22R-Hydroxycholesterol and 9-cis-Retinoic Acid Induce ATP-binding Cassette Transporter A1 Expression and Cholesterol Efflux in Brain Cells and Decrease Amyloid β Secretion*

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The ATP-binding cassette transporter A1 (ABCA1) is a major regulator of peripheral cholesterol efflux and plasma high density lipoprotein metabolism. In adult rat brain we found high expression of ABCA1 in neurons in the hypothalamus, thalamus, amygdala, cholinergic basal forebrain, and hippocampus. Large neurons of the cholinergic nucleus basalis together with CA1 and CA3 pyramidal neurons were among the most abundantly immunolabeled neurons. Glia cells were largely negative. Because cholesterol homeostasis may have an essential role in central nervous system function and neurodegeneration, we examined ABCA1 expression and function in different brain cell types using cultures of primary neurons, astrocytes, and microglia isolated from embryonic rat brain. The basal ABCA1 mRNA and protein levels detected in these cell types were increased markedly after exposure to oxysterols and 9-cis-retinoic acid, which are ligands for the nuclear hormone liver X receptors and retinoic X receptors, respectively. Functionally, the increased ABCA1 expression caused by these ligands was followed by elevated apoA-I and apoE-specific cholesterol efflux in neurons and glia. In non-neuronal and neuronal cells overexpressing a human Swedish variant of amyloid precursor protein, 22R-hydroxycholesterol and 9-cis-retinoic acid induced ABCA1 expression and increased apoA-I-mediated cholesterol efflux consequently decreasing cellular cholesterol content. More importantly, we demonstrated that these ligands alone or in combination with apoA-I caused a substantial reduction in the stability of amyloid precursor protein C-terminal fragments and decreased amyloid β production. These effects of 22R-hydroxycholesterol may provide a novel strategy to decrease amyloid β secretion and consequently reduce the amyloid burden in the brain.

ATP-binding cassette transporter A1 (ABCA1) is a member of a large superfamily of evolutionarily conserved transmembrane proteins that transport lipids, proteins, and drugs across cellular membranes (1). Mutations in the ABCA1 gene cause high density lipoprotein (HDL) deficiency syndromes, such as Tangier disease, which are characterized by the virtual absence of HDL and apolipoprotein A-I (apoA-I), cholesterol deposition in tissue macrophages, and prevalent atherosclerosis (2, 3). ABCA1 mediates cholesterol efflux and secretion of excess cholesterol from cells to lipid-free apolipoproteins and is a major determinant of plasma HDL concentration (4). Thus, ABCA1 controls reverse cholesterol transport, a metabolic pathway whereby excess cholesterol in peripheral tissues is removed and transported to the liver (5). Because of its ability to deplete macrophages of cholesterol and to raise plasma HDL levels, ABCA1 has been studied mainly for its role in the pathogenesis of atherosclerosis (5, 6). Nonetheless, ABCA1 mRNA and protein are widely distributed among multiple tissues including brain (7, 8), suggesting a more generalized role for ABCA1. Data regarding the possible physiological function of ABCA1 in the central nervous system, however, are limited. ABCA1 expression and ABCA1-mediated lipid secretory pathway are regulated by hydroxysterols and 9-cis-retinoic acid, ligands for nuclear hormone liver X receptors (LXRs) and retinoid X receptors (RXRs), respectively (9). The LXRs function as heterodimers with RXRs, and these dimers can be activated by ligands for either receptor (10). In conditions that result in high cholesterol levels, LXR activation increases the mRNA levels of several target genes primarily involved in lipid metabolism including ATP-binding transporters. Nuclear receptors LXR and RXR are also expressed in neurons and glia (7, 11).

Cholesterol plays major structural and functional role in the central nervous system. Although the central nervous system comprises less than 10% of the total body mass, it contains approximately one quarter of all of the unesterified cholesterol (12). Almost all of the central nervous system cholesterol is derived from in situ biosynthesis and is transported by lipoproteins similar to plasma HDL (13). The main apolipoproteins are apoE, produced by astrocytes (14) and microglia (15), and apoA-I from the systemic circulation or from brain endothelial cells (16). Mutations that affect the synthesis and intracellular traffic of cholesterol in neurons lead to neurodegeneration like that seen in Smith-Lemli-Opitz syndrome (17) and Niemann-Pick type C disease (18), and disturbances in brain cholesterol
metabolism may contribute to the pathogenesis of Alzheimer’s disease (AD) (19). AD is a senile dementia, characterized by extracellular deposits of amyloid β (Aβ) peptide, derived from the amyloid precursor protein (APP) cleavage (20). To generate Aβ, a small percentage of APP is cleaved by two enzymes: β-secretase-1 (21) and a still unidentified γ-secretase, producing two secreted products, Aβ and soluble APPβ (sAPPβ). Most of the APP is cut by a protease named α-secretase-generating soluble APPα (sAPPα). The residual C-terminal APP fragment (CTF-α) remains within the plasma membrane and can be cleaved by γ-secretase activity yielding p3 fragment and thus precluding Aβ production. Previous in vitro studies have shown that cellular cholesterol modulates APP processing in cell lines and primary neurons. Aβ production and secretion are reduced dramatically when cellular cholesterol levels are decreased by inhibiting de novo synthesis with hydroxymethylglutaryl-CoA reductase inhibitors (22) alone or in combination with the cholesterol-extracting agent methyl-[β]-cyclodextrin and statins (23). Furthermore, elevated cellular cholesterol levels decrease α-secretase activity (24) and increase β- and γ-secretase activity (25, 26).

To clarify further the central nervous system function of ABCA1, we first examined ABCA1 expression and transcriptional regulation in brain cells. We found high expression of ABCA1 in neurons in the cortex, basal forebrain, and hippocampus of the rat brain. We then demonstrated ABCA1 transcriptional up-regulation by established ligands for the LXRs/RXRα and a functional increase in apolipoprotein-mediated cholesterol efflux in primary neuronal and glial cultures. More importantly, we showed that in non-neuronal and neuronal cells overexpressing a human Swedish variant of APP (APPsw), ligand-activated LXRα/RXRβ, alone or in combination with ABCA1-mediated cholesterol depletion, caused substantial reduction in the stability of APP C-terminal fragments and decreased Aβ production.

MATERIALS AND METHODS

Chemicals

The following were purchased from Sigma and used in all experiments: delipidated calf serum, β-mercaptoethanol, 1-glutamine, 22R-hydroxycholesterol (22R), 9-cis-retinoic acid (RA), 22S-25hydroxycholesterol (22S), leupeptin, aprotinin, and AEBSF. We obtained 1,2-3H]cholesterol (specific activity 25 Ci/mol) from PerkinElmer Life Sciences. Tissue culture flasks and plates were from Corning (Corning, NY) and Falcon (Lincoln, NJ). 22R, RA, and 22S were dissolved in ethanol.

Antibodies

Microtubule-associated protein 2 (MAP-2) and rat integrin (CD11b) mouse antibodies (Abs) were from Chemicon International (Temecula, CA). Anti-gliial fibrillary acidic protein Ab was from Sigma. Rabbit polyclonal anti-ABCA1 was from Novus (Littleton, CO). Goat anti-ABCA1 was from Santa Cruz Biotechnology (Santa Cruz, CA). TAU-5 Ab was from NeoMarkers (Fremont, CA). Mouse anti-β-tubulin Ab was from Santa Cruz Biotechnology. The 6E10 monoclonal Ab (Signet, Dedham, MA) recognizes the first 17 amino acids of the Aβ peptide. 6E10 Ab was used for Western blotting to detect full-length APP (APPP) and sAPPα and for immunoprecipitation of total Ab. Rabbit C8 polyclonal Ab (generous gift from Dr. Denis Selkoe, Harvard University) was used to detect CTF resulting from α- or β-secretase cleavages. Secondary Abs conjugated to horseradish peroxidase and to alkaline phosphatase were from Jackson Immunoresearch (West Grove, PA). Alexa-labeled secondary Abs and Di-Ac-LDL were from Molecular Probes (Eugene, OR).

Cell Culture

Primary Neuronal Cultures—Primary neuronal cultures were made from dissociated cortices and hippocampi of 17–19-day-old Sprague-Dawley rat embryos as described previously (27). Briefly, cortices and hippocampi were dissected and incubated with 1× trypsin/EDTA (Invitrogen) for 10 min at room temperature. The trypsin was inactivated with complete defined neurobasal medium supplemented with B27, GlutaMax II, 10% horse serum, 5% fetal bovine serum, and antibiotics (medium and additives were from Invitrogen). A neuronal cell suspension was obtained after filtering through a 70-μm Falcon cell strainer (BD Biosciences, Franklin Lakes, NJ). For all experiments except immunostaining, neurons were plated at a high density (2 × 10^5/ml, 2 ml/well) on 100 μg/ml poly-n-lysine-coated six-well Costar plates (Stony Brook, NY). For immunostaining neurons were plated at low density (2 × 10^4/ml, 250 μl/well) on 200 μg/ml poly-o-lysine-coated Nunc Permanox chamber slides (Nalge, Naperville, IL). One hour after plating the medium was changed with complete neurobasal medium as above, without serum. At day in vitro 2 the neuronal cultures were treated with cytokine-β-2-arabinoside (4 μM final concentration) to suppress proliferation of non-neuronal dividing cells.

Primary Glial Cultures—Mixed glial cultures were isolated from the cerebral cortices of 21-day-old Sprague-Dawley rat embryos or newborn pups as described previously (28). The cortices were minced and dissociated by trituration in 0.25% trypsin and 0.01% DNase-I. For the addition of Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum and antibiotics (growth medium), the supernatant was passed through a 70-μm cell strainer, resuspended in growth medium, and plated in poly-l-lysine-coated T75 Falcon flasks at a density of 1.5–2.0 × 10^5 cells/flask. The mixed glial cultures were grown for 1 week before collecting the microglia and astrocytes. To isolate microglia, we cultured the cells for 7 days and then vigorously agitated the confluent cultures on a rotary shaker for 15 h (37 °C, 180 rpm). The resulting cell suspension, which was rich in microglia and oligodendroglia, was placed in T75 plastic flasks (1 × 10^7/ml, total volume of 10 ml) and allowed to adhere at 37 °C. After a 1–3-h adhering interval, loosely adhering cells (most of which were oligodendroglia) were removed by gently shaking the flasks at room temperature. The strongly adhering microglia were then released by vigorous shaking in defined medium with 0.2% trypsin, resuspended, and 15% fetal bovine serum was added. The purity of microglial cultures was assessed by the uptake of DiL-Ac-LDL according to the original protocol (28) and the manufacturer’s instructions. More than 95% of the cells harvested from the medium after shaking took up Dil-Ac-LDL. To isolate astrocytes from the mixed glial culture, we harvested the adherent astroglial cells, which were detached by tryptic/EDTA treatment. These cells were collected, pelleted by centrifugation, and resuspended in fresh growth medium. Antibodies to the following markers were used to identify the specific cell types: MAP-2 for neurons, glial fibrillary acidic protein for astrocytes, and CD11b for microglia. The purity of these enriched neuronal, astroglial, and microglial cultures as determined by the specific staining of the markers was greater than 95%. All cells were incubated at 37 °C, 5% CO₂, and 95% air. Cells were seeded in six-well plates or T25 Corning flasks, and then astrocytes and microglia were grown to 80–90% confluence before use. Astrocytes were used up to six passages.

CHOAPPsw Cells—CHOAPPsw cells (a generous gift from Dr. Ruth Perez, University of Pittsburgh) were maintained in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum and 500 μg/ml G418 (Invitrogen) as described before (29).

H4 (Human Neuroglioma) Cells—H4 cells were obtained from ATCC and transfected with pcDNA1.1HygroAPPSw. A stable cell line was established after a 1-month selection with 250 μg/ml hygromycin (Invitrogen) (30).

RNA Isolation and RT-PCR

To determine the expression of ABCA1 in neurons and glia, we used reverse transcription followed by PCR amplification (RT-PCR). Total RNA from enriched primary embryonic rat neuronal, astrocytic, and mixed glial cell cultures was isolated using RNaseasy Kit (Qiagen, Valencia, CA). RNA concentrations were determined spectrophotometrically using a DU640 spectrophotometer (Beckman). Mouse forward ABCA-1 341F (5'-TCCCCGGACGGGTCCCGGTGTT-3') and reverse ABCA-1 899R (5'-CAGCTTCTGGGCAAGGGCCC-3') primers (NCBI accession NM_013454) were used for semiquantitative RT-PCR. The primers detect ABCA1 mRNA. GAPDH (Clontech Laboratories, Palo Alto, CA) was used as an internal control. Semiquantitative RT-PCR for rat ABCA1 levels was performed using 0.5 μg of total RNA and two-step RT-PCR. The reverse transcription reaction and synthesis of the first-strand cDNA were run at 42 °C for 60 min using Advantage RT-For-PCR (Clontech) and random primer hexamer. The PCR was carried out four cycles (denaturation at 95 °C for 15 s, annealing at 95 °C for 1 min, and extension at 60 °C for 1 min) using a PCR kit (Promega) and the above ABCA1 primers. Data quantification and analysis were calculated relative to the level of the control. Each sample was assayed in triplicate during two independent experiments.
Northern Blotting

Northern blotting was performed as reported previously (30) with the following modifications. Total RNA (~5 μg) was separated on 1% denaturing agarose gel containing 2.2 M formaldehyde (Ambion, Austin, TX), transferred to NytranSuperCharge membrane (Schleicher & Schuell), UV cross-linked, and processed for detection of mRNA using NorthernMaxTM system (Ambion). A 518-bp digoxigenin-labeled antisense single stranded DNA probe was generated by asymmetric PCR amplification using a DIG Probe Synthesis kit (Roche Molecular Biochemicals). ABCA1–879R primer and 2 μl of the first strand cDNA reaction as generated for the RT-PCR. Overnight hybridization of the probe to the immobilized RNA was carried out in ULTRAhyb™ Ultrasensitive Hybridization Buffer (Ambion), and the membrane was processed in digoxigenin Wash and Block Buffer set (Roche). To verify equal loading of RNA, we stripped the ABCA1 probe from the membranes and rehybridized them with a rodent GAPDH control probe. The hybridized probe/anti-IG-alkaline phosphatase complex was visualized on x-ray film (Kodak) after incubation of the membrane with CDP-Star ultra-sensitive chemiluminescent substrate for alkaline phosphatase (Roche). The relative intensities of the hybridization signals were determined by densitometry (Molecular Dynamics, model 300A) and quantified.

Radioactive Labeling and LXR/RXR Ligand Stimulation

For cholesterol efflux studies, monolayers of cells were washed and then incubated for 24 h in complete culture medium containing 2 μCi/ml 1,2-[3H]cholesterol, antibiotics, and 10% fetal bovine serum, as described previously (30, 31). Parallel cultures incubated with nonradioactive cholesterol were used to measure and compare the amount of ABCA1 protein and its mRNA levels. After the 24-h labeling period, cells were washed and then incubated with medium plus 1% delipidated calf serum (Sigma) plus or minus the indicated ligands (10 μM 22R and 10 μM RA). After this 8-h incubation, some of the wells were washed with phosphate-buffered saline (PBS) and the cells lysed in 0.5 M NaOH. These wells provided a baseline (time zero = T0) value for total 1,2-[3H]cholesterol content for time course experiments. Unlabeled monolayers were harvested for total RNA isolation or protein analysis at different time points.

Measurement of Cholesterol Efflux

Cells containing 1,2-[3H]cholesterol were treated with LXR/RXR ligands 22R (10 μM) and RA (10 μM). These cells and vehicle control cells were washed with medium and incubated for 24 h or in the time course experiments for 4–24 h in the presence or absence of cholesterol accep-
tors (apoA-I or apoE). ApoA-I from human plasma and recombinant apoE3 (Sigma) were used at concentrations of 15–30 μg/ml. After incubation, the medium was centrifuged to remove any cells. The cells were washed and lysed in NaOH. Aliquots of medium and cell lysates were assayed by liquid scintillation counting. The percent efflux was calculated by dividing the radioactivity in the medium by the sum of the

![Image of ABCA1 expression and cholesterol efflux in primary neurons.](http://www.jbc.org/)

**A**. Northern blotting for ABCA1. Primary neurons were treated for 24 h with vehicle (C) or 22R + RA, and total RNA was isolated. Northern blotting was performed using a single stranded antisense DIG-labeled ABCA1 probe. Membranes were probed with a GAPDH cDNA probe to ensure equivalent RNA loading and its integrity. **B**. Neurons were treated as in A, lysed in lysis buffer, and Western blot analysis was performed using anti-ABCA1 polyclonal Ab. Western blotting with β-tubulin Ab was used as a loading control. Lane 1, control; lane 2, 22R and RA. **C and D**, ABCA1 mediates cholesterol efflux in primary neurons. Cells were labeled with [3H]cholesterol, and cholesterol efflux was determined after a 24-h incubation with 30 μg/ml apoA-I (C) or 15 and 30 μg/ml apoE3 (D). 22R + RA was present during the incubation with apolipoproteins. Apolipoprotein-specific efflux was determined as indicated under “Materials and Methods.” Data (mean ± S.E.) are the result of two (in C) or one (in D) experiment(s) in triplicate. ***, p < 0.001; **, p < 0.002; and *, p < 0.01 compared with control nontreated with LXR/RXR ligands. **E**, 24 h treatment with 22R + RA and 20 μg/ml apoA-I decreased cholesterol content in neurons. Cellular lipids were extracted with chloroform/methanol, and the total cholesterol (free cholesterol and cholesteryl esters) concentration was determined by enzymatic assay as described under “Materials and Methods.” The cholesterol mass was normalized to the total protein (μg of cholesterol/mg of protein) and presented as a -fold of control. n = 5, *, p < 0.05.
dependent cholesterol efflux in neurons. Were performed as in Fig. 3, Western blot (A) and quantification of ABCA1 mRNA and protein levels. Protein extracts were prepared at 4-h intervals. Northern blot (B) and Aβ. Data for Northern blotting are the result of one experiment in triplicate and for Western blotting, a single experiment.

**Measurement of Cholesterol Content**

Cells were washed in PBS and divided in half for determination of cholesterol content and for protein extraction. Cholesterol mass was determined as described previously (32) with a slight modification. Extraction was performed with chloroform/isopropanol (3:1), and after centrifugation clear supernatant was decanted. The solvent was evaporated under nitrogen, the residue dissolved, and total cholesterol (free cholesterol and cholesteryl esters) concentration determined by enzymatic assay (Infinity cholesterol reagent, Sigma) using a standard curve with cholesterol standards from Sigma. The cholesterol mass was normalized to the total protein (µg of cholesterol/mg of protein).

**Protein Isolation and Western Blotting Analysis**

For Western blot analysis cellular extracts were prepared from primary neurons, microglia, and astrocytes. Cells were washed and scraped in PBS and lysed in 10 mM Tris-HCl, pH 7.3, 1 mM MgCl₂, and 0.25% SDS, 1% Triton X-100 in the presence of protease inhibitors (10 µg/ml leupeptin, 10 µg/ml aproptin, and 10 µg/ml 4-(2-aminophenyl)benzenesulfonyl fluoride hydrochloride). Cellular extracts were centrifuged to remove debris. For ABCA1 Western blot analysis, extracts containing 20–50 µg of total protein were reduced with 2-mercaptoethanol in NuPAGE™ loading buffer (without boiling), loaded, electrophoresed on 3–8% NuPAGE™ Tris acetate gels (Novex, San Diego), and transferred to nitrocellulose membranes. ABCA1 was detected with a rabbit primary Ab raised against human ABCA1 protein. For Western blot analyses of APPfl, CTF, sAPPa, and Aβ, we used cellular extracts and conditioned media and 10–20% Tricine gels. Membranes were then incubated with a goat anti-rabbit IgG conjugated to horseradish peroxidase and processed for visualization by enhanced chemiluminescence ECL Plus™ (Amersham Biosciences) according to the manufacturer’s protocol. Western blotting with anti-β-tubulin Aβ was used as an internal standard. The relative intensities of the bands were quantified by densitometry (Molecular Dynamics).

**Immunoprecipitation and ELISA for Aβ**

Immunoprecipitation and ELISA for Aβ were performed essentially as before (33, 34). Briefly, Aβ was immunoprecipitated from the conditioned medium and immunoblotted using the 6E10 Ab. ELISA for Aβ was performed using the same 6E10 as the capture Ab and anti-Ab40 and anti-Ab42 polyclonal Abs (BioSource International, Camarillo, CA) to detect Ab₁₋₄₀ and Ab₁₋₄₂, respectively. The amount of Aβ was normalized either to the total protein or to the expression of APPfl as measured by Western blotting.

**Tissue Preparation for Immunohistochemistry**

The study fully conformed to the guidelines outlined in the Guide for the Care and Use of Laboratory Animals from the U.S. Department of Health and Human Services and was approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Young mid-age (250–275 g) Harlan Sprague-Dawley rats were deeply anesthetized with pentobarbital (Nembutal, 80–100 mg/kg; Abbott Laboratories, North Chicago, IL) and perfused transcardially with 100 ml 0.1 M PBS (pH 7.4) followed by 500 ml of 4% paraformaldehyde with 15% saturated picric acid in 0.1 M phosphate buffer (pH 7.4). After perfusion, the brain was removed and placed into the same fixative for 30 min, then immersed in 4% paraformaldehyde in neutral 0.1 M phosphate buffer at 4°C overnight. The brain was transferred to 15% sucrose in 0.1 M

Fig. 4. Time course of ABCA1 expression and apolipoprotein-dependent cholesterol efflux in neurons. Cells were treated with 22R or RA alone or combination of 22R and RA, and total RNA and protein extracts were prepared at 4-h intervals. Northern blot (A) and Western blot (B) and quantification of ABCA1 mRNA and protein levels were performed as in Fig. 3, A and B. Data for Northern blotting are the result of one experiment in triplicate and for Western blotting, a single experiment. C, time course of apolipoprotein-dependent cholesterol efflux in primary neurons treated with LXR/RXR ligands. Cells were labeled for 24 h with [³H]cholesterol, incubated for 8 h with 22R + RA and then with apoA-I (30 µg/ml) plus 22R and RA. Aliquots of the efflux medium were taken at 4-h intervals, and apoA-I-dependent cholesterol efflux was determined as indicated under “Materials and Methods.” Data are presented as a percentage (mean ± S.E.) of the total radioactivity in cells and medium; each point is an average of triplicate experiments. * < 0.05 compared with control nontreated with LXR/RXR ligands.
phosphate buffer (pH 7.4) at 4 °C for 24 h, then to 30% sucrose in 0.1 M phosphate buffer (pH 7.4) at 4 °C until it sank. The cryoprotected brain was frozen and cut in coronal plane on a cryostat (Jung CM 1800; Brodersen Instrument, Valencia, PA) at a 40-μm thickness.

**ABCA1 Immunohistochemistry**

Immunohistochemistry for ABCA1 was conducted using the free floating technique as described previously (35). Sections were pre-blocked with 10% normal goat serum and 0.1% Triton X-100 in 0.1M PBS and incubated overnight at 4 °C with a rabbit anti-ABCA1 polyclonal Ab (1:750) in 0.1 M PBS with 5% normal serum and 0.1% Triton X-100. Affinity-purified, biotinylated goat anti-rabbit IgG (1:50; Jackson ImmunoResearch Laboratories) was incubated as secondary Ab with 5% normal serum and 0.1% Triton X-100 in 0.1M PBS at 4 °C for 2 h at room temperature. Between all steps, the tissue was rinsed three times with 0.1% Triton X-100 in 0.1M PBS, for 10 min each time. The peroxidase reaction was developed by a DAB Substrate Kit (Vector, Burlingame, CA) with the addition of 2.5% nickel as a reaction intensifier. Sections were rinsed several times in 0.1M Tris-buffered saline, mounted on gelatinized slides, dehydrated in alcohols, defatted in xylenes, and placed on cover slips for analysis with an Olympus Vanox AH-2 microscope. Three sections of brain tissue from three animals were processed for immunoreactivity. To control for nonspecific immunostaining, we incubated additional sections in the absence of primary Ab or with an irrelevant secondary Ab. In both control paradigms, there was no detectable immunostaining of tissue sections.

**ABCA1 Immunostaining of Primary Neuronal Cultures**

Cells were plated on four-well poly-l-lysine-coated Permanox slide chambers. Primary neuronal cultures, plated at low density (5 × 10⁴ cells), were fixed between day in vitro 2 and day in vitro 7. The cells were fixed with 4% paraformaldehyde at room temperature for 30 min, permeabilized with 0.2% Triton X-100 for 10 min, and blocked for 1 h in PBS containing 5% bovine serum albumin, 0.2% Tween 20, and 5% goat serum. After blocking the cells were incubated overnight with primary Abs followed by a 1-h incubation with a secondary Ab. Two different anti-ABCA1 Abs were used to detect ABCA1: goat anti-ABCA1 primary Ab (Santa Cruz) and rabbit anti-ABCA1 (Novus). Secondary Abs for detection of anti-ABCA1 primary Abs were donkey anti-goat and goat anti-rabbit Alexa488-labeled secondary Abs. For MAP-2 and TAU-5 we used anti-mouse Alexa548-labeled secondary Ab. The slides were examined with a fluorescent microscope (Olympus IX-70), and images taken through a SPOT-2 CCD camera were processed using Image software (National Institutes of Health). The following negative controls were used to prove the specificity of ABCA1 immunostaining: (a) staining with primary anti-ABCA1 Ab preincubated with the blocking peptide (Santa Cruz); and (b) staining only with secondary Abs. There was no staining detected in any of the negative control experiments.

**Statistical Analysis**

Results are reported as the mean ± S.E. Statistical significance was determined by two-tailed Student’s t test (GraphPad Prism version 3.0, GraphPad Software, San Diego).

**RESULTS**

**Distribution and Localization of ABCA1 in Rat Brain**—ABCA1 immunostaining was detected throughout the adult rat brain. In the cerebral cortex, the Ab exclusively labeled neuronal cells. ABCA1-positive neurons were observed in all examined cortical layers (Fig. 1A). In these cells, localization of the immunoreaction product was restricted to the cell membrane (Fig. 1B). In subcortical regions, ABCA1 immunoreactive cells were found in the hypothalamus, thalamus, amygdala, and cholinergic basal forebrain (Fig. 1, C–F). Among thalamic nu-
clei, the reticulate nucleus displayed the most abundant cellular ABCA1 immunostaining. Large neurons of the cholinergic nucleus basalis were one of the most abundantly immunolabeled neurons. In addition, both neurophil and neuronal staining was particularly intense in the hippocampus. Dentate granule cells as well as hilar neurons showed strong ABCA1 immunoreactivity that was restricted to the cell periphery (Fig. 2, A and B), similar to the staining pattern observed in cortical neurons. The CA1 and CA3 pyramidal cells displayed even more intense immunoreaction, with some immunoreactive apical dendrites. Stratum oriens of both fields showed a dense neurophil staining (Fig. 2, C and D). These results showed that ABCA1 is expressed predominantly in the neuronal cells of adult rat brain.

**ABCA1 Expression in Primary Neurons**—To determine whether ABCA1 was transcribed in neurons, we performed Northern blotting on total RNA extracted from embryonic rat neurons. Fig. 3A (lanes 1 and 2) illustrates a band of ~8 kb corresponding to the predicted size of ABCA1 mRNA. To examine whether ligands for the nuclear receptors LXR and RXR activate ABCA1 transcription in neurons similar to what has been shown in macrophages, we treated primary neurons for 24 h with 22R and RA, which are ligands for LXR and RXR, respectively. Fig. 3A (lanes 3 and 4) showed a substantial increase in ABCA1 mRNA with this combination, which was accompanied by a marked increase in ABCA1 protein (Fig. 3B). It should be noted that we and others (30, 36) detected several bands by Western blotting, suggesting the possibility of post-translational modification with lower molecular mass species migrating at ~210 kDa.

We found that application of 10 μM 25-hydroxycholesterol, which occurs naturally in the brain (37), also increased ABCA1 mRNA and protein levels (data not shown). Previously, it was shown in macrophages and fibroblasts that increased cholesterol efflux to lipid-free apolipoproteins correlated with increased levels of ABCA1 expression. To examine the potential connection between ABCA1 expression and function in neurons, we labeled the cells with radioactive cholesterol for 24 h and induced the expression of ABCA1 for additional 8 h. Cholesterol efflux was activated with the addition of apoA-I and measured 24 h later. Because ABCA1 was shown to mediate cholesterol transport not only to apoA-I but also to other exchangeable apolipoproteins in non-neuronal cells (38), we compared apoE- and apoA-I-specific cholesterol efflux after treatment with ligands for LXR/RXR (Fig. 3D). ApoE was chosen because it is the main apolipoprotein in the central nervous system and the ApoE3 isoform, in particular, because it has the highest allelic frequency in the population. The increase in
ABCA1 protein level was concomitant with the marked up-regulation in apolipoprotein-dependent cholesterol efflux, which was consistent with increased ABCA1 functionality. The treatment with ligands for LXR/RXR increased cholesterol efflux to lipid-free apoA-I and apoE3 in a similar fashion, with apoA-I showing slightly higher efflux than apoE3 (Fig. 3, C and D). As expected, the cholesterol efflux was dependent on the exogenous apolipoprotein concentration (Fig. 3D).

Next we examined whether the increased cholesterol efflux after treatment with LXR/RXR ligands affected the cholesterol content in neurons. Fig. 3E shows that treatment with 22R + RA, and apoA-I, resulted in a small but significant decrease in cholesterol content compared with the control cells (no ligands, no apoA-I). It should be noted that ABCA1-mediated cholesterol efflux requires the addition of lipid-free/lipid-poor apolipoproteins (4), and therefore, in this experiment we compared ABCA1-dependent (cells treated with ligands and apoA-I) versus ABCA1-independent (control cells, no ligands, no apoA-I) reduction in cholesterol content. Therefore, we concluded that ABCA1 was expressed in neurons, and its expression and function were regulated by LXR/RXR ligands. We demonstrated also that stimulation of apolipoprotein-specific cholesterol efflux decreased cholesterol concentration in neurons.

Time Course of 22R and RA Up-regulation of ABCA1 mRNA, Protein Expression, and Cholesterol Efflux—We examined further the kinetics of ABCA1 mRNA expression, protein synthesis, and cholesterol efflux (Fig. 4). A 5-fold increase in ABCA1 mRNA was detected within the first 4 h after treatment with 22R and RA. There was no increase in ABCA1 mRNA after treatment with 22R or RA alone at this time point. The ABCA1 mRNA level continued to increase steadily after 22R and RA treatment, and at the 24 h time point it was more than 30-fold greater compared with the control. A 24-h treatment with any of the ligands alone resulted in a significant but lower increase in ABCA1 mRNA, consistent with previous results in non-neuronal cells, indicating that ligands for LXR/RXR act synergistically to increase ABCA1 expression (39, 40).

Immunolocalization of ABCA1 in Neurons—To characterize further ABCA1 localization in neurons, we used primary cultures of cortical and hippocampal neurons that showed high levels of ABCA1 expression. To determine in which of the nerve processes ABCA1 was localized, we costained neurons with anti-MAP-2 and anti-tau Abs. In neurons, MAP-2 was present in soma and dendrites with little or no immunostaining detectable in axons (41). Fig. 5, A and B, shows that ABCA1 and MAP-2 were expressed in the same cells, although the distribution of these two proteins differed to some extent. ABCA1 and MAP-2 colocalized in the cell body and in some of the neurites, whereas ABCA1 staining was extended to processes not immunostained for MAP-2 (Fig. 5C). To determine whether ABCA1 was present specifically in axons, we costained neuronal cultures with the anti-tau Ab TAU-5, which recognizes both the phosphorylated and unphosphorylated forms of tau.

Microtubule-associated protein tau plays an important role in axonal morphology, growth, and polarity and, in contrast to MAP-2, is localized to axons (41). As visible from Fig. 5, D and E, in day in vitro 3 neurons, ABCA1 and tau colocalized to axons and specifically to the growth cones of developing axons (Fig. 5E, inset). There was no staining with primary anti-ABCA1 Ab preincubated with the blocking peptide (data not shown). Fig. 5F is a higher magnification of 6-day-old neurons and demonstrates that ABCA1 also localized to the plasma membrane (Fig. 5F, arrow) and in intracellular compartments. Similar patterns of distribution were observed previously in HeLa cells (42). From these experiments we concluded that in neurons ABCA1 was localized to cell body, dendrites, and axons.

**ABCA1 Expression in Primary Astrocytes—**Immunohistochemistry experiments showed very little ABCA1 immunostaining of glial cells in the white matter and cortex in agreement with a previous report (8). Considering the important role of astrocytes in the formation of brain lipoprotein complexes and transport of cholesterol (43), we next examined ABCA1 expression in these cells using a semiquantitative RT-PCR method. Astrocytic cultures were prepared from 21-day-old embryos or newborn pups and used after several passages to ensure the absence of surviving neurons. Fig. 6A, lane 1, shows a 584-bp ABCA1 product generated by the two-step RT-PCR
ABCA1 expression in microglial cells—Finally, we determined ABCA1 expression in microglial cells, which express markers usually found in tissue macrophages and activated monocytes and, thus, may assume macrophage functionality in the central nervous system. Because ABCA1 is highly expressed in macrophages, we were interested in whether microglia also expressed ABCA1. Like astrocytes, microglia participate in the formation of central nervous system lipoproteins but to a lesser extent. RT-PCR using total RNA from primary microglial cells confirmed a low expression level of ABCA1 (Fig. 6A, lane 3). ABCA1 expression was also detected by Northern blotting, although the basal expression of ABCA1 was very low (Fig. 6B, lane 1). The expression of ABCA1 mRNA and protein was increased considerably after treatment with 22R (Fig. 7, B and C, lanes 3). The combination of 22R and RA resulted in a 2-fold increase of ABCA1 mRNA and protein over a single treatment with 22R, replicating the findings in neurons and astrocytes (Fig. 7, B and C, lanes 2). The increased ABCA1 expression in microglial cells correlated with the increase in apolipoprotein-specific efflux (Fig. 7D). The -fold increase after LXR/RXR ligand treatment was highest in the microglia (Fig. 7D, 5.5-fold).

Aβ secretion in CHOAPPsw cells treated with 22R and RA—Previous studies suggest that decreasing the amount of cholesterol in the cells shifts APP processing toward the a-secretase pathway (24) and reduces Aβ production in neurons (25). In contrast, high cholesterol concentration in the medium of cultured cells inhibits the secretion of APPs (44, 45). To determine whether the LXR/RXR-mediated increase in ABCA1 expression affects cholesterol concentration and consequently influences APP processing we analyzed CHO cells stably expressing the Swedish variant of APP695 (CHOAPPsw). Treatment of CHOAPPsw cells with 22R + RA resulted in significant increase in ABCA1 protein (Fig. 8B, upper panel) and did not change the steady-state level of APP (Fig. 8A, lower panel). To induce cholesterol efflux, 100 μg/ml apoA-I was added to the cells stimulated with LXR/RXR ligands, but not to control cells. After 24 h, culture medium was collected for measuring Aβ and sAPPα. Cells were washed in PBS and divided for cholesterol content measurement and protein extraction. Cholesterol mass was determined as described above and normalized to the total protein. The cholesterol content in cells treated with 22R + RA but not in control cells decreased after the addition of apoA-I (Fig. 8B, lower panel), confirming astrocytic ABCA1 expression. RT-PCR using GAPDH-specific primers generated a 960-bp product (lower panel) and showed preserved RNA integrity and equal RNA load in all samples.

Treatments with 22R (Fig. 6A, lane 3) or the combination of 22R and RA (lane 2) for 24 h resulted in a significant increase of ABCA1 mRNA. To supplement the semiquantitative RT-PCR analysis, we used Northern blotting to quantify better the transcriptional regulation of ABCA1 by oxysterols. We found a 3-fold increase in ABCA1 expression in astrocytes (Fig. 6B, lane 2) after treatment of astrocytes with LXR/RXR ligands. ABCA1 protein was also increased more than 7-fold after 22R alone (Fig. 6C, lane 4) or after a combination of 22R and RA (lane 3).

ABCA1 expression in CHOAPPsw cells after LXR/RXR ligand treatment. A, Western blotting for ABCA1 and APP after treatment with 20 μM 22R + RA. APP695 protein expression was determined by immunoblotting using 6E10 Ab. B and C, cells were treated with 22R + RA, and cholesterol efflux was initiated with 100 μg/ml apoA-I. Control cells (C-apo) did not receive apoA-I. After 24 h, medium was collected and used for determination of Aβ. Cells were washed in PBS and divided by half for determination of cholesterol content and for protein extraction. B, total cholesterol content in CHOAPPsw cells after treatment with 22R + RA. Total cholesterol was determined as in Fig. 3E and normalized to the total protein (TP). C, ELISA for Aβ1–40 was performed as explained under “Materials and Methods.” The amount of Aβ was normalized to the amount of total protein and presented as pg/ml/mg total protein. *, p < 0.05, **, p < 0.005, ***, p < 0.001 versus control. +, p < 0.005 versus 22R + RA. 22R + RA is 10 μM 22R and RA, and 2 X 22R is 20 μM 22R and RA. D, total Aβ was immunoprecipitated from the medium using the 6E10 Ab and the samples immunoblotted with the 6E10 Ab as described under “Materials and Methods.” Below is the quantification of the band intensity presented as a -fold of control after normalization to APP. Data (mean ± S.E.) are the results of triplicate determinations from one experiment.

Fig. 8. ABCA1 expression, cholesterol content, and Aβ secretion in CHOAPPsw cells after LXR/RXR ligand treatment. A, Western blotting for ABCA1 and APP after treatment with 20 μM 22R + RA. APP695 protein expression was determined by immunoblotting using 6E10 Ab. B and C, cells were treated with 22R + RA, and cholesterol efflux was initiated with 100 μg/ml apoA-I. Control cells (C-apo) did not receive apoA-I. After 24 h, medium was collected and used for determination of Aβ. Cells were washed in PBS and divided by half for determination of cholesterol content and for protein extraction. B, total cholesterol content in CHOAPPsw cells after treatment with 22R + RA. Total cholesterol was determined as in Fig. 3E and normalized to the total protein (TP). C, ELISA for Aβ1–40 was performed as explained under “Materials and Methods.” The amount of Aβ was normalized to the amount of total protein and presented as pg/ml/mg total protein. *, p < 0.05, **, p < 0.005, ***, p < 0.001 versus control. +, p < 0.005 versus 22R + RA. 22R + RA is 10 μM 22R and RA, and 2 X 22R is 20 μM 22R and RA. D, total Aβ was immunoprecipitated from the medium using the 6E10 Ab and the samples immunoblotted with the 6E10 Ab as described under “Materials and Methods.” Below is the quantification of the band intensity presented as a -fold of control after normalization to APP. Data (mean ± S.E.) are the results of triplicate determinations from one experiment.
22R + RA + apo column) and doubling the 22R + RA concentration did not produce any further increase in the amount of cellular cholesterol (Fig. 8B, 2 X 22R + RA + apo column).

To determine the effect of 22R and RA on APP processing, we measured Aβ_{1–40} secretion by sandwich ELISA. Conditioned media from CHOAPPsw cells used to determine intracellular cholesterol content were centrifuged and aliquots utilized for media from CHOAPPsw cells used to determine intracellular cholesterol content and apoA-I decreased total Aβ secretion more than 5-fold (Fig. 8D). There was no significant change in the secreted sAPPα (data not shown). These results clearly showed that in non-neuronal cells 22R and RA, ligands for LXR/RXR induced ABCA1 expression, reduced cholesterol content, and in the presence of apoA-I decreased Aβ secretion.

Role of 22R and RA on APP Processing and Aβ Secretion in Neuronal Cells—To examine whether the ligands for LXR/RXR have the same effect on Aβ secretion in neuronal cells, we used H4 human neuroglioma cells stably expressing APPsw (H4APPsw). We found that treatment of H4APPsw cells with 10 μM 22R + RA increased ABCA1 protein expression (Fig. 9A) in a manner similar to that seen with CHO cells. The increase in ABCA1 expression was accompanied by more than a 2-fold increase in cholesterol efflux to 50 μg/ml apoA-I (Fig. 9B, 22R + RA + apo column). Treatment with 22R + RA alone resulted in a small but significant increase in cholesterol efflux compared with control cells not treated with ligands and apolipoproteins (Fig. 9B, 22R + RA-apo without C-apo, p < 0.05). As expected, apoA-I alone did not change ABCA1 expression and caused a small but statistically significant increase in cholesterol efflux over control cells without apoA-I (Fig. 9, A and B, compare C – apo with C + apo). Incubation of H4APPsw cells with LXR/RXR ligands did not change the APP precursor level either with or without apoA-I (Fig. 9C). In contrast, 22R + RA considerably decreased the level of intracellular APP-CTF, which is a product of α- and β-secretase cleavage (total APP-CTF), regardless of the presence or absence of apoA-I (Fig. 9D).

To examine the effect of LXR/RXR on Aβ secretion, we immunoprecipitated and immunoblotted total Aβ with 6E10 Ab. Fig. 10A (22R + RA + apo lane) shows that 10 μM 22R + RA and apoA-I decreased total Aβ secretion more than 2-fold. 22R + RA treatment alone resulted in a small but significant decrease in total Aβ secretion (~35%, 22R + RA lane), and apoA-I had no statistically significant effect (C – apo lane). The decrease in total Aβ secretion caused by 22R + RA and apoA-I was accompanied by small but significant increase in the secretion of sAPPα (Fig. 10B, 22R + RA + apo lane), which was confirmed in multiple independent experiments. Surprisingly, 22R + RA alone increased sAPPα secretion even more profoundly (Fig. 10B, 22R + RA lane) compared with the 22R + RA treatment with the addition of apoA-I.

To determine the effect of LXR/RXR treatment specifically on the secretion of Aβ_{1–40} and Aβ_{1–42}, we applied sandwich ELISA. The levels for Aβ_{1–40} and Aβ_{1–42} were normalized to the level of APPfl and presented as a -fold of the control not treated with ligands and apoA-I (C – apo). Fig. 10, C and D, shows that treatment with 22R + RA and apoA-I produced a statistically significant decrease in Aβ_{1–40} and Aβ_{1–42}, respectively (compare C – apo and 22R + RA + apoA-I column).
Aβ secretion and sAPPα production in H4APPsw cells treated with 22R and RA. Cells were treated with 10 or 20 μM 22R + RA, and cholesterol efflux was initiated with 50 μg/ml apoA-I. A, total doubling in the 22R + RA concentration (2X + apo column) reduced Aβ_{1-40} secretion more than 3-fold (Fig. 10C) and Aβ_{1-42} more than 2-fold (Fig. 10D), thus demonstrating a concentration-dependent effect. The results from these experiments provide important evidence that ligand activation status of LXR/RXR influences APP processing by possibly two distinct mechanisms: one related to ABCA1-mediated cholesterol efflux and depletion of cellular cholesterol content, and another independent of changes in cholesterol concentration.

**DISCUSSION**

ABCA1 acts as a major regulator of peripheral cholesterol efflux and plasma HDL metabolism. Because of its ability to deplete macrophages of cholesterol and to raise plasma HDL levels, ABCA1 has been studied mainly for its role in the pathogenesis of atherosclerosis. Interestingly, approximately half of Tangier disease patients exhibit a nonspecific peripheral neuropathy that may be motor, sensory, or mixed in type (46). Morphologically, the neuropathy is characterized by lipid inclusion in Schwann cells, neuronal loss, axonal degeneration, and demyelination of peripheral nerves. Thus, we believe it is reasonable to hypothesize that ABCA1 could have an important role in neuronal functionality and viability.

ABCA1 mRNA is widely distributed among multiple tissues including brain (7). In this study, we have found ABCA1 mRNA and protein expression in neurons, astrocytes, and microglial cells from embryonic rat brain. In adult rat brain we found high expression of ABCA1 in neurons from different brain regions. Large neurons of the cholinergic nucleus basalis together with CA1 and CA3 pyramidal neurons were among the most abundantly immunolabeled cells. In all cell types examined ABCA1 expression was increased by LXR/RXR ligands. Here we have also demonstrated that these ligands increase apolipoprotein-specific cholesterol efflux in neurons, astrocytes, and microglia in parallel with the increased ABCA1 expression. In a previous study, Whitney et al. (7) reported that LXR/RXR ligands increased the expression of ABCA1 mRNA in neurons and astrocytes prepared from embryonic mouse brain. In contrast to our observations, however, they found that increased ABCA1 expression correlated with the increased cholesterol efflux in astrocytes but not in neurons. An important difference between our and their studies, however, is that we measured apolipoprotein-specific, cholesterol efflux rather than efflux without the addition of apolipoproteins. According to the current model, the first step of ABCA1-regulated cholesterol efflux depends on the association of lipid-free/lipid-poor apolipoproteins with the cell surface (47, 48). Apolipoproteins bind either directly to ABCA1 or to other receptors on the plasma membrane (49) to activate ABCA1-mediated lipid secretion. Thus, ABCA1 is unique among ABC transporters in that it requires cellular apolipoprotein interaction before lipids are translocated to its site in the membrane (4). In astrocytes the addition of apolipoproteins may be less critical for ABCA1-dependent cholesterol efflux because they secrete apoE, and LXR/RXR
lignands were shown to increase the expression of apoE (50). As mentioned above, we found that values for cholesterol efflux in astrocytes and microglia were much higher than in neurons, consistent with their role in the secretion of cholesterol and apolipoproteins and brain cholesterol transport.

Our observations suggest a possible important role for ABCA1 in the efflux, transport, and redistribution of cholesterol in the central nervous system. Cholesterol in nerve cells comes mainly from de novo synthesis. Recent data, however, suggest that the ability of central nervous system neurons to form synapses is limited by the availability of exogenous cholesterol (51). Mauch et al. (51) showed that neurons produce enough cholesterol to survive, but massive synaptogenesis requires large amounts of cholesterol and, therefore, is dependent on cholesterol production by astrocytes. In contrast to glia, neurons do not engage in secretion of brain apolipoproteins and formation of lipoprotein complexes. Instead, they express numerous low density lipoprotein receptors to acquire lipoproteins (52). Furthermore, the cholesterol produced by glia and delivered via apoE/apoA-I-containing lipoproteins to neurons may be of particular importance during neuronal injury and remodeling (43). In fact, a recent study demonstrated that ABCA1 mRNA was dramatically up-regulated in neurons and glia in areas of damage by hippocampal AMPA lesion (53). The regulation of ABCA1 expression by oxysterols through LXRs is physiologically relevant in vivo because the brain has a mechanism for the production of oxysterols, and LXR/XXRxs are expressed in neurons and glia (7, 11). Moreover, a recent study demonstrated that LXRxs have an important function in lipid homeostasis in the brain and that loss of these receptors results in severe brain abnormalities and neurodegeneration (11).

Cholesterol may also contribute to the pathogenesis of AD (54–56). Cholesterol is an important lipid that controls membrane fluidity in neurons, and its distribution throughout the membrane is not uniform: some patches of membrane termed lipid rafts contain high densities of cholesterol and are characterized by low membrane fluidity (57). There is an evidence that Aβ production is associated with lipid rafts and the probable explanations are that β-secretase-1 and γ-secretase activities are localized in rafts or that a fraction of APP is sorted to the axonal membrane of neurons and in particular to lipid rafts (25, 26). Reducing the amount of cholesterol in the membrane increases fluidity of the membrane and may shift APP processing toward the α-secretase pathway thus reducing Aβ production in neurons (22, 24). Recent genetic data have linked AD to ABCA1; carriers of a common genetic isoform of ABCA1 associated with increased HDL-cholesterol levels showed delayed age at onset of AD (58, 59). Our results reveal that treatment of both non-neuronal and neuronal cells with 22R and RA induced ABCA1 expression and increased apoA-I-mediated cholesterol efflux, consequently decreasing cellular cholesterol content. Importantly, Apo1-40 and Apo1-42 were also decreased. In contrast, treatment with ligands without apolipoproteins induced only a small increase in the cholesterol efflux. Surprisingly, in both treatments the steady-state level of CTF was reduced considerably, whereas the secretion of sAPPα increased. These results suggest that the effects of 22R and RA on APP processing might depend not only on ABCA1-mediated cholesterol efflux but on additional factors. Oxysterols regulate other genes affecting cholesterol homeostasis, including ABCG1, sterol regulatory element-binding protein processing, and hydroxymethylglutaryl-CoA reductase (60, 61). Furthermore, oxysterols and cholesterol decrease the expression of low density lipoprotein receptors including low density receptor-related protein (LRP) (62). It is important that LRP has been implicated in the pathogenesis of AD, and the absence of LRP affects APP processing (63).

It is interesting to contrast our results with those recently published by Fukumoto et al. (53), who demonstrated a modest increase in the secretion of Apo1-40 and Apo1-42 peptides derived from proteolytic processing of endogenous mouse wild type APP. It is possible that the species used in the two studies are important in the 22R-RA effects on Aβ secretion: we used CHO and human cells and Fukumoto et al. (53) used mouse cells. In addition, we examined the processing of the human Swedish mutant form and not the endogenous murine APP. Perhaps most important, we studied Aβ processing in the presence of the physiologically relevant ApoA-1. We, therefore, suggest that the role of the extracellular environment might be critical for LXR/XXR lignand effects.

In conclusion, the results presented here support the hypothesis that ABCA1 regulates cholesterol efflux in brain cells and the formation of brain lipoproteins. Accordingly, ABCA1 may prevent the accumulation of excess cholesterol in neurons by increasing the internal cycling of brain cholesterol among glial cells and neurons or by stimulating cholesterol flux out of central nervous system. Thus, ABCA1 may affect APP processing, decrease Aβ secretion, and consequently decrease amyloid burden in the brain.

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22R-Hydroxycholesterol and 9-cis-Retinoic Acid Induce ATP-binding Cassette Transporter A1 Expression and Cholesterol Efflux in Brain Cells and Decrease Amyloid β Secretion

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