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EXPERT REVIEW

Vectorial Ligand Transport Through Mammalian Choroid Plexus

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ABSTRACT In the last decade, there has been substantial progress in understanding vectorial ligand transport through rodent and human choroid plexus (CP), the locus of the blood-CSF interface. In this Review, we enumerate the experimental data required to establish vectorial transport through CP and describe transporters involved in vectorial transport across CP. We also note how these transporters differ from those at the blood-brain barrier. The ligand (substrate) examples presented are methyltetrahydrofolate, methotrexate, leukotriene C_4, nucleosides, thiamine monophosphate, prostaglandins, and digoxin. Our focus is on more definitive experiments, including animal and human transporter "knock-outs." Finally, we discuss the neurochemical implications of vectorial transport through CP and the clinical implications of transporter polymorphisms and knockouts. Examples include descriptions of how vectorial transport through the CP for several micronutrients (e.g., methyltetrahydrofolate) nourishes the brain and how knowledge of CP vectorial transport can lead to important treatments.

KEY WORDS brain nourishment · cerebrospinal fluid · multidrug resistance proteins · neuroprotection · organic acid transporting polypeptides

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INTRODUCTION

Over 25 years ago, we and other investigators observed that many endogenous substances, e.g., nucleosides (1), prostaglandins (2) and leukotriene C_4 (3), and certain drugs, including penicillin (4), fluorescein (5) and methotrexate (6), were transported out of CSF into blood by saturable, inhibitable carriers or transporters. These transporters were very likely located in the choroid plexus (CP), since, *in vitro*, the isolated CP, the locus of the blood-CSF barrier (BCSFB), accumulated these substances in a fashion consistent with the *in vivo* data. Anatomically, the single layer of tight-junctioned choroid plexus epithelial cells has two sides (7): an apical side facing the CSF and a basolateral side facing the choroid plexus capillary endothelium, which is not joined by tight junctions (1,8). Moreover, there was a widespread and correct belief that for vectorial transport there must be separate transporters at the apical (luminal) and basolateral (interstitial) sides of the CP, as was initially shown for glucose in the tight-junctioned cerebral capillaries that make up the blood-brain barrier (BBB) (9). (Like the CP, the BBB has an apical/luminal side facing the blood and a basolateral/interstitial side.) In other words it was unlikely that passive simple diffusion, depending on molecular size, lipid solubility and charge, could explain the distribution and transfer of many molecules into and out of the CNS. As data became available, several putative transporters were found to change the distributive concentration of transported ligands in CSF by an order of magnitude or more. For example, the efflux rate of penicillin from the CSF compartment in rabbits was 14 times the influx rate (4). Moreover, it was also clear that certain micronutrients (e.g., ascorbic acid (AA) and methyltetrahydrofolate (MeTHF)) were "pumped" from blood through the basolateral CP

membrane into the epithelium and then via the apical CP membrane into CSF (9). Moreover, with riboflavin and fluorescein, which are pumped out of CSF, one could actually observe their apical-to-basolateral transport through CP *in vitro* in the isolated CP. Using fluorescence microscopy, we and others photographed fluorescent riboflavin (10) and fluorescein (5,11) being concentrated in the CP epithelial cells from the apical (CSF) side and then being further concentrated (by a putative transporter at the basolateral membrane) in the stroma on the basolateral side—clearly a saturable two-step sequential transport process. This phenomenologically explained the efflux transport of these two substances out of CSF. However, at the time of these initial observations and for many years subsequently, there was little mechanistic or molecular knowledge of these CP transport systems except for the purification and characterization of the choroid plexus folate receptor alpha (FRα) transporter (12).

Since the original observations, there has been tremendous progress in recent years using both older and new molecular and immunological techniques. In previous publications, we and others have described these techniques in detail (1,8,9,13). Now we understand many of these transport processes at both the BBB and the CP. These two loci of the CNS barrier systems work in concert (14) to both nourish the brain and protect it from noxious substances, both endogenous and exogenous (9,13). In many cases they also provide homeostatic functions as described below. Moreover, there have been human experiments of nature that have confirmed the results of experimental studies in animals (see below).

However, the BBB and BCSFB have, in some cases, different transporters. For example, P-glycoprotein (P-gp; ABCB1) and breast cancer resistance protein (BCRP; ABCG2) are located at the luminal surface of the BBB (13,15) but not within the CP, whereas within the CP, the multi-drug resistance protein one (MRP-1; ABCC1) and the AA transporter (sodium-dependent vitamin C transporter 2; SVCT2) are located at the basolateral surface of the CP but not at the BBB (9,15). Consequently, these CNS transport interfaces need separate discussion. In this review, we focus on the rodent CP vectorial transporters plus information on the human CP transporters where available and relevant. We will also comment on the transporters at the BBB where appropriate.

Until recently, much of the understanding of the transport through the CP has been focused on individual transporters in the CP. For example, we know in great detail, indeed on a molecular level, the apical membrane localization and characteristics of the organic acid transporting polypeptide 3 (Oat 3) that transports penicillin, riboflavin, PAH, and cimetidine from the CSF into CP. On the other hand, the basolaterally located SVCT2 takes up AA from the interstitial fluid for subsequent transport of the vitamin C through the cell and then out of CP into CSF (9,13). However, we do not know how the penicillin and vitamin C substrates, respectively, following uptake by the choroidal epithelium, are then transferred out of CP at the other side of the epithelium. Recently, we have published rigorous criteria necessary to conclude that a transporter for organic molecules is, in fact, doing what it is thought to do (13).

The overall objectives of this paper are to define the criteria necessary to conclude that a two-step vectorial transport process exists in the CP, i.e., to describe the location, molecular and kinetic bases of the two steps. This is now possible in some cases with recent data coupled to the older data, and in several cases, due to experiments of nature (see below). We will also categorize conclusions as *definite* or *probable* depending on the strength of the data meeting the vectorial criteria, and point out where there are missing data. The specific examples that we cover are listed in Table I.

There are three general types of transporters covered (Table II). These include secondary-type active transporters and facilitated diffusion-type transporters, receptor-mediated transporters and finally primary active transporters that require ATP.

As noted above, we recently published the criteria necessary to conclude that a transporter exists and is active in CP (13). In Table III of the current paper we enumerate the additional criteria necessary to establish that two transporters, one on the apical and the other on the basolateral side, are involved in vectorial CP ligand transport.

Unlike the straightforward criteria to establish a single transporter (13) in CP (Table III), the criteria to establish vectorial transport are not absolute, especially with the promiscuous (multi-ligand) transporters involved in the vectorial transport described in this paper. However, when all the criteria are met, the evidence becomes very strong. Obviously the transporter must be present and located directionally on the correct side (Table III). Visual observation of ligand transport can be quite revealing (5,10). The kinetics of uptake and release by the isolated CP

Table I Examples of Vectorially Transported Substances Through CP and Directionality

1) Methyltetrahydrofolate (MeTHF)—into CSF
2) Methotrexate (Mtx)—out of CSF
3) Leukotriene C_4 (LTC_4)—out of CSF
4) Nucleosides—out of CSF
5) Thiamine monophosphate (TMP)—into CSF
6) Prostaglandins (PG)—out of CSF
7) Digoxin—into CSF ?

Table II Transport Types and Classification

1) Non-ATP Requiring Transporters (SLC[a]- transporters)
a) Secondarily active
b) Facilitated diffusion
1) Directional
2) Equilibrative
2) Receptor-Mediated Transporters
3) Primary Active Transporters—(ABC[b]—type transporters)
a) Requires ATP

[a] *SLC* Solute transporter family

[b] *ABC* ATP-binding cassette transporter family

in vitro compared with *in vivo* data can be helpful. Also, the efficacy ($V_{max} \div K_T$) may be revealing *in vivo* and *in vitro* (Table III); the higher the efficacy, the more likely the putative transporter transports the ligand, all other matters being equal, an assumption termed "efficacy theory." However, because most of these receptors are promiscuous, the efficacy may be lower *in vivo* because of competing ligands that, where known, must be taken into account. (For example, rabbit CSF contains many endogenous substances that alter the apparent efficacy of the CP apical uptake system for thymidine as discussed below (16)).

Knock-down, -out or -up, cells and animals with one or the other or both transporters missing or enhanced, in CP, can be very revealing. However, in some cases, other transporters (transporter redundancy) can replace a missing transporter, or the KO'ed transporter may not be quantitatively important—thus making a KO study unrevealing. Also cell/tissue culture CP studies must be interpreted with extreme caution for possible up- or down-regulation of the investigated transporter. For example, Mrp1 and Scvt2 are normally essentially absent in cerebral capillaries but are up-regulated *in vitro* (9,15). However, in some cases, CP cells grown *in vitro* on perforated filters can show directionality and kinetics of transfer in normal and KO cells consistent with data from *in vitro* studies in intact CP and *in vivo* data (13). Sometimes, *in vivo* distribution studies with exogenous ligands in KO mice can be revealing (see below) when CSF, brain, CP and plasma are sampled.

Finally, in many cases, several transporters have some affinity for a putative ligand. For example, Mrp4 and Mrp1 (both on the basolateral side of CP; Fig. 1) have affinity for methotrexate (K_T~0.2 mM and >1 mM, respectively) (17,18). In such a case, the transporter with the greater efficacy (Mrp4) would be more likely to transport the bulk of the methotrexate (Table III), all other things being equal. But this criterion is soft because of the assumptions made. For example, there could be unknown competing ligands for Mrp4 and not Mrp1 that neutralize the efficiency advantage for Mrp4. Finally, crystallization and x-ray analysis at ~0.3 nm would provide the final structural data. None of the transporters in this Review have been crystallized and studied at 0.3 nm yet, although P-glycoprotein at the BBB has recently been crystallized and studied at 0.38 nm (13).

THE TRANSPORTERS

Examples of transporters that we discuss and their location and characteristics in CP are shown in Fig. 1. FRα, PCFT and RFC transporters in animals and humans have recently been discussed at length (9). See Table IV for abbreviations, nomenclature and gene identification. In short, MeTHF, the principal folate in plasma, is sequentially transported from plasma via CP basolateral FRα, cytosolic PCFT and then apical RFC into CSF as discussed below

Table III Criteria to Establish Vectorial Transporter Transport

1) Presence and location of putative transporters on opposite sides of CP i.e., apical and basolateral sides with appropriate orientation
2) Visualization of ligand transfer, if possible, e.g., riboflavin and fluorescein transport
3) Kinetics of uptake in isolated CP consistent with *in vivo* kinetics
4) Efflux transporter release of ligand kinetically consistent in CP *in vitro* with *in vivo* data
5) Effect of isolated CP ligand uptake in "knock-down" or "knock-out" rodents (or humans) with one or the other transporters, or both "out"
a) Dose-response curves with "dose" (Vmax) of transporter
6) Inhibitor studies against one or the other transporters with appropriate kinetics
7) Through-put directional *in vitro* studies with choroid plexus epithelial monolayers in chambers
8) *In vivo* distribution studies with exogenous labeled compounds in KO rodents
9) Where possible, the efficacy[a] of transporter for ligand in vesicles, isolated CP *in vitro* and choroidal tissue *in vivo* are all consistent
10) Efficiency theory—all other things being equal (see text)
11) Crystallization of transporter and X-ray analysis at ~0.3 nm

[a] Efficacy defined as the Vmax (maximal transport velocity) divided by the K_T (concentration that one-half saturates the transporter)

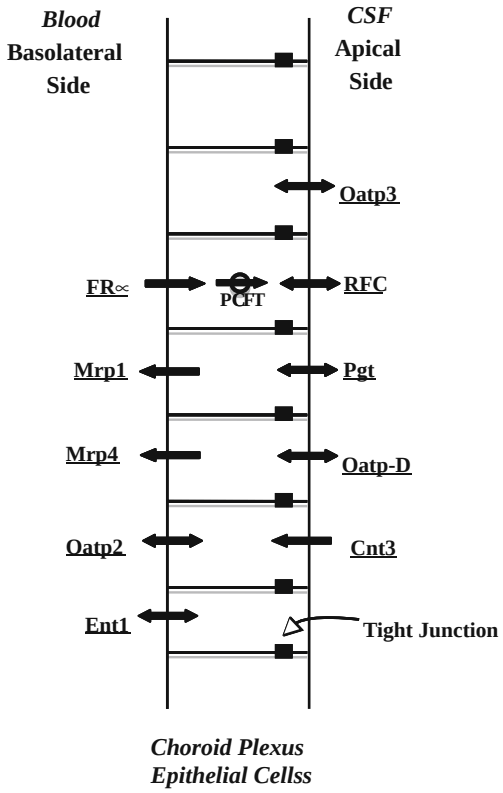

Fig. 1 Established transporter locations in rodent CP. Arrows indicate *potential* directionality of transport. Arrows with two arrowheads are facilitated diffusion transporters; with one arrowhead, primary or secondary active transport transporters. In humans, the genes and transporter proteins are denoted by capital letters. For abbreviations, see text and Table IV.

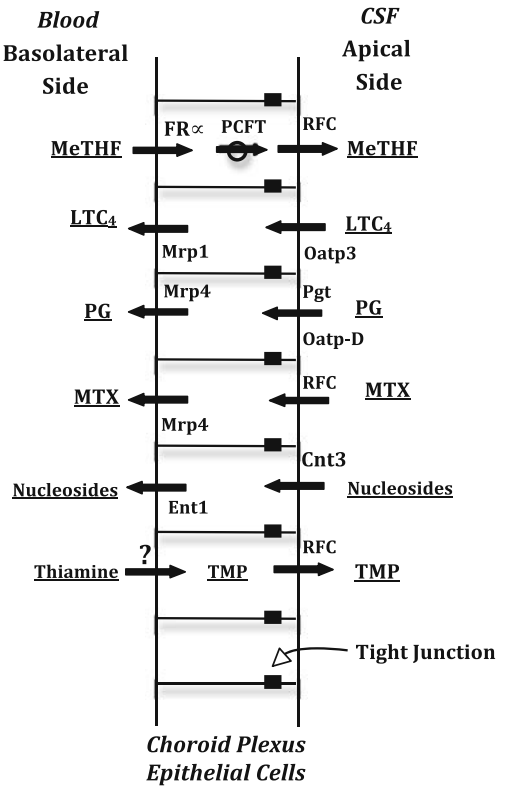

Fig. 2 Vectorial ligand transport through CP. Arrows indicate *actual in vivo* directionality of transported ligand. For abbreviations see Tables I and IV.

(Fig. 2) (9). The concentration of MeTHF in CSF is normally four times that in plasma (9). These transporters, working together, also provide MeTHF homeostasis in CSF and secondarily in brain (9).

Organic acid transporting polypeptide 2–Oatp2 (Slco1a4; new protein name Oatp1a4) is a facilitated diffusion basolateral glycoprotein transporter of 661 amino acids and has a molecular weight (MW) of ~73 kD (Fig. 1) (19,20). It has 12 putative trans-membrane domains and is trans-stimulated by intracellular glutathione; thus, Oatp2 tends to take up molecules from blood into CP since glutathione levels in CP are high. It has a high affinity for digoxin (K_T~0.2 μM) but also has affinity (~ 10–30 μM) for some bile acids, conjugated sulfates and glucuronides. There is no clear homologue yet found in humans.

Organic acid transporting polypeptide 3–Oatp3 (gene Slco1a5; new protein name Oatp1a5) is a facilitated diffusion apical glycoprotein transporter of 670 amino acids with a MW of ~74 kD (21,22). It has 12 putative trans-membrane

Table IV Rodent Transporter Abbreviations and Nomenclature[a]

Common transporter name (abbreviation)	Rodent gene name	Other names
Folate receptor-α (FRα)	Folate receptor α	
Proton-coupled folate transporter (PCFT)	Slc 46a1	
Reduced folate carrier (RFC)	Slc 19a1	
Multidrug resistance protein 1 (Mrp1)	Abcc1	
Multidrug resistance protein 4 (Mrp4)	Abcc4	
Organic acid transporting polypeptide 2 (Oatp2)	Slco1a4	Oatp1a4
Organic acid transporting polypeptide 3 (Oatp3)	Slco1a5	Oatp1a5
Prostaglandin transport protein (Pgt)	Slco2al	Oatp2a1
Organic acid transporting polypeptide D (OatpD)	Slco3a1	Oatp3a1
Concentrative nucleoside transporter 3 (Cnt3)	Slc28a3	
Equilibrative nucleoside transporter 1 (Ent1)	Slc29a1	

[a] Homologous human genes and transporters are portrayed in capital letters.

domains and transports LTC_4 with an affinity >1 μM. Oatp1 through 4 do not transport para-aminohippurate (PAH) and methotrexate (21). Oatp-3 also transports taurocholate (K_T=34 μM) and thyroid hormones (5–7 μM). There is no clear homologue yet discovered in humans.

The prostaglandin (PG) transporter–Pgt (Slco2al; protein Oatp2a1) is a facilitated diffusion transporter that can exchange PGs for lactate (23–25). Since lactate is higher in cells, Pgt is primarily a PG uptake transporter. Pgt, a putative 12 transmembrane glycoprotein, probably an apical transporter, has 643 amino acids, and like all Oatp proteins is not dependent on ATP, sodium or chloride. Pgt has a high affinity for PGs (e.g., ~ 0.1 μM for PGE_1, PGE_2 and $PGF_{2\alpha}$). Pgt has a similar homologue in humans.

Oatp-D (Slco3al; protein Oatp3al), a facilitated diffusion transporter, probably has 12 trans-membrane domains (26). It is also an apical glycoprotein transporter and has 710 amino acids with MW of ~76 kD. It has a very high affinity for PGs (K_T for PGE_1, and PGE_2 of ~0.05 μM), but also has very weak affinity for weak organic acids (e.g., penicillin G). The human homologue is 98% similar.

Multi-drug resistant protein 1 (Mrp1/ Abcc1) located basolaterally in CP is a primary active transporter that requires two ATP molecules to effect transport. It is a ~190 kD glycoprotein with 1541 amino acids, 17 putative trans-membrane domains and a MW of ~190 kD (27,28). It has a very high affinity for LTC_4 (~ 0.1 μM) and unconjugated bilirubin (~0.01 μM) but also transports glucuronidated and sulfated conjugates (which can be formed *in situ* in CP by conjugating enzymes or transported into CP via the apical membrane) with much lower affinity. Mrp1 is inhibited by probenecid. Its concentration in CP is >25 times that in brain capillaries (15). The human homologue MRP1 is similar. Both Mrp1 and Mrp4 transport many chemotherapeutic drugs and thus are relevant to clinical medicine (see below).

Mrp4 (Abcc4) is an active transporter located basolaterally in CP and on the analogous apical side of cerebral capillaries at the BBB (18,29,30). In this case, at both loci, Mrp4 pumps certain molecules from the CNS into blood. In other words, Mrp4 at both the BBB and BCSFB work in 'excretory' harmony. Mrp4 (MW~135 kD) is smaller than Mrp1, has putatively 12 trans-membrane domains and is a glycoprotein that, like Mrp1, requires two ATP molecules for activity. It is promiscuous and transports glucuronidated and sulfated compounds as well as certain nucleoside analogues and methotrexate and is inhibited by probenecid. Unlike Mrp1, Mrp4 transports prostaglandins (K_I for PGE_1, PGE_2, $FGF_{2\alpha}$~2 μM). It is noteworthy that both Mrp1 and Mrp4 KO mice are healthy and reproduce normally (29). Only when exposed to toxic transported ligands does the KO abnormality become apparent (30). Mrp1 and Mrp4 evidently either play no important role in normal physiology, or other transporters can take their place.

Cnt3, a sodium-dependent energy-requiring (active) transporter (Table IV), and Ent1, a nitrobenzylthioinosine (NBTI)-sensitive facilitated diffusion transporter are located on the apical and basolateral membranes of CP, respectively (1,31,32). Cnt3 and the Ent1 sequentially transfer both ribo- and deoxyribonucleosides across CP from CSF into blood (see below). These transporters have recently been reviewed in detail (1).

UNIDIRECTIONAL ONE-STEP TRANSPORTERS

Mrp transporters, located in the basolateral membrane of CP (Fig. 1), can act unilaterally (just basolaterally) or as a component of a two-step basolateral plus apical transfer process. This is because Mrp transporters can "grasp" the ligand from the intracellular milieu and then expel the ligand from the CP into the blood by an ATP-dependent process. Thus, Mrp can take through-put molecules from the apical side or molecules that diffuse into CP from blood and directly eject these ligands back into the blood. As a consequence, in Mrp1 KO mice, etoposide (a chemotherapeutic ligand for Mrp1) from blood reaches a concentration in CSF ten times higher than in control mice in distribution studies. In both control and KO mice, the plasma concentration of etoposide is comparable (28). Similarly, in Mrp4 KO mice, the CSF concentration of topotecan (a chemotherapeutic ligand for Mrp4) is ten times higher than in control mice (30).

DIRECTIONAL TRANSPORT OF LIGANDS THROUGH CP INVOLVING TWO OR MORE STEPS (Table I)

Methyltetrahydrofolate

As noted above, in rodents, MeTHF is transferred from blood into CSF by a complex 3-tiered concentrating system with CSF having a ~4 times higher MeTHF concentration than plasma (Fig. 2) (9). There is also compelling evidence that the CP is the major route for MeTHF entry into human CSF and brain. Homozygous KO humans with absent PCFT genes have a very low CSF MeTHF concentration and suffer severe neurological defects (9). Similarly, children with homozygous FRα receptor KO have a deficient CSF MeTHF concentration and develop devastating epilepsy, mental retardation and movement disorders in their second year (33). It is crucial to note that FRα is present in CP but not in BBB (9). Moreover, mice with FRα KO lack viability and are reabsorbed in utero (9).

Thus, in both animals and humans, the evidence for the CP MeTHF transport system (Fig. 2) and its critical importance is very strong. Further strengthening the importance of the CP folate transport mechanism is the observation that massive daily doses of reduced folates can ameliorate both PCFT and FRα KO patients' signs and symptoms (9,33). Apparently, enough folate can enter brain by either RFC and/or simple diffusion if sufficiently large doses are given. Finally, although RFC is a bidirectional facilitated diffusion transporter (Fig. 1), *in vivo*, its primary (actual) direction for net MeTHF transport is into CSF (Fig. 2).

Methotrexate

Unlike MeTHF that is transported *into* CSF and brain, methotrexate, an important chemotherapeutic drug, is transported *out of* CSF by a concentrating system in CP (6). This system both *in vivo* and *in vitro* in the isolated CP is probenecid-sensitive (6). The most likely route of transport (Figs. 1 and 2) is methotrexate uptake into CP from the apical side by RFC (K_T~20 μM) and subsequent release into the blood on the basolateral side by ATP-requiring Mrp4 (K_T~0.2 mM) (Fig. 2). Methotrexate, in contrast to MeTHF, has relatively low affinity for FRα (K_T~1.0 μM *vs* K_T~0.02 μM for MeTHF) (34). When the extant data for methotrexate transport via CP are reviewed against the required data in Table III, conclusive data for the RFC-Mrp4 transport hypothesis in CP are missing. For example, although KO mice for Mrp4 exist, the effect on the distribution of methotrexate in Mrp4 KO mice *versus* wild-type mice is unknown. Similarly, other important data required in Table III are not extant. However, it is unlikely that RFC KO mice can be created, since RFC is an essential transport system for many cells in the body. Clearly, much more work has to be done to prove the methotrexate CP vectorial transport hypothesis (Fig. 2).

Leukotriene C_4

LTC_4 is synthesized in brain by LTC_4-synthetase with a CSF concentration of ~0.1 nM (35). LTC_4 does not penetrate into the CNS from blood (35). Children born with absent CNS LTC_4-synthetase activity and no detectable LTC_4 in CSF have a severe developmental disorder with devastating neurological symptoms and die within the first year (35). However, in normal rodents, LTC_4 is rapidly cleared from CSF and brain *in vivo* by a probenecid-sensitive process (3). *In vitro* in the isolated CP, LTC_4 is vigorously accumulated by a probenecid-sensitive energy-requiring transport system, not by binding or intracellular metabolism. Accumulated LTC_4 is readily released from the CP *in vitro* (3). It is worth noting that CP contains a high concentration of LTC_4-synthetase (36) but apparently no 5-lipoxigenase. Therefore, LTC_4 can only by synthesized in CP if there is a source of the precursor LTA_4, e.g., from white blood cells.

The most likely route of transfer of LTC_4 from CSF through the CP membranes is via apical Oatp3 and basolateral Mrp1 (Fig. 2). Oatp3 has a K_T for LTC_4 > 1 μM. However, Mrp1 (unlike Mrp4) has a very high affinity for LTC_4 (K_T~0.1 μM) and would presumably pump LTC_4 out of CP into blood by a probenecid-sensitive mechanism quite efficiently (18,37). In fact, Mrp1 has the highest affinity for LTC_4 of all molecules tested. Consequently, the efficiency argument is very strong support for Mrp1 transporting LTC_4 out of CP. However, it is possible that *in vivo* other molecules compete for Mrp1 transport and lower the apparent efficiency. Data for the distribution and clearance of exogenous and/or endogenous LTC_4 in extant Mrp1 KO mice (expected to have an elevated CSF concentration of LTC_4) would bolster the argument for a role of Mrp1 in transporting LTC_4 in CP.

Nucleosides

Both ribo- and deoxyribonucleosides in blood equilibrate with these nucleosides in CSF and presumably the extracellular space of brain via equilibrative transporters at the BBB (1). The concentrations of plasma and CSF ribonucleosides and deoxyribonucleosides are tightly controlled *in vivo*. There is also a powerful active transporter (Cnt3) at the CP apical membrane that transports both ribo- and deoxyribonucleosides into CP (K_T ~5–20 μM) from CSF; then these nucleosides are released at the basolateral membrane by the equilibrative transporter 1 (Ent1), which is sensitive to inhibition by nitrobenzylthioinosine (NBTI) as noted above (Fig. 2) (1,31). Shown in Fig. 3 is thymidine uptake by the isolated choroid plexus in artificial CSF containing 1 μM thymidine with and without 1 μM NBTI (inhibitor of Ent1) in the CSF medium (M) (38). This uptake is not related to metabolism or binding in CP. As can be seen, with efflux blocked by 1 μM NBTI (Fig. 3), there is steady linear uptake of thymidine by a very powerful pump (Cnt3); in fact, with efflux blocked, the pump concentrated thymidine in the *in vitro* CP~65 times at 15 min (38). *In vivo* uridine and other ligands in CSF (transported by Cnt3) not only inhibit thymidine transport but are also vigorously accumulated by the CP *in vitro* (1). The exact role of the CP active sodium-dependent efflux nucleoside transport system is not clear. It probably regulates nucleoside concentrations in CSF and brain and prevents excess nucleosides in CSF from building up due to over-production in brain by thymidine synthetase, ribonucleotide reductase, hypoxanthine-guanine phosphoribosyltransferase and other enzymes (1).

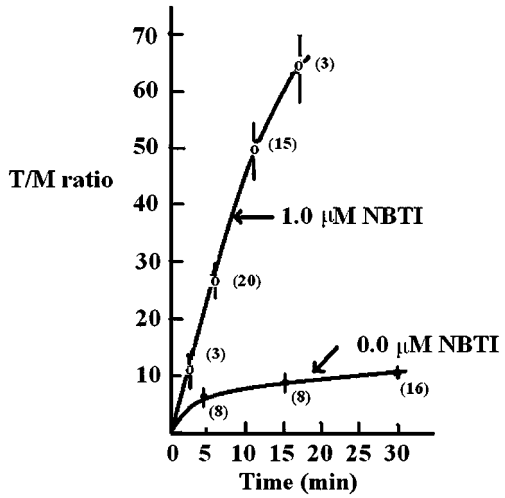

Fig. 3 Uptake of (^{3}H)thymidine by isolated CP as a function of time and inhibitory nitrobenzylthioinosine (NBTI) concentration. Rabbit choroid plexuses were incubated for various times in 3 ml artificial CSF containing 1 μM (^{3}H)thymidine at 37°C under 95 % O_2: 5 % CO_2 in a metabolic shaker with or without 1 μM NBTI in the medium. At the end of the incubation, the tissue (T)-to-medium (M) ratios (T/M) were determined and are shown as a function of time; the reader is referred back to the *Nucleosides* subsection and Fig. 2 for complementary information. With both 1 μM thymidine and 1 μM thymidine plus NBTI in the medium, greater than 95% of the (^{3}H) in CP was (^{3}H)thymidine at 15 min of uptake.

Thiamine monophosphate (TMP)

Roughly two-thirds of thiamine in plasma and CSF is TMP. When the isolated CP is incubated in thiamine-containing artificial CSF, it picks up thiamine by an active transport system and then phosphorylates and releases it (presumably on the apical side) as mainly TMP via RFC ($K_T \sim 25$ μM) (8). This is presumably a source of TMP in CSF (Fig. 2). The exact reason for TMP in CSF is unclear, but we hypothesize that thiamine enters and leaves CP and brain cells by one mechanism and that TMP enters and leaves by a separate mechanism (RFC) (8). This bifunctional system guarantees adequate thiamine for intracellular requirements with the RFC–TMP system acting as a buffer/reservoir.

Prostaglandins (PG)

In vivo, prostaglandins are not metabolized in the CNS but instead are rapidly cleared from the CSF by a probenecid-sensitive system (2). *In vitro*, PGs are accumulated in the isolated CP by a probenecid-sensitive, Na^+- and ATP-dependent system. PGs have high affinity (~0.05–0.1 μM) for both Pgt and OatpD, two facilitated diffusion systems present apically in CP. Since Pgt efficiently exchanges lactate for PG, it is primarily an inward-directed transporter, since the lactate concentration is generally higher intracellularly (Fig. 2) (39).

PGE_1 and PGE_2 both have a $K_T \sim 2$ μM for Mrp4, which almost certainly transports intracellular PGs out of CP basolaterally. The best current model is that PGs in CSF are transported apically into CP by Pgt and/or OatpD and then out (basolaterally) to the choroidal interstitium by Mrp4. It is the lower affinity Mrp4 that requires energy, is probenecid-sensitive and has a much lower affinity than the apical transporters—thus determining the *in vitro*/*in vivo* CP kinetic behavior where the K_T is ~20 μM for PGE. Many of the critical experiments in Table III have not yet been carried out to prove this hypothesis (e.g., measuring PG transport from CSF to blood in Mrp4 KO mice). Such experiments are readily possible in principle.

Digoxin

Digoxin, a drug used to treat heart conditions, is efficiently kept out of brain by P-glycoprotein (ABCB1)-mediated efflux at the BBB. However, in P-glycoprotein KO mice or animals born without P-glycoprotein activity (e.g., Collie dogs), digoxin readily enters/accumulates in brain (13). A leading hypothesis is that Oatp2—present on the basolateral CP and both sides of brain capillaries—is responsible for digoxin entry/accumulation into brain and CSF in P-glycoprotein KO animals with compromised extrusion capacity. Oatp2, as noted above, is a facilitated diffusion transporter with substantial affinity for digoxin (0.2 μM). This working hypothesis remains to be proven.

DISCUSSION

In this Review, we emphasize six general points. First, it is now possible to devise convincing scientific criteria (Table III) to study and establish vectorial transport in CP. Second, perhaps not surprisingly, there are different transporters on both the apical and basolateral CP membranes for some single ligands, unlike the situation with the glucose transporter (glut 1) at the BBB (9). Third, both CP and the endothelium at the BBB have different transport duties, although some overlap (e.g., Mrp4). Fourth, the mechanisms and/or affinities of these transporters differ greatly. Moreover, some facilitated diffusion transporters even have directionality *in vivo*. Fifth, knowledge of vectorial ligand transport in CP and brain has important implications for neurochemistry and neuropharmacology. Finally, there are polymorphisms, including KO animals and humans, for some of these transporters with clinical consequences.

In recent decades, we have systematically increased our knowledge of the transport and distribution of endogenous and exogenous substances in brain and CSF. For the nutrient transporters, such as the glucose transporter at the

BBB (glut1) and the AA transporter at the CP (SVCT2) (9), this has been relatively straightforward because these transporters are quite specific in their ligand preferences. Thus, one can study a specific ligand *in vivo* and not worry too much about promiscuity. For the promiscuous transporters in Table IV and Fig. 1, especially where vectorial transport of a ligand is being studied, strict criteria are needed to draw reasonable conclusions (Table III) with some certainty. Even high efficacy (Table III) provides only relatively strong evidence (since it is always possible that an unknown ligand with affinity for the putative transporter exists *in vivo*). Moreover, it is often difficult to find ligands specific for the transporters.

As noted above, in several of the examples discussed (Fig. 2), there are missing data (e.g., with LTC_4) for vectorial transport through CP. Unfortunately, we do not know what happens to LTC_4 distribution in Mrp1 KO mice (Table III). When these data become available, we can be more certain that our tentative conclusions (Fig. 2) are correct. However, in some cases, we can be almost certain that the models proposed in Fig. 2 are correct. For example, the CP vectorial transport systems for MeTHF (which concentrates MeTHF in CSF for use by brain) is necessary for brain function, since human homozygous KOs of FRα or PCFT lead to devastating, indeed fatal, neurological syndromes (9,33). Transport through the BBB cannot prevent the neurological damage unless massive doses of reduced folates are given.

It is interesting that the protective and nourishing roles for brain are split between the BBB and CP of the BCSFB. Certain critical transport functions occur at the BBB (e.g., macronutrient transport), whereas others occur at the BCSFB (e.g., micronutrient transport including AA and MeTHF (9), and probably LTC_4 efflux transport). However, together these two barriers work in concert to both protect and nourish the brain in a stable environment. As noted above, the systems involved in CP vectorial transport are very complex. At present, although one can often make a reasonable hypothesis about vectorial transport, to be certain about a specific ligand, one must measure its characteristics using the rigorous criteria outlined in Table III. Moreover, for many molecules, there will be overlapping specificities (e.g., digoxin and methotrexate as described above). The challenge then becomes to identify which is the dominant pathway *in vivo*, i.e., is it at the BBB or CP? If at the BCSFB, what transporters are involved?

Perhaps not surprising, some of the facilitated diffusion transporters turn out to have directionality *in vivo* (Fig. 1 *vs.* Fig. 2). For example, Pgt seems to transport PG into cells since it can exchange lactate for PG (39). Lactate is normally present at higher concentrations inside cells than outside (39). Thus, the lack of dependence on ATP and Na (co-transport), or other evidence for primary or secondary active transport, does not rule out directionality *in vivo*.

Scientific knowledge of the specific vectorial transport systems in the blood-brain-CSF interfaces has important implications for the both normal physiology and neuropharmacology. For example, if it can be established that a specific transporter (e.g., Mrp4) is responsible for efflux transport (out of brain) of a required CNS drug, one could make blockers of that system. In the Mrp4 case, there would be substantial assurance that this blockade will not harm the patient in a mechanism-based fashion, since Mrp1 and Mrp4 KO mice develop and reproduce normally.

Finally, where studied in animals and humans, there are many genetic polymorphisms or even KOs (in some cases created) for these CNS transporters (9,33). Almost certainly, many more CP transporter polymorphisms in humans will be discovered. Consequently, many humans will have large variability in ligand and drug distribution in the CNS. We predict that future studies of the BCSFB will explain much of the variability in human responses to neurotoxic agents.

Undoubtedly, there are many more transporters to be found and characterized in CP for complete characterization of vectorial transport at the BCSFB. Indeed, the CP is an exceedingly complex organ with its ability to metabolize, synthesize (e.g., transthyretin), and unidirectionally and vectorially transport molecules with so many diverse and complicated systems. However, notwithstanding the complexity, the future is bright for the neurochemistry and neuropharmacology of the CP-CSF system since the questions raised in this Review are now in principle answerable. The answers have important toxicological and therapeutic implications.

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