i values for the uptake of PAH and benzylpenicillin by the CP, as well as the uptake of benzylpenicillin by the CP and rOat3. Correlation between the K_m and K_i values for the uptake of benzylpenicillin and PAH by the isolated rat CP (a), as well as those for the uptake of benzylpenicillin by the isolated rat CP and rOat3-expressing cells (b). The solid lines represent the regression line of the K_m and K_i values for the uptake of PAH and benzylpenicillin by the isolated rat CP (a; r=0.956, p<0.01), and the uptake of benzylpenicillin by the isolated rat CP and by rOat3 (b; r=0.985, p<0.01). Panel a suggests that benzylpenicillin and PAH share the uptake mechanism at the brush border surface of the CP. The K_m and K_i values for benzylpenicillin uptake by the isolated rat CP were comparable to those found in rOat3-expressing cells, suggesting that rOat3 is responsible for the uptake of benzylpenicillin by the isolated rat CP. Taken from Nagata et al. [55].

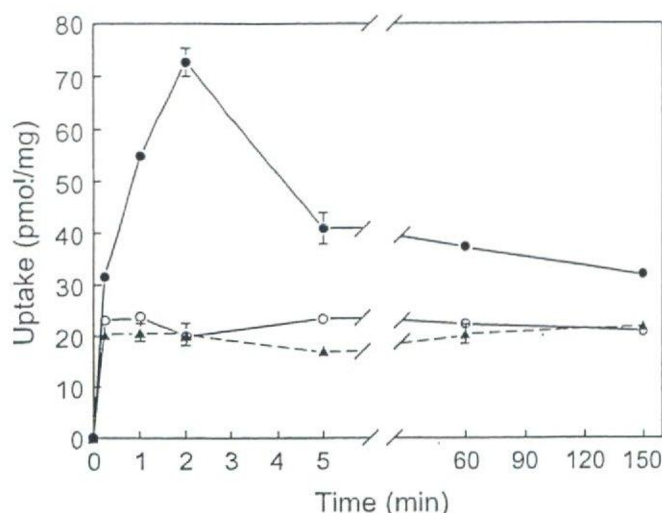

Fig. 5. Stimulatory effect of extracellular sodium and glutarate on the uptake of 2,4-dichlorophenoxyacetate by the brush border membrane of the bovine CP. Vesicles contained 100 mM mannitol, 100 mM KCl, 1 mM $MgSO_4$, 20 mM Tris–HEPES, pH 7.4. They were diluted 10-fold with transport buffer containing 10 μM [^{3}H]2,4-dichlorophenoxyacetate and either 100 mM NaCl (○) or 100 mM NaCl plus 20 μM glutarate (●). The effect of 500 μM probenecid was tested in the presence of 100 mM NaCl plus 20 μM glutarate in the external buffer (▲). Means±S.E., n=3. Taken from Pritchard et al. [142].

and glutarate at the BBM of the CP. Therefore, an outward concentration gradient of dicarboxylate produced by a sodium-dependent dicarboxylate transporter can serve as a driving force for the transporter responsible for 2,4-D uptake by the CP. 2,4-D is a poor substrate of rOat3 [60], but whether rOat3 accounts for the uptake of 2,4-D by the isolated CP remains to be examined.

The transporter involved in the basolateral excretion of hydrophilic organic anions remains unknown. Breen et al. [143] have characterized the subsequent basolateral excretion mechanism of fluorescein using confocal microscopy. They quantified the amount of fluorescein associated with the intracellular compartment and the basolateral side and demonstrated that substitution of K^+ for Na^+ reduced the accumulation of fluorescein in the basolateral compartment without affecting the amount associated with the intracellular compartment, indicating a reduction in the basolateral excretion clearance by this treatment. Since perturbation of the K^+ gradient directly or pharmacologically causes depolarization of the membrane voltage, the basolateral excretion of fluorescein is likely to be membrane voltage-dependent. The substrate specificity and molecular characteristics of this transporter remain unknown.

5. Efflux transport mechanism for organic cations in the choroid plexus

Miller and Ross [144] measured the extraction of NMN in vivo using the ventriculocisternal perfusion technique. The extraction of NMN during perfusion from the lateral ventricles to the cisternal magna was greater than that of inulin and was reduced by the addition of mepiperhenidol to the perfusate, suggesting involvement of an organic cation transporter in the extraction [144]. Other organic cations, such as cimetidine, choline, and TEA, typical substrates of renal organic cation transporters, have been shown to undergo carrier-mediated uptake at the brush border surface of the CP. The transporters for organic cations expressed in the CP are illustrated in Fig. 6.

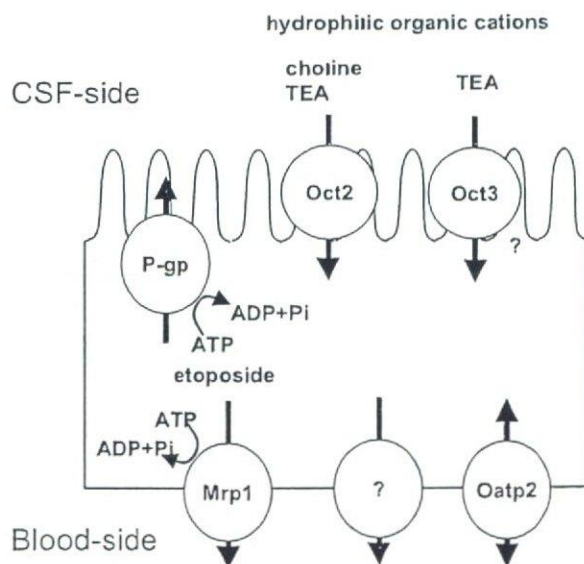

Fig. 6. Schematic diagram of the efflux transport systems for organic cations in the CP. The uptake of choline at the BBM of the CP has been suggested to be mediated by Oct2, although its expression in the CP is controversial. In addition, Oct3 mRNA was shown in the CP, but its membrane localization in the CP remains unknown. P-glycoprotein shows vesicular compartment localization in the CP, especially in the subapical region and its role in the CP remains unclear. The excretion mechanisms across the BLM remain unknown. Mrp1 has been suggested to be involved in the excretion of etoposide across the basolateral membrane. Oatp2 accepts type II organic cations as substrates, as well as amphipathic organic anions, and it may be involved in their uptake/excretion across the BLM.

5.1. Cimetidine

Cimetidine is a histamine H_2 receptor antagonist and a weak base. It is a bi-substrate, which is recognized by both organic anion and cation transporters. Ullrich et al. [145] examined the inhibitory potency of a series of compounds on the renal uptake of PAH and NMN using the stop-flow peritubular capillary microperfusion method and found that H_2 receptor antagonists, such as cimetidine, famotidine, and ranitidine, inhibit both organic anion and cation systems. It has been shown that saturable a mechanism accounts for the CSF elimination of cimetidine after i.c.v. injection [146]. Suzuki et al. [147] demonstrated saturable uptake of cimetidine by the isolated rat CP (K_m 53 μM). Organic anions, such as PAH and benzylpenicillin, produced a significant inhibition as did organic cations, such as histamine, creatinine, quinidine and quinine, while compounds, such as NMN, choline or TEA, had only a minimal effect even at 20 mM (Fig. 7) [147]. These results suggest the involvement of an organic anion transporter, rather than an organic cation transporter, as far as the uptake of cimetidine by the CP is concerned. Taking into consideration the fact that other H_2 receptor antagonists, such as famotidine and ranitidine, have been classified as bisubstrates [145], their uptake by the CP may be accounted for by the same transporter. Since cimetidine is a good substrate of rOat3 with a K_i value similar to its K_m previously determined in the CP [55,57,147], rOat3 is one of the candidates.

5.2. Tetraethylammonium and choline

Villalobos et al. [148] examined the involvement of a transporter in the uptake of TEA by isolated and primary cultured CP through the BBM of the CPE. The uptake of TEA by the isolated rat CP and primary cultured CPE was inhibited by tetrapentylammonium (TePA) (Fig. 8). Furthermore, the uptake of TEA by primary cultured cells was saturable with a K_m value of 350 μM and inhibited by NMN, darstine, choline and cimetidine, but not by PAH (Fig. 9). A preloading of unlabeled TEA into the cells prior to starting the uptake experiment stimulated the uptake of TEA by

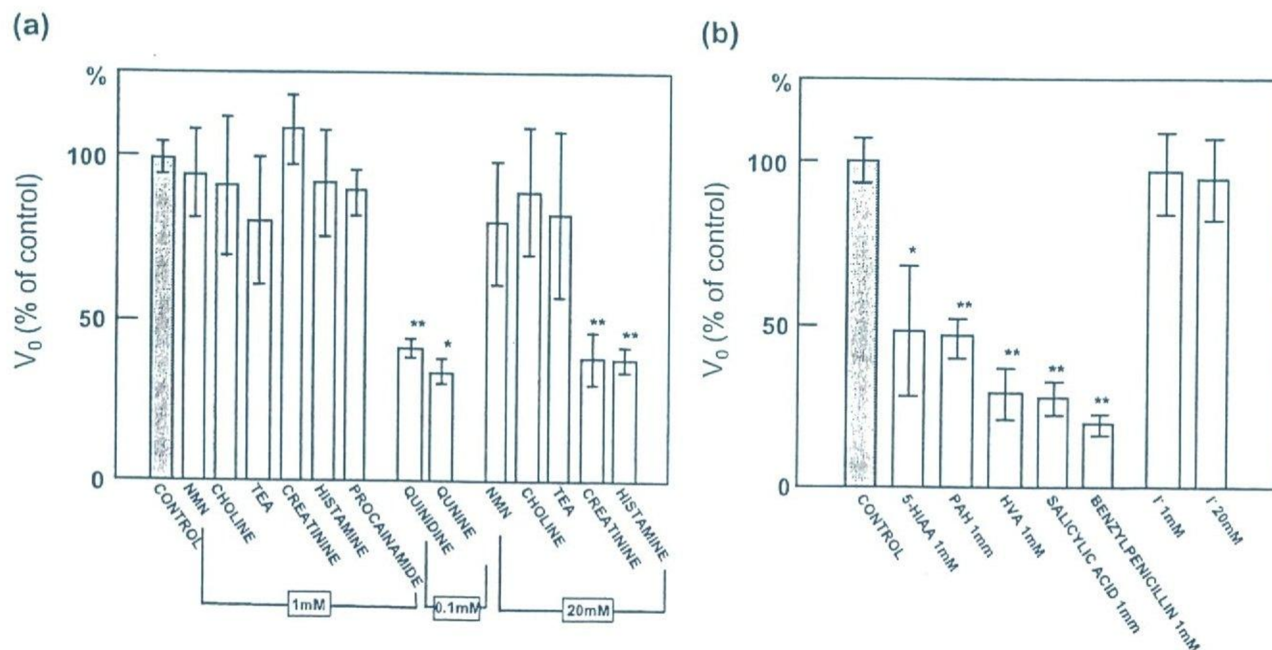

Fig. 7. Effect of organic cations and anions on the uptake of cimetidine by the isolated rat CP. [^{3}H]Cimetidine was incubated with various inhibitors at the concentrations indicated for 3 min at 37 °C. Although cimetidine is a weak base, it has been shown to be a bisubstrate which is recognized by both organic anion and cation transporters. The uptake by the isolated CP was inhibited by organic anions, such as PAH and benzylpenicillin, suggesting an involvement of an organic anion transporter, but minimal inhibition by TEA suggests a minor contribution of an organic cation transporter. Each bar represents the mean±S.E. of three independent experiments. **$P<0.01$; *$P<0.05$, by student's t-test. 5-HIAA, 5-hydroxyindoleacetate; HVA, homovanillic acid. Taken from Suzuki et al. [147].

primary cultured cells, suggesting that the transporter responsible for TEA uptake is characterized by its bidirectional nature.

Villalobos et al. [149] characterized the uptake mechanism using primary cultured CPE, in which a saturable uptake of choline was observed with a K_m value of 50 μM. It has been suggested that the transporter responsible for choline uptake in the primary cultured rat CPE also accepts NMN and

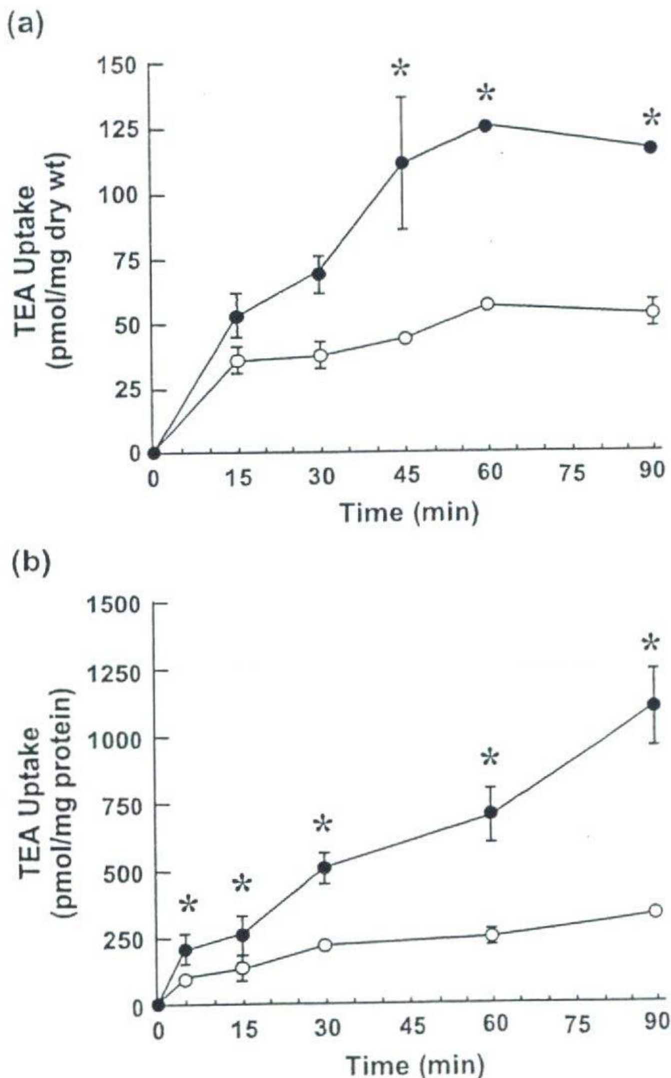

Fig. 8. Effects of TePA on the time-dependent uptake of TEA by isolated rat choroid plexus and primary cultures of rat choroid plexus epithelial cells. Isolated CP was incubated with 10 μM [^{14}C]TEA in the presence (●) or absence of tetrapentylammonium (TePA) (○, 100 μM). Similarly, cultured cells were incubated with 10 μM [^{14}C]TEA in the presence or absence of TePA (○, 1 mM). *P<0.05 vs. uptake in the absence of TePA. Taken from Villalobos et al. [148].

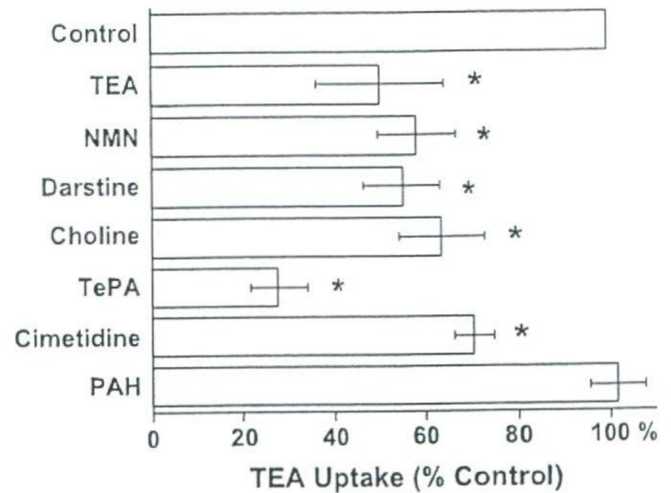

Fig. 9. Effects of organic ions on the 30 min TEA uptake by primary cultures of rat choroid plexus epithelial cells grown on a solid support. Cells were incubated with 10 μM [^{14}C]TEA in the absence (control) or presence of each inhibitor (1 mM). *P<0.05 vs. control uptake. TePA: tetrapentylammonium. Taken from Villalobos et al. [148].

TEA as substrates, since the preloading of primary cultured cells with NMN and TEA stimulated the uptake of choline 1.5-fold (*trans*-stimulation). The organic cation, hemicholinium-3, is a moderate inhibitor for choline uptake and NMN showed a weak inhibition. The choline transporter in the CP has been suggested to be a facilitative transporter, which depends on the membrane voltage. The uptake of choline was not affected by extracellular Na^+, whereas increasing the K^+ concentration markedly reduced the uptake, causing depolarization of the intracellular potential from −70 to −15 mV. This K^+ effect was further supported by the results that treatment of the primary cultured CPE with Na^+/K^+ ATPase (ouabain) or K^+ channel inhibitor (Ba^{2+}), causing perturbation of K^+ gradient pharmacologically, reduced the choline uptake to a degree similar to that produced by a high K^+ buffer.

Sweet et al. [71] carried out further characterization of choline uptake in the CP. According to the RT-PCR analysis by Sweet et al., both rOct2 and rOct3 mRNA are expressed in the CP, while Choudhuri et al. [26] could observe expression of rOct1 and rOct3 mRNA in the CP. Choline uptake was only observed in rOct2-expressing oocytes with a K_m value of 440 μM and not in rOct3-expressing oocytes. The K_m value of choline for rOct2 was greater than the previously reported value by Vallalobos et al. (50 μM), but

comparable with the K_m value for choline uptake by the isolated rat CP, which was determined to be 180 μM in this report [71]. rOct2 may be a candidate transporter for choline uptake in the CP. Further studies are necessary to identify the localization of rOct3 in the CP and, if it is expressed at the BBM of the CP, the contribution to the uptake of hydrophilic organic cations, such as TEA, should be examined.

6. Discussion and future aspects

The present review summarizes the current status of the efflux transport mechanisms for organic ions in the CP. The many published studies have provided molecular insights into the uptake systems operating at the BBM of the CP. Due to limitations in methodology, the excretion process for organic ions has not been fully characterized yet and the molecular characteristics of the transporters involved in this process remain unknown. ABC transporters, such as MRPs and/or alternatively membrane voltage-sensitive mechanisms, are current candidates to account for this process for organic anions, while OCTNs may be involved in the excretion of hydrophilic organic cations as proposed in the kidney [82,90]. Gene knockout of the candidate gene will give us insight into the basolateral excretion of organic ions.

Evidence from many studies has shown that the primary cultured CPE retains the efflux transport systems for organic anions and cations. In addition, Kitazawa et al. have developed an immortalized cell line of rat CPE which is prepared from a transgenic rat harboring the temperature-sensitive SV40 large T antigen [24,150,151]. These models will allow us to investigate the transport mechanisms for small compounds across the monolayer of the CPE. It is necessary to compare the expression levels of transporter genes in these models with those in freshly isolated CP in future studies. RNA interference has been developed and shown to efficiently suppress target genes even in mammalian cells [152]. This new methodology will help us to investigate the transport mechanisms in the CP from a genetic viewpoint.

Once a transporter involved in the efflux transport has been identified, cDNA-transfectants expressing human transporters serve as a screening system for the selection of drugs with suitable pharmacokinetic properties. Recently, Sasaki et al. [153] and Cui et al. [154] established double transfectants, which express uptake and efflux transporters (OATP-C or OATP 8, and MRP2, respectively) in the basal and apical membrane of MDCK II cells, respectively. The basal-to-apical transport of their common substrates is greater in OATP-C/MRP2 or OATP8/MRP2 than that in control or single gene transfected cells and this transport mimics the hepatobiliary transport in humans [153,154]. The same approach can be used as an in vitro model of the CPE after the transporters involved in the uptake and efflux have been identified. Further studies are necessary to discover whether human orthologs of the transporters identified in the rodent CP are expressed in the human tissues. Furthermore, the interindividual differences in the transport activities due to up- or down-regulation in disease states and genetic polymorphisms are important topics which also need to be investigated in future studies.

References

[1] T. Speake, C. Whitwell, H. Kajita, A. Majid, P.D. Brown, Mechanisms of CSF secretion by the choroid plexus, Microsc. Res. Tech. 52 (2001) 49–59.

[2] M.B. Segal, The choroid plexuses and the barriers between the blood and the cerebrospinal fluid, Cell. Mol. Neurobiol. 20 (2000) 183–196.

[3] B. Gao, P.J. Meier, Organic anion transport across the choroid plexus, Microsc. Res. Tech. 52 (2001) 60–64.

[4] N. Strazielle, J.F. Ghersi-Egea, Demonstration of a coupled metabolism–efflux process at the choroid plexus as a mechanism of brain protection toward xenobiotics, J. Neurosci. 19 (1999) 6275–6289.

[5] R. Spector, Drug transport in the mammalian central nervous system: multiple complex systems, Pharmacology 60 (2000) 58–73.

[6] N. Mizuno, T. Niwa, Y. Yotsumoto, Y. Sugiyama, Impact of drug transporter studies on drug discovery and development, Pharmacol. Rev. 55 (2003) 425–461.

[7] H. Kusuhara, Y. Sugiyama, Efflux transport systems for drugs at the blood–brain barrier and blood–cerebrospinal fluid barrier: Part 1, Drug Discov. Today 6 (2001) 150–156.

[8] J.F. Ghersi-Egea, N. Strazielle, Brain drug delivery, drug metabolism, and multidrug resistance at the choroid plexus, Microsc. Res. Tech. 52 (2001) 83–88.

[9] F.G. Russel, R. Masereeuw, R.A. van Aubel, Molecular aspects of renal anionic drug transport, Annu. Rev. Physiol. 64 (2002) 563–594.

[10] H. Koepsell, V. Gorboulev, P. Arndt, Molecular pharmacology of organic cation transporters in kidney, J. Membr. Biol. 167 (1999) 103–117.

[11] K.I. Inui, S. Masuda, H. Saito, Cellular and molecular aspects of drug transport in the kidney, Kidney Int. 58 (2000) 944–958.

[12] B. Hagenbuch, P.J. Meier, The superfamily of organic anion transporting polypeptides, Biochim. Biophys. Acta 1609 (2003) 1–18.

[13] 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 (2001) 397–421.

[14] G. Burckhardt, N.A. Wolff, A. Bahn, Molecular characterization of the renal organic anion transporter 1, Cell Biochem. Biophys. 36 (2002) 169–174.

[15] H. Suzuki, Y. Sugiyama, Excretion of GSSG and glutathione conjugates mediated by MRP1 and cMOAT/MRP2, Semin. Liver Dis. 18 (1998) 359–376.

[16] J.M. Collins, R.L. Dedrick, Distributed model for drug delivery to CSF and brain tissue, Am. J. Physiol. 345 (1983) R303–R310.

[17] H. Suzuki, T. Terasaki, Y. Sugiyama, Role of efflux transport across the blood–brain barrier and blood–cerebrospinal fluid barrier on the disposition of xenobiotics in the central nervous system, Adv. Drug Deliv. Rev. 25 (1997) 257–285.

[18] H. Dai, W.E. Elmquist, Drug transport studies using quantitative microdialysis, Methods Mol. Med. 89 (2003) 249–264.

[19] H. Kusuhara, T. Terasaki, Y. Sugiyama, Brain efflux index method. Characterization of efflux transport across the blood–brain barrier, Methods Mol. Med. 89 (2003) 219–231.

[20] M. Ogawa, H. Suzuki, Y. Sawada, M. Hanano, Y. Sugiyama, Kinetics of active efflux via choroid plexus of beta-lactam antibiotics from the CSF into the circulation, Am. J. Physiol. 266 (1994) R392–R399.

[21] J.E. van Montfoort, B. Hagenbuch, G.M. Groothuis, H. Koepsell, P.J. Meier, D.K. Meijer, Drug uptake systems in liver and kidney, Curr. Drug Metab. 4 (2003) 185–211.

[22] P.J. Meier, B. Stieger, Bile salt transporters, Annu. Rev. Physiol. 64 (2002) 635–661.

[23] R.H. Angeletti, P.M. Novikoff, S.R. Juvvadi, J.M. Fritschy, P.J. Meier, A.W. Wolkoff, The choroid plexus epithelium is the site of the organic anion transport protein in the brain, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 283–286.

[24] S. Ohtsuki, T. Takizawa, H. Takanaga, N. Terasaki, T. Kitazawa, M. Sasaki, T. Abe, K. Hosoya, T. Terasaki, In vitro study of the functional expression of organic anion transporting polypeptide 3 at rat choroid plexus epithelial cells and its involvement in the cerebrospinal fluid-to-blood transport of estrone-3-sulfate, Mol. Pharmacol. 63 (2003) 532–537.

[25] H. Kusuhara, Z. He, Y. Nagata, Y. Nozaki, T. Ito, H. Masuda, P.J. Meier, T. Abe, Y. Sugiyama, Expression and functional involvement of organic anion transporting polypeptide subtype 3 (Slc21a7) in rat choroid plexus, Pharm. Res. 20 (2003) 720–727.

[26] S. Choudhuri, N.J. Cherrington, N. Li, C.D. Klaassen, Constitutive expression of various xenobiotic and endobiotic transporter mRNAs in the choroid plexus of rats, Drug Metab. Dispos. 31 (2003) 1337–1345.

[27] B. Gao, B. Stieger, B. Noe, J.M. Fritschy, P.J. Meier, Localization of the organic anion transporting polypeptide 2 (Oatp2) in capillary endothelium and choroid plexus epithelium of rat brain, J. Histochem. Cytochem. 47 (1999) 1255–1264.

[28] D. Sugiyama, H. Kusuhara, H. Taniguchi, S. Ishikawa, Y. Nozaki, H. Aburatani, Y. Sugiyama, Functional characterization of rat brain-specific organic anion transporter (Oatp14) at the blood–brain barrier: high affinity transporter for thyroxine, J. Biol. Chem. 278 (2003) 43489–43495.

[29] T. Abe, M. Kakyo, H. Sakagami, T. Tokui, T. Nishio, M. Tanemoto, H. Nomura, S.C. Hebert, S. Matsuno, H. Kondo, H. Yawo, Molecular characterization and tissue distribution of a new organic anion transporter subtype (oatp3) that transports thyroid hormones and taurocholate and comparison with oatp2, J. Biol. Chem. 273 (1998) 22395–22401.

[30] H.C. Walters, A.L. Craddock, H. Fusegawa, M.C. Willingham, P.A. Dawson, Expression, transport properties, and chromosomal location of organic anion transporter subtype 3, Am. J. Physiol.: Gasterointest. Liver Physiol. 279 (2000) G1188–G1200.

[31] N. Li, D.P. Hartley, N.J. Cherrington, C.D. Klaassen, Tissue expression, ontogeny, and inducibility of rat organic anion transporting polypeptide 4, J. Pharmacol. Exp. Ther. 301 (2002) 551–560.

[32] V. Cattori, J.E. van Montfoort, B. Stieger, L. Landmann, D.K. Meijer, K.H. Winterhalter, P.J. Meier, B. Hagenbuch, Localization of organic anion transporting polypeptide 4 (Oatp4) in rat liver and comparison of its substrate specificity with Oatp1, Oatp2 and Oatp3, Pflugers Arch. 443 (2001) 188–195.

[33] B. Noe, B. Hagenbuch, B. Stieger, P.J. Meier, Isolation of a multispecific organic anion and cardiac glycoside transporter from rat brain, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 10346–10350.

[34] C. Reichel, B. Gao, J. Van Montfoort, V. Cattori, C. Rahner, B. Hagenbuch, B. Stieger, T. Kamisako, P.J. Meier, Localization and function of the organic anion-transporting polypeptide Oatp2 in rat liver, Gastroenterology 117 (1999) 688–695.

[35] J.E. van Montfoort, B. Hagenbuch, K.E. Fattinger, M. Muller, G.M. Groothuis, D.K. Meijer, P.J. Meier, Polyspecific organic anion transporting polypeptides mediate hepatic uptake of amphipathic type II organic cations, J. Pharmacol. Exp. Ther. 291 (1999) 147–152.

[36] B. Gao, B. Hagenbuch, G.A. Kullak-Ublick, D. Benke, A. Aguzzi, P.J. Meier, Organic anion-transporting polypeptides mediate transport of opioid peptides across blood–brain barrier, J. Pharmacol. Exp. Ther. 294 (2000) 73–79.

[37] L. Li, P.J. Meier, N. Ballatori, Oatp2 mediates bidirectional organic solute transport: a role for intracellular glutathione, Mol. Pharmacol. 58 (2000) 335–340.