ABCA1 and Scavenger Receptor Class B, Type I, Are Modulators of Reverse Sterol Transport at an in Vitro Blood-Brain Barrier Constituted of Porcine Brain Capillary Endothelial Cells*

Received for publication, July 29, 2002, and in revised form, August 28, 2002
Published, JBC Papers in Press, August 28, 2002, DOI 10.1074/jbc.M207601200

Ute Panzenboeck, Zoltan Balazs, Andrea Sovic, Andelko Hrzenjak, Sanja Levak-Frank, Andrea Wintersperger, Ernst Malle, and Wolfgang Sattler‡

From the Institute of Medical Biochemistry and Medical Molecular Biology, University Graz, Harrachgasse 21, A-8010 Graz, Austria

The objective of the present study was to investigate the involvement of key players in reverse cholesterol/24(S)OH-cholesterol transport in primary porcine brain capillary endothelial cells (pBCEC) that constitute the BBB. We identified that, in addition to scavenger receptor class B, type I (SR-BI), pBCEC express ABCA1 and apolipoprotein A-I (apoA-I) mRNA and protein. Studies on the regulation of ABCA1 by the liver X receptor agonist 24(S)OH-cholesterol revealed increased ABCA1 expression and apoA-I-dependent [3H]cholesterol efflux from pBCEC. In unpolarized pBCEC, high density lipoprotein, subclass 3 (HDL3)-dependent [3H]cholesterol efflux, was unaffected by 24(S)OH-cholesterol treatment but was enhanced 5-fold in SR-BI overexpressing pBCEC. Efflux of cellular 24(S)-[3H]OH-cholesterol was highly efficient, independent of ABCA1, and correlated with SR-BI expression. Polarized pBCEC were cultured on porous membrane filters that allow separate access to the apical and the basolateral compartment. Addition of cholesterol acceptors to the apical compartment resulted in preferential [3H]cholesterol efflux to the basolateral compartment. HDL₃ was a better promoter of basolateral [3H]cholesterol efflux than lipid-free apoA-I. Basolateral pretreatment with 24(S)/OH-cholesterol enhanced apoA-I-dependent basolateral cholesterol efflux up to 2-fold along with the induction of ABCA1 at the basolateral membrane. Secretion of apoA-I also occurred preferentially to the basolateral compartment, where the majority of apoA-I was recovered in an HDL-like density range. In contrast, 24(S)-[3H]OH-cholesterol was mobilized efficiently to the apical compartment of the in vitro BBB by HDL₃, low density lipoprotein, and serum. These results suggest the existence of an autoregulatory mechanism for removal of potentially neurotoxic 24(S)/OH-cholesterol. In conclusion, the apoA-I/ABCA1- and HDL/SR-BI-dependent pathways modulate polarized sterol mobilization at the BBB.

In the past few years substantial evidence has accumulated for some neurodegenerative disorders being tightly coupled to lipid and/or lipoprotein metabolism in the peripheral circulation. At the same time it has become generally accepted that high density lipoproteins (HDL)¹ protect against atherosclerosis and possibly against neurodegenerative diseases by modulating sterol flux. For instance, a defect in intracellular cholesterol trafficking might be etiologically important in progressive neurodegeneration observed in Niemann-Pick type C disease (1). Studies performed in cell culture, animal models, and on human post-mortem material indicate that cholesterol is a major determinant affecting the severity of Alzheimer’s disease and the deposition of intraneuronal amyloid β (reviewed in Ref. 2). In line with this, the outcome of retrospective studies demonstrated a strong decrease in the incidence of Alzheimer’s disease and dementia for patients that were treated with statins, inhibitors of endogenous cholesterol biosynthesis (3, 4). Moreover, decreased serum HDL cholesterol and apolipoprotein A-I (apoA-I) concentrations correlate with the severity of Alzheimer’s disease (5). Several subtypes of neuropathies observed in patients suffering Tangier disease, a disorder characterized by severe deficiency or the absence of circulating HDL, further underline the importance of functional HDL metabolism for normal function of the central nervous system. Tangier disease is caused by mutations in the ATP-binding cassette transporter (ABC) A1 gene, and the absence of HDL is because of defective assembly of cholesterol and phospholipids with apoA-I (reviewed in Refs. 6 and 7).

One of the most striking differences between cholesterol metabolism in the brain and the periphery is the slow turnover of cerebral cholesterol, accounting for 0.1–1% of the turnover observed in the periphery (8). Because the blood-brain barrier (BBB) restricts exchange with plasma lipoproteins, the brain covers a major part of its own cholesterol demand by de novo synthesis (9). In addition, the integrity of the BBB itself strongly depends on cellular cholesterol homeostasis. Another major difference is a unique strategy of the brain to secrete cholesterol. The removal of excess cholesterol from the brain is partly accounted for by the conversion to the more polar metabolite 24(S)/OH-cholesterol by cytochrome P46 (cholesterol 24(S)-hydroxylase) (10) and subsequent secretion across the BBB for elimination by the liver (11). Consistent with the

¹ The abbreviations used are: HDL, high density lipoproteins; ABCA1, ATP-binding cassette transporter A1; apoA-I, apolipoprotein A-I; BBB, blood-brain barrier; DIDS, diisothiocyanostilbene-2,2-disulfonic acid; LDL, low density lipoproteins; LXR, liver X receptor; pBCEC, porcine brain capillary endothelial cells; PBS, phosphate-buffered saline; RCT, reverse cholesterol transport; RXR, retinoid X receptor; SR-BI, scavenger receptor class B, type I; HRP, horseradish peroxidase; HUVEC, human umbilical vein endothelial cells; RT, reverse transcriptase.

* This work was supported by the Austrian National Bank Grants OENB 9622 (to W. S.) and 8778 (to E. M.) and the Austrian Science Foundation (FWF) Granta SFB 007-716 (to W. S.), P14186-MED, and P15404-MED (to E. M.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Institute of Medical Biochemistry and Medical Molecular Biology, University Graz, Harrachgasse 21, 8010 Graz, Austria. Tel.: 43-316-380-4188; Fax: 43-316-380-9615; E-mail: wolfgang.sattler@kfunigraz.ac.at.

Published, JBC Papers in Press, August 28, 2002
Cholesterol and 24(S)OH-Cholesterol Transport at the BBB

conversion to 24(S)OH-cholesterol being the major pathway for the maintenance of brain cholesterol homeostasis, 24(S)OH-cholesterol levels are elevated in cerebrospinal fluid of Alzheimer's patients (12) and in plasma correlate with the severity of dementia (13), probably as a consequence of increased cholesterol turnover. Thus far, the underlying mechanisms that contribute to sterol transport and homeostasis in the brain and at the BBB are relatively obscure.

In contrast, in peripheral tissues many steps of the protective pathway that prevent the excess accumulation of cholesterol, a process termed reverse cholesterol transport (RCT), have been elucidated. ABCA1 has been identified as the primary gatekeeper for eliminating tissue cholesterol, because it mediates the apolipoprotein-dependent transfer of intracellular cholesterol and phospholipid to lipid-free apoA-I (14–16). Partially lipidated apoA-I matures into spherical HDL via esterification of cholesterol by plasma lecithin-cholesterol acyltransferase, and HDL particles are processed and remodeled by the combined actions of cholesterol ester and phospholipid transfer proteins and of hepatic lipase (17). Scavenger receptor class B type I (SR-BI), highly expressed in liver parenchymal cells, takes up cholesteryl esters selectively, i.e. without concomitant HDL particle endocytosis (18), and cholesterol and its catabolites are finally excreted into bile. Depending on the concentration gradient of cholesterol, SR-BI also promotes cholesterol efflux from peripheral cells to HDL but not to lipid-free apoA-I (19, 20).

The endothelial cell lineage of the BBB is able to synthesize a number of proteins exhibiting key functions during RCT. As recently reported by our group (21, 22), primary porcine brain capillary endothelial cells (pBCEC) express SR-BI, and SR-BI contributes to selective uptake of HDL-associated lipids by these cells. In addition, porcine brain endothelial cells have been reported to synthesize apoA-I (23), and apoA-I abundantly present in the central nervous system is obviously transported across the BBB (8).

With the present study we aimed to elucidate the mechanisms underlying cholesterol and 24(S)OH-cholesterol transport at the BBB by studying the functions and regulation of key players in RCT that are expressed by pBCEC, i.e. SR-BI, apoA-I, and ABCA1 (Refs. 21 and 23 this study). We investigated the regulation of ABCA1 and apoA-I-dependent cholesterol efflux by 24(S)OH-cholesterol, which, like other oxysterols, represents a specific ligand for the nuclear receptor that regulates ABCA1 expression, liver X receptor (LXR; Ref. 24). We studied the impact of SR-BI expression levels on HDL-dependent efflux of cholesterol and of 24(S)OH-cholesterol. To verify results obtained with pBCEC monolayers, we investigated polarized sterol flux in the presence of apically added acceptors and the polarized regulation of ABCA1 expression by 24(S)OH-cholesterol, using an in vitro model of the BBB (25).

EXPERIMENTAL PROCEDURES

Materials—Earle's medium M199, Dulbecco's modified Eagle's Ham's F-12 (1:1, v/v) medium, penicillin/streptomycin, gentamycin, L-glutamine, and trypsin were obtained from Biochrom (Berlin, Germany). Pronase and dispase were purchased from Sigma. Ox serum was from PAA Laboratories (Linz, Austria). Plasticware for cell culture and Transwell® inserts (polycarbonate membrane, 0.4-µm pore size) were from Costar (Vienna, Austria). [3H]Cholesterol (1.48–2.22 TBq/ mmol) was from PerkinElmer Life Sciences; [24(S)]-24(S)OH-cholesterol (2.07 GBq/mmol) was from Biotrend (Köln, Germany), and [4,24(S)]-cholesterol was from Steraloids (Newport, CT). Opti-MEM® cell culture medium was from Life Technologies (Karlsruhe, Germany). 150 nM hydrocortisone, 1% penicillin/streptomycin, and 0.35% glutamine (v/v) were added to Dulbecco's modified Eagle's Ham's F-12 medium, containing 150 mM hydrocortisone, 1% penicillin/streptomycin, and 0.35% glutamine (v/v). After 1–2 days of induction, dishes with 300–1000 cells/cm² were used for experiments.

Isolation of Lipoproteins and of ApoA-I—Human apoE-free HDL₃ and low density lipoproteins (LDL) were prepared by density gradient ultracentrifugation of plasma obtained from normalplasmic donors who have donated plasma in a TL120 tabletop ultracentrifuge (350,000 × g; Beckman Instruments, Vienna, Austria) (27). Lipoproteins recovered by direct aspiration were desalted by size-exclusion chromatography on PD-10 columns and purified via SDS-PAGE and identification of apolipoproteins. ApoA-I was isoalted as described (28).

SDS-PAGE and Immunoblotting—SDS-PAGE was performed on 10% or 12% acrylamide gels (29). We studied the impact of ABCA1 expression, liver X receptor (LXR; Ref. 21) on the maintenance of brain cholesterol homeostasis, 24(S)OH-cholesterol, using an

http://www.jbc.org/
1 × PCR buffer, and 1 unit of Finnzyme DyNAzyme II DNA polymerase. The reaction mix was heated at 94 °C for 4 min, and amplification was carried out for 35 cycles (denaturation, 30 s at 94 ℃; annealing, 30 s at 57 ℃; extension 1 min at 72 ℃). Oligonucleotide primers used for amplification of ABCA1 are as follows: forward primer 5'-GCTTCA-GATGCTCCGAAAGCTTCTT and reverse primer 5'-GACAATTAAGACA- CACACGCTGTTAG (MWG Biotech; Ebersberg, Germany). A 609-bp fragment was obtained. cDNA for ABCA1 was amplified using human ABCA1-specific primers. Oligonucleotide primers used for amplification of apoA-I are as follows: forward primer 5'-CTGACCTTGCTG-TGCTCT and reverse primer 5'-acatcttcggtgactcctg (MWG Biotech; Ebersberg, Germany). A 410-bp cDNA fragment was obtained. RT-PCR products were separated on 1% agarose gels.

Cellular Cholesterol and 24(S)OH-Cholesterol Efflux Studies—Efflux of cellular sterols was analyzed as described previously (30). In brief, sterol pools of pBCEC monolayers were metabolically labeled in medium containing 10% ox serum and 0.5 M sodium containing 10% ox serum and 0.5...
ApoA-I-dependent cholesterol efflux from pBCEC is mediated by ABCA1 and regulated by the LXR ligand 24(S)-OH-cholesterol. A, [3H]cholesterol efflux from pBCEC monolayers in the presence of increasing concentrations of apoA-I was determined at 6 h, subsequent to a 16-h treatment in the absence ("basal") or presence of 10 μM of either or both the nuclear receptor ligands 9cis-retinoic acid (9cis-RA) or 24(S)-OH-cholesterol (24(S)OH-C), as described under “Experimental Procedures.” Asterisks denote values statistically different from basal conditions, as assessed by two-way analysis of variance (*, p < 0.05; ***, p < 0.0005). B, protein expression of ABCA1 (~250 kDa) and LXRα (~57 kDa) in response to a 16-h treatment with the indicated concentrations of 24(S)-OH-cholesterol was determined by immunoblotting of proteins from whole cell lysates (30 μg of total protein/lane). C, pBCEC were incubated in the absence or presence of 24(S)-OH-cholesterol (10 μM, 16 h), and cellular [3H]cholesterol efflux was then determined after a 6-h incubation in the presence of apoA-I (10 μg/ml) and increasing concentrations of DIDS (0–0.5 mM; *, p < 0.05; **, p < 0.005; ***, p < 0.0005). From pBCEC was relatively high as compared with RBE4 cells (2.9 ± 0.6% versus 0.9 ± 0.12% for pBCEC and RBE4, respectively; data for RBE4 not shown). Since, in contrast to pBCEC, RBE4 did not secrete apoA-I (as determined by immunoblotting, data not shown), it is reasonable to assume that endogenous secretion of apoA-I contributes to cholesterol removal from pBCEC under basal conditions.

In line with increased cholesterol efflux to lipid-free apoA-I, pretreatment of pBCEC with 24(S)-OH-cholesterol induced the expression of ABCA1 protein (4- and 6-fold, at 2.5 and 10 μM 24(S)-OH-cholesterol, respectively) and of LXRα (3- and 4-fold, at 2.5 and 10 μM 24(S)-OH-cholesterol), as determined by densitometric evaluation of immunoblots (Fig. 2B).

To support the possibility that ABCA1 is responsible for apoA-I-dependent cholesterol removal, we tested the effect of the ABC transporter inhibitor DIDS that dose-dependently inhibited both basal (27.5 ± 12.9% inhibition) and 24(S)-OH-cholesterol-induced (51.3 ± 6.7% inhibition) [3H]cholesterol efflux (Fig. 2C). It is important to note that DIDS inhibits cholesterol efflux in the absence of exogenous apoA-I, indicating that the ABCA1 pathway in pBCEC is constitutively active. By contrast, the specific P-glycoprotein inhibitor PSC833 (0.1–10 μM) failed to inhibit [3H]cholesterol efflux (data not shown), confirming that P-glycoproteins are not involved. These results together are consistent with a major role for ABCA1 in apoA-I-mediated cholesterol efflux from pBCEC, a process that is regulated by 24(S)-OH-cholesterol via an LXR-dependent pathway.

HDL₃-mediated Cholesterol Efflux from pBCEC Relates to SR-BI—In addition to ABCA1 and apoA-I, SR-BI plays a major role in RCT. SR-BI mediates selective uptake of HDL-associated lipids, but it may also mediate HDL-dependent cholesterol efflux from peripheral tissues, presumably depending on the gradient of the chemical potential of the lipid between cell surface and acceptor particle (19). The expression of SR-BI and its ability to mediate selective uptake of HDL-associated lipids in pBCEC has recently been reported by our group (22). Here we analyzed HDL₃-mediated cholesterol efflux from pBCEC and found an efficient dose response (Fig. 3A), which was not yet saturated at the highest HDL₃ concentration used (100 μg of protein/ml), comparable with what has been reported for other endothelial cell types (32). In contrast to apoA-I-dependent cholesterol efflux (Fig. 2A), pretreatment of pBCEC with 24(S)-OH-cholesterol did not affect HDL₃-dependent cholesterol efflux, despite that SR-BI protein expression was decreased by 50% (Fig. 3A, inset).

In order to evaluate a potential role of SR-BI in HDL₃-dependent cholesterol removal from pBCEC, SR-BI was overexpressed using an adenoviral approach. Transfection of pBCEC with a control virus (containing the human β-galactosidase reporter gene) was without significant effect on HDL₃-mediated cholesterol efflux (Fig. 3B), whereas adenoviral transfection with SR-BI resulted in a 5.5-fold enhancement as compared with non- or mock-transfected cells (Fig. 3B). Densitometric evaluation of immunoreactive protein bands revealed that SR-BI expression was increased 8-fold after adenovirus transduction (inset to Fig. 3B) indicating that HDL₃-dependent cholesterol efflux from pBCEC is mediated by SR-BI.

Efflux of 24(S)-[3H]OH-Cholesterol from pBCEC Monolayers—To date little is known about the mechanism(s) underlying transport of the brain-specific cholesterol metabolite 24(S)-OH-cholesterol across the BBB. Thus, in analogy to cholesterol efflux experiments, experiments were performed using radioactively labeled 24(S)-OH-cholesterol. Initially, the most potent acceptors for the oxysterol were assessed by time and dose-response studies (Fig. 4). In contrast to what was
found for cholesterol efflux, apoA-I only slightly promoted the efflux of 24(S)-[3H]OH-cholesterol over the relatively high amount mobilized already in the absence of an acceptor (control, 18.5 ± 1.8% of total radioactivity at 3 h; Fig. 4). The addition of HDL 3 led to a further, time- and dose-dependent increase of cellular cholesterol efflux (33.5 ± 1.2 and 60 ± 2.3% of the total radioactivity; 5 and 50 μg/ml total HDL 3 protein/ml corresponding to 10 and 100 μg/ml HDL 3, respectively; Fig. 4). The acceptor properties of LDL (not shown) appeared to be at least as efficient as HDL 3 with 81.1 ± 0.7% 24(S)-[3H]OH-cholesterol efflux (3 h, 50 μg/ml LDL-protein corresponding to 250 μg/ml total LDL). By contrast, fatty acid-free bovine serum albumin (not shown) reached the efflux-promoting capacity of HDL 3 only at 20-fold higher concentrations, i.e., at 1 mg/ml. It thus appears that the lipid content of the acceptor particle correlates with the acceptor capacity for 24(S)-OH-cholesterol. As one would expect, human serum (5% v/v) removed the oxysterol most efficiently (100% after a 30-min incubation; not shown).

pBCEC transduced with SR-BI were used to investigate a potential role of SR-BI in HDL 3-dependent 24(S)-OH-cholesterol removal (Fig. 4B). Transfection of pBCEC with the β-galactosidase control virus was without major effect on control and SR-BI-mediated 24(S)-[3H]OH-cholesterol efflux but was enhanced 5-fold after adenoviral transduction with SR-BI. Efflux in the absence of an acceptor (control) increased in SR-BI overexpressing cells but was 7-fold lower as compared with medium containing HDL 3 as acceptor. It thus appears that comparable with cholesterol efflux (Fig. 3B), efflux of 24(S)-OH-cholesterol depends on the expression level of SR-BI.

Sterol Efflux from Polarized pBCEC in an in Vitro BBB Model—The data presented above imply major roles for ABCA1.
and SR-BI, respectively, in apoA-I- and HDL₃-mediated removal of cellular cholesterol from pBCEC monolayers. In addition we could show that 24(S)OH-cholesterol is removed highly efficiently by exogenous HDL₃, LDL, albumin, and serum. In order to elucidate the potential physiological roles of these sterol transport pathways, we next investigated sterol flux from polarized pBCEC cultured in the Transwell® system.

To study cholesterol mobilization, cells grown on Transwell® filters were labeled from the basolateral side with [³H]cholesterol and equilibrated as described under “Experimental Procedures.” The efflux rates determined after a 2.5-h incubation in the presence of medium alone (control), apoA-I, or HDL₃ in the apical chamber were comparable with the rates obtained during the corresponding monolayer experiments (Fig. 5A). Pretreatment with 24(S)OH-cholesterol (applied to the basolateral compartment) did not or only slightly enhanced apical apoA-I-dependent cholesterol efflux (variations were observed between individual experiments). Interestingly, the amount of radioactive tracer accumulating in the basolateral compartment was significantly higher as compared with the proportion mobilized to the apical compartment when apoA-I (basolateral: apical = 2.4), apoA-I after 24(S)OH-cholesterol pretreatment (basolateral:apical = 3.6), or HDL₃ (basolateral:apical = 2.7) was present as acceptor in the apical compartment. In addition, pretreatment with 24(S)OH-cholesterol resulted in a 1.7-fold enhancement of apoA-I-dependent basolateral cholesterol efflux. Thus, apical cholesterol acceptors mobilized cellular cholesterol preferentially to the basolateral compartment, and 24(S)OH-cholesterol induces cholesterol mobilization to the basolateral compartment.

In analogy to cholesterol efflux (Fig. 5A), we investigated polarized 24(S)-[³H]OH-cholesterol mobilization in Transwell® cultures (Fig. 5B). The apical acceptors HDL₃, LDL, serum, and fatty acid-free albumin most efficiently promoted the accumulation of the radiotracer into the apical compartment (between 55 and 60%). 24(S)-[³H]OH-cholesterol efflux in the presence of apoA-I was undistinguishable from control conditions (22%). Basolateral efflux, by contrast, accounted for only ~20% and did not differ significantly between the different incubation conditions. These data confirm that 24(S)OH-cholesterol (in contrast to cholesterol) is mobilized efficiently to the apical compartment by lipoprotein acceptors.

The Effect of 24(S)OH-Cholesterol on the Regulation of ABCA1 in Polarized pBCEC—Results obtained with both monolayers (Fig. 2A) and polarized pBCEC Transwell® cultures (Fig. 5A) demonstrate that 24(S)OH-cholesterol regulates the ABCA1/apoA-I-mediated removal of cellular cholesterol from pBCEC. Induction from the basolateral side, i.e. under physiological conditions representing the extracellular fluid of brain parenchymal tissue where 24(S)OH-cholesterol is formed, enhances cholesterol efflux to the basolateral compartment when apoA-I is present in the apical compartment (Fig. 5A).

We thus investigated a potential role of ABCA1 in polarized cholesterol efflux. For this, DIDS was added to both chambers during efflux experiments with apoA-I as apical acceptor (Fig. 6A). As observed in monolayer experiments, the addition of the LXR ligand 24(S)OH-cholesterol led to a significant increase in cholesterol mobilization to the apical (1.5-fold) and the basolateral compartment (2-fold). The addition of the ABC transporter inhibitor DIDS (0.5 mM) led to a significant reduction of cholesterol mobilization to the apical (22%) and the basolateral compartment (16%) under basal conditions. This effect was more pronounced in 24(S)OH-cholesterol-treated pBCEC (37 and 38% inhibition of efflux to the apical and basolateral compartment, respectively). No inhibitory effect was observed when PSC833 (10 μM) was used as inhibitor, and neither DIDS nor PSC833 inhibited cholesterol efflux when HDL₃ was added as apical acceptor (data not shown).

The next experiments were designed to study the plasma membrane distribution of ABCA1 in polarized pBCEC (Fig. 6B). ABCA1 was expressed at apical and basolateral membranes, consistent with the bi-directional cholesterol flux observed. Notably, the amount of biotinylated ABCA1 visualized on the basolateral membrane after immunoprecipitation and streptavidin-HRP detection was 3-fold induced after basolateral incubation with 24(S)OH-cholesterol (10 μM, 16 h), consistent with the high basolateral efflux rates observed for cellular

![Fig. 5. Apical sterol acceptors mobilize [³H]cholesterol from polarized pBCEC Transwell® cultures preferentially to the basolateral compartment but mobilize 24(S)-[³H]OH-cholesterol very effectively to the apical compartment. A, pBCEC were cultured on Transwell® filters and labeled with [³H]cholesterol (0.5 μCi/ml, 24 h). During the last 16 h of incubation, inducing medium containing 0.1% albumin and 24(S)OH-cholesterol (10 μM) was added basolaterally where indicated. Cholesterol efflux was determined after a 2.5-h incubation in inducing medium without additions (control) or in medium containing apoA-I (20 μg/ml) or HDL₃ (50 μg of protein/ml). Aliquots of the apical and basolateral chambers were collected and counted. Data shown are means ± S.D. from a single experiment representative of three, performed in triplicate. B, pBCEC were cultured on Transwell® filters and labeled with 0.1 μCi/ml 24(S)-[³H]OH-cholesterol as described in A. In analogy to monolayer experiments (Fig. 4) Transwell® cultures were then equilibrated for 1 h in inducing medium containing 0.1% albumin (BSA). apoA-I (20 μg/ml), HDL₃ (50 μg protein/ml), LDL (50 μg protein/ml), serum (1%, v/v), or fatty-acid free albumin (1 mg/ml) were added to the apical chamber, and 24(S)OH-cholesterol efflux was determined after 2.5 h as described for A. Data shown are means ± S.D. from a single experiment representative of three, performed in triplicate.](https://hbc.org/Downloaded from http://www.jbc.org/July 24, 2018)
cholesterol. In contrast, the expression levels of apical ABCA1 were induced to a lesser extent (1.5-fold) upon 24(S)OH-cholesterol treatment.

Taken together these data demonstrate that 24(S)OH-cholesterol induces ABCA1 at the basolateral membrane along with ABCA1-dependent basolateral cholesterol efflux and that the inhibition of ABCA1 with DIDS reduces 24(S)OH-cholesterol-induced cholesterol efflux.

Polarized Secretion of ApoA-I by pBCEC May Contribute to Basolateral Cholesterol Efflux—As shown in Fig. 1, pBCEC grown in monolayers secrete substantial amounts of apoA-I that at least partially originate from endogenous synthesis. It cannot be excluded, however, that some apoA-I may be taken up from serum HDL (prior to switching the medium to serum-free conditions) and is then re-secreted by pBCEC. Whatever mechanism prevails, it is likely that the secretion of apoA-I facilitates cholesterol efflux.

Therefore, the polarized secretion of apoA-I from Transwell® cultures was analyzed. In order to clarify whether secreted apoA-I is present in lipid-free and/or lipid-associated form, media from the apical and basolateral compartments were collected after 24 h and subjected to ultracentrifugation in KBr density gradients. Three major fractions (1.006–1.075, 1.075–1.175, and 1.175–1.235 g/ml) were collected, and proteins were precipitated and subjected to immunoblotting. As is evident from Fig. 7, the medium in the apical compartment contained only small amounts of immunoreactive apoA-I, whereas the majority was detected in the basolateral compartment. Apically secreted apoA-I was detected almost exclusively in fraction 3 (1.18–1.24 g/ml), indicating the presence of lipid-poor/free apoA-I. In contrast, basolaterally secreted apoA-I was detected predominantly in fraction 2 (1.07–1.18 g/ml), which corresponds to the density range of plasma total HDL. A smaller proportion was present in fraction 3, indicative of lipid-poor/free apoA-I. These data support an important role for apoA-I and/or HDL in basolateral cholesterol efflux.

DISCUSSION

Epidemiological, biochemical, and genetic evidence link cholesterol metabolism with neurodegenerative diseases, in particular with Alzheimer’s disease (3, 5, 33, 34). This prompted us to study sterol transport mechanisms at the polarized interface of an in vitro model of the BBB.

Cholesterol Mobilization in pBCEC Monolayers—ABCA1 expression by pBCEC is up-regulated in response to 24(S)OH-cholesterol and accompanied by elicited cholesterol efflux to apoA-I (Figs. 1B and 2). This is similar to what has been reported earlier for other oxysterols (35–38). 24(S)OH-cholesterol is a high affinity ligand for LXRα and β (39), and cerebral 24(S)OH-cholesterol concentrations can be as high as 30 μM (40). Our findings that LXRα and ABCA1 expression and cholesterol efflux are inducible by 24(S)OH-cholesterol (≤10 μM, i.e. below cytotoxic levels; Ref. 41) clearly suggest a role for this compound as endogenous LXR ligand at the BBB. These findings, together with reduced cholesterol efflux in the presence of DIDS, strongly support the notion that ABCA1 is in control of apoA-I-dependent cholesterol mobilization from pBCEC.

SR-BI is a high affinity receptor for HDL, stimulating the bi-directional transfer of lipids between HDL and cells (20). Results obtained during the present study revealed that SR-BI mediates efficient cholesterol efflux to HDLβ (Fig. 3), with a rate comparable with 24(S)OH-cholesterol-induced apoA-I/ABCA1-mediated cholesterol efflux. In other tissues, cellular cholesterol levels have been shown to regulate SR-BI expression (42, 43) via sterol regulatory element-binding protein transcription factor-binding sites (44, 45). In line with recent observations (46, 47), we have observed down-regulation of SR-BI synthesis in the presence of 24(S)OH-cholesterol. One of the physiological tasks of SR-BI at the BBB is facilitation of selective α-tocopherol uptake (22, 48), an indispensable micronutrient for proper neurological function; it is noteworthy, however, that no abnormalities in the central nervous system have been
reported for mice genetically deficient in SR-BI (49).

The relative contribution of the apoA-I/ABCA1 versus HDL/SR-BI-dependent pathway to cholesterol efflux will presumably depend on the presence of the respective acceptor particles and the availability of 24(S)OH-cholesterol. ApoA-I is synthesized and secreted by pBCEC (Fig. 1, B and C) (23), which makes the possibility of regulated cholesterol flux at the BBB even more likely. It is conceivable that 24(S)OH-cholesterol up-regulated expression of ABCA1 in pBCEC results in enhanced efflux of cellular cholesterol to extracellular apoA-I. The fact that 24(S)OH-cholesterol up-regulates and DIDS down-regulates cholesterol efflux under basal conditions indicates that this pathway is constitutively active in pBCEC and could facilitate the formation of HDL particles at the BBB.

**Cholesterol Mobilization in Polarized pBCEC Cultures**—One of the most intriguing findings of the present study is the fact that from polarized pBCEC, apical acceptors mobilized cholesterol efflux predominantly to the basolateral compartment (Fig. 6A). The underlying mechanisms of HDL-dependent basolateral cholesterol efflux are presently unclear. Preliminary results revealed predominant SR-BI expression at the apical membrane, suggesting the involvement of SR-BI during apical cholesterol efflux.2 Other unpublished results showed that HDL₃ traverses the in vitro BBB by transcytosis and could promote cholesterol efflux from the basolateral membrane.2 Recently it was demonstrated (50) that HDL, being internalized via SR-BI, undergoes a novel process of selective transcytosis, leading to polarized cholesterol transport in hepatocytes. Whether this pathway is also active in pBCEC is currently under investigation.

Our experimental settings revealed pronounced basolateral expression of ABCA1 in response to 24(S)OH-cholesterol treatment and favor an important contribution of ABCA1 to basolateral cholesterol efflux. These results are reminiscent of polarized, apoA-I-dependent cholesterol mobilization in gallbladder epithelial and intestinal cells (51–53). In our experimental system both endogenous apoA-I synthesis and apoA-I transcytosis appear to contribute to basolateral cholesterol efflux for the following reasons. (i) At the end of short time incubations in the presence of apical apoA-I, a small fraction of apoA-I was immunodetected in the basolateral compartment, incubations in the presence of apical apoA-I, a small fraction of apoA-I was present in the density range of plasma HDL, suggesting that HDL₃ traverses the in vitro BBB is the recently cloned apoA-I-binding protein (data not shown). This suggests that transcytosis is likely to occur. One candidate potentially involved in transcytosis of apoA-I across the BBB is the recently cloned apoA-I-binding protein (54). (ii) During long term incubations (24 h) in serum-and acceptor-free medium, pBCEC grown on Transwell® filters secrete substantial amounts of apoA-I preferentially to the basolateral compartment (Fig. 7), suggesting endogenous apoA-I production. The majority of basolaterally secreted apoA-I was present in the density range of plasma HDL, suggesting ABCA1-dependent formation of lipidated particles. Alternatively, lipidated apoA-I-containing particles could be assembled intracellularly. Again, it is possible that both pathways participate, probably in a similar manner as reported for hepatoma cells (55).

**24(S)OH-Cholesterol Efflux from Monolayers and Polarized Cultures**—The removal of excess cholesterol from the brain via conversion to the more polar metabolite 24(S)OH-cholesterol by cytochrome P456 is thought to represent the major pathway in brain cholesterol homeostasis (10, 11). Our results on 24(S)OH-cholesterol efflux from pBCEC (Figs. 4, 5B, and 6B) are consistent with the observation that 24(S)OH-cholesterol associates mainly with HDL and LDL in human plasma (56). The observations of the present study are in line since human serum efficiently mobilized the majority of 24(S)OH-cholesterol to the apical compartment (75% of released tracer). Interestingly, the ratio of 24(S)OH-cholesterol to cholesterol has been reported to be higher in HDL than in other lipoprotein fractions, indicating that HDL may be the preferential physiological carrier of this oxysterol (56). The fact that HDL₃-dependent 24(S)OH-cholesterol efflux was strongly enhanced in response to SR-BI overexpression supports these conclusions. In summary, these results imply that an SR-BI/HDL-dependent pathway provides a major route for efficient 24(S)OH-cholesterol mobilization across the BBB to the apical (plasma) compartment.

Our data further suggest that sterol flux at the BBB is a delicately balanced process that facilitates removal of 24(S)OH-cholesterol to the peripheral circulation, thus preventing the accumulation of neurotoxic 24(S)OH-cholesterol concentrations in the brain. In contrast, cholesterol is transported into the opposite direction via an ABCA1/apoA-I-dependent pathway that is enhanced by the LXR agonist 24(S)OH-cholesterol. The acceptor molecule, apoA-I, can originate either from the peripheral circulation or from endogenous synthesis by BVEC, facilitating the assembly of substantial amounts of HDL-like particles at the basolateral side of the BBB. Thus, in an autoregulatory fashion, the 24(S)OH-cholesterol/LXR/ABCA1/apoA-I cholesterol efflux pathway that operates in the basolateral direction may serve to produce lipiddated, HDL-like particles in the brain parenchymal fluid. These particles likely contribute to reverse 24(S)OH-cholesterol transport to the BBB. SR-BI at the apical membrane of BVEC supports the efflux of 24(S)OH-cholesterol across the BBB to plasma lipoprotein acceptors, thereby contributing to reverse sterol transport across the BBB.

**Acknowledgments**—We thank Dr. M. Vadon (Department of Blood Transfusion, LKH, Graz, Austria) for providing human plasma. We also thank B. Hirschmugl (Graz, Austria) for the expert technical assistance.

**REFERENCES**

1. Xie, C., Turley, S. D., and Dietschy, J. M. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 11992–11997
2. Sparks, D. L., Martin, T. A., Gross, D. R., and Hunsaker, J. C., III (2000) Microsc. Res. Tech. 50, 283–293
3. Wolozin, B. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 5371–5373
4. Jick, H., Zarnberg, G. L., Jick, S. S., Seshadri, S., and Drachman, D. A. (2000) Lancet 356, 1627–1631
5. Merched, A., Xia, Y., Vivvikis, S., Soret, J. M., and Sies, G. (2000) Neurobiol. Aging 21, 27–30
6. Schmitz, G., and Langmann, T. (2001) Curr. Opin. Lipidol. 12, 129–140
7. Santamarina-Fojo, S., Remaley, A. T., Neufeld, E. B., and Brewer, H. B., Jr. (2000) J. Lipid Res. 42, 1339–1345
8. Dietschy, J. M., and Turley, S. D. (2001) Curr. Opin. Lipidol. 12, 105–112
9. Turley, S. D., Burns, D. K., and Dietschy, J. M. (1998) Am. J. Physiol. 274, E1099–E1105
10. Lund, E. G., Guilleard, J. M., and Russell, D. W. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 7238–7243
11. Björkhem, I., Andersson, U., Ellis, E., Alveus, G., Ellestad, D., Diczfalusy, U., Sjovall, J., and Einarsson, C. (2001) J. Biol. Chem. 276, 37004–37010
12. Papassotiriopoulos, A., Lutjohann, D., Bagli, M., Locatelli, S., Jessen, F., Ullinghoff, R., Pot, U., Björkhem, I., Von Bergmann, K., and Heun, R. (2002) J. Psychiatr. Res. 36, 27–32
13. Lutjohann, D., Papassotiriopoulos, A., Björkhem, I., Locatelli, S., Bagli, M., Oehring, R. D., Schlegel, U., Jessen, F., Rao, M. L., von Bergmann, K., and Heun, R. (2000) J. Lipid Res. 41, 185–198
14. Bodzioch, M., Orso, E., Klucken, J., Langmann, T., Bottcher, A., Diederich, W., Oettl, B. F., Fichter, K., Ashbourne-Excoffon, K. J., Sensen, C. W., Scherer, S., Mott, S., Denis, M., Martindale, D., Frohlich, J., Morgan, K., Koop, B., Patonake, S., Kastelein, J. J., Hayden, M. R., and et al. (1999) Nat. Genet. 22, 336–345

2 Z. Balazs, U. Panzenboeck, and W. Sattler, unpublished observations.
Cholesterol and 24(S)OH-Cholesterol Transport at the BBB
ABCA1 and Scavenger Receptor Class B, Type I, Are Modulators of Reverse Sterol Transport at an in Vitro Blood-Brain Barrier Constituted of Porcine Brain Capillary Endothelial Cells

Ute Panzenboeck, Zoltan Balazs, Andrea Sovic, Andelko Hrzenjak, Sanja Levak-Frank, Andrea Wintersperger, Ernst Malle and Wolfgang Sattler

J. Biol. Chem. 2002, 277:42781-42789.
doi: 10.1074/jbc.M207601200 originally published online August 28, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M207601200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 55 references, 30 of which can be accessed free at http://www.jbc.org/content/277/45/42781.full.html#ref-list-1