Quantitation of two pathways for cholesterol excretion from the brain in normal mice and mice with neurodegeneration

Chonglun Xie,* Erik G. Lund,[1,†] Stephen D. Turley,* David W. Russell,† and John M. Dietschy[2,*]

Departments of Internal Medicine* and Molecular Genetics,† University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75390-8887

Abstract **Although the pool of cholesterol in the adult central nervous system (CNS) is large and of constant size, little is known of the process(es) involved in regulation of sterol turnover in this pool. In 7-week-old mice, net excretion of cholesterol from the brain equaled 1.4 mg/day/kg body weight, and from the whole animal was 179 mg/day/kg. Deletion of cholesterol 24-hydroxylase, an enzyme highly expressed in the CNS, did not alter brain growth or myelination, but reduced sterol excretion from the CNS 64% to 0.5 mg/day/kg. In mice with a mutation in the Niemann-Pick C gene that had ongoing neurodegeneration, sterol excretion from the CNS was increased to 2.3 mg/day/kg. Deletion of cholesterol 24-hydroxylase activity in these animals reduced net excretion only 22% to 1.8 mg/day/kg. Thus, at least two different pathways promote net sterol excretion from the CNS. One uses cholesterol 24-hydroxylase and may reflect sterol turnover in large neurons in the brain. The other probably involves the movement of cholesterol or one of its metabolites across the blood-brain barrier and may more closely mirror sterol turnover in pools such as glial cell membranes and myelin.**—Xie, C., E. G. Lund, S. D. Turley, D. W. Russell, and J. M. Dietschy. **Quantitation of two pathways for cholesterol excretion from the brain in normal mice and mice with neurodegeneration.** ***J. Lipid Res.*** **2003.** 44: **1780–1789.**

Supplementary key words Niemann-Pick type C disease • dementia • glial cells • oxysterols • cholesterol 24-hydroxylase • sterol 27-hydroxylase • neurons

The cells of all extrahepatic tissues outside of the central nervous system (CNS) continuously acquire cholesterol from two sources, de novo synthesis and the uptake of sterol carried in LDLs. In the mouse, as in other species (1), the predominant source for this sterol is de novo synthesis (~100 mg/day/kg body weight), while cellular uptake of LDL cholesterol through the clathrin-coated pit/lysosomal pathway contributes only a small amount (~5–10 mg/day/kg) to this acquisition process (2–4). Within the cells of these tissues, this newly acquired sterol is transported outwardly to the cell surface, where it apparently continuously replaces cholesterol within the bulk phase and sphingolipid-rich microdomains of the plasma membrane (5, 6). Because the pool of cholesterol in these extrahepatic tissues remains constant (~2,200 mg/kg), each day an amount of sterol equal to that newly acquired must be removed from these cells and carried by lipoproteins back to the liver for excretion from the body as either neutral, i.e., cholesterol or acidic, i.e., bile acid, sterols. While it is not entirely clear why the integrity of these cells depends upon this continuous flow of sterol from the endoplasmic reticulum and lysosomes to the plasma membrane, several lines of evidence suggest this movement may be involved in the regulation of certain proteins and in the function of a number of specific transporters on the cell surface (7–11). Because of this continuous replacement, in a species with a very high metabolic rate like the mouse, ~7–9% of the total body pool of cholesterol is turned over each day (2, 4).

Much less is understood about cholesterol turnover in the brain. As in most of the other extrahepatic organs, sterol in the brain is essentially all unesterified, but unlike in these other tissues, this cholesterol is present in two functionally distinct pools, the plasma membranes of glial and nerve cells, and the multi-layered myelin sheaths elaborated by support cells that surround the processes of neurons. While the concentration of unesterified cholesterol in most organs varies from ~1.4 mg/g wet weight (muscle) to ~5.0 mg/g (lung) (2), in the brain, these concentrations vary from ~8 mg/g (gray matter) to ~40 mg/g (spinal cord). In the mouse, the pool of cholesterol in the brain (~330 mg/kg) accounts for about 15% of the total body pool, but in the primate, over 20% of the body sterol pool is located in the CNS (12). Nevertheless, the turnover of this large pool of unesterified cholesterol within

Manuscript received 21 April 2003 and in revised form 10 June 2003.

Published, JLR Papers in Press, June 16, 2003.
DOI 10.1194/jlr.M300164-JLR200

Abbreviations: CNS, central nervous system; LDLR, LDL receptor; NPC1, Niemann-Pick type C; SR-BI, scavenger receptor class B, type I.

[1] Present address for E. G. Lund: Merck Research Laboratories, Rahway, NJ 07090.

[2] To whom correspondence should be addressed.
e-mail: john.dietschy@utsouthwestern.edu

the CNS is apparently very slow. In the mouse, for example, where 7–9% of the whole animal cholesterol pool is replaced each day, only about 0.4% of the pool in the brain is turned over (13). Similarly slow rates of sterol turnover have been reported in the brains of the baboon and human (13–15). The processes responsible for this turnover, however, are poorly understood, and it is not clear whether these overall rates of turnover apply equally to the pools of cholesterol in the plasma membranes and myelin sheaths within the CNS.

In contrast to other tissues of the body, acquisition of cholesterol within the brain appears to come entirely from de novo synthesis. With the development of methods that allowed measurement of absolute rates of synthesis in vivo (16), it was found in several species that the rate of accumulation of sterol in the developing CNS could be fully accounted for by the rate of de novo synthesis (17–20). Furthermore, there was a very close correlation between the rate of synthesis and the ultimate concentration of sterol found in different regions of the CNS (20, 21). In contrast, repeated attempts to demonstrate LDL cholesterol (or HDL cholesterol) uptake into the brain, even in the fetus before closing of the blood-brain barrier, failed to show net transfer of sterol from the plasma into the CNS (2, 21, 22). Thus, the tissues of the CNS have very high rates of cholesterol synthesis during brain development in the fetus and newborn, and these rates decline with maturation of the animal (23). However, cholesterol synthesis continues, albeit at a low rate, even after mature brain size is achieved and the pools of sterol in the CNS become constant (1, 23).

Several lines of evidence suggest that this newly synthesized cholesterol is involved in an internal circulation of sterol among different cell types within the brain. The glia and neurons of the CNS express several members of the LDL receptor (LDLR) family, including the LDLR itself and the LDLR related protein, and the cerebrospinal fluid contains a number of apolipoproteins including apolipoprotein E (apoE), apoA-I, and apoA-IV (24–26). In one study, for example, it was shown that synapse formation between neurons in vitro required the presence of glial cells; however, this requirement could be obviated by providing the nerve cells with cholesterol complexed to apoE (27). That neurons take up such apoE-cholesterol complexes is also consistent with the observation that unesterified cholesterol accumulates in these cells when Niemann-Pick type C (NPC1), a protein required for the processing of sterol entering cells through the clathrin-coated pit pathway, is mutated (28, 29).

If, as these observations suggest, sterol is being continuously synthesized within the CNS even in the mature animal where the pool of sterol in the brain has become constant, it follows that mechanisms must be available to continuously transport cholesterol across the blood-brain barrier into the plasma for excretion. In principle, such movement might involve the transfer of cholesterol itself or the movement of this sterol after hydroxylation within the CNS. The observations that the brain contains both cholesterol 24-hydroxylase and sterol 27-hydroxylase activities (30–32), and that there is net transfer of 24(*S*)-hydroxycholesterol from the brain into the venous outflow in humans (33) indicate that formation of these oxysterols may be one of the major pathways for the movement of sterol out of the CNS. With the availability of mice lacking cholesterol 24-hydroxylase activity (*Cyp46a1*$^{-/-}$) (34, 35), as well as those lacking cholesterol 7α-hydroxylase (*Cyp7a1*$^{-/-}$) and sterol 27-hydroxylase (*Cyp27a1*$^{-/-}$) activities (36, 37), it is now possible to measure the quantitative importance of each of these pathways for cholesterol turnover in the brain. The current studies, therefore, were undertaken *1*) to explore the effect of age and gender on cholesterol metabolism in the mouse brain; *2*) to measure the effect of deletion of CYP46A1 and the other sterol hydroxylases on cholesterol metabolism in different regions of the CNS; *3*) to quantitate net sterol turnover in the CNS and to measure the role of CYP46A1 in this process; and, finally, *4*) to measure the rate of net cholesterol turnover in animals with ongoing neurodegeneration and to define the role of CYP46A1 in this excretory process.

EXPERIMENTAL PROCEDURES

Animals and diets

The mice with deletion of either cholesterol 24-hydroxylase (*Cyp46a1*$^{-/-}$) or cholesterol 7α-hydroxylase (*Cyp7a1*$^{-/-}$) activity were generated as described previously (35, 36, 38). The sterol 27-hydroxylase knockout mice (*Cyp27a1*$^{-/-}$) were originally obtained from Dr. Eran Leitersdorf (37, 39). Mice with a mutation in NPC1 protein (*Npc1*$^{-/-}$) were obtained from a colony at the National Institutes of Health (28, 40) and were then bred into a mixed background of C57BL/6 and 129S6/SvEv. All genetically modified animals were maintained in the same mixed background of C57BL/6 and 129S6/SvEv, as were matching control mice (*Cyp46a1*$^{+/+}$, *Cyp7a1*$^{+/+}$, *Cyp*27*a1*$^{+/+}$, *Npc1*$^{+/+}$). The animals were fed ad libitum a low-cholesterol (0.02%, w/w), low-fat (4%, w/w) basal rodent diet (No. 7001, Harlan Teklad, Madison, WI) after weaning at the end of the third week. With the exception of one experiment in which animals were studied at ages varying from 7 to 210 days, all studies were carried out when the mice were exactly 7 weeks of age and in the fed state near the end of the 12 h dark phase of the light cycle (41). The experimental groups contained nearly equal numbers of males and females because there were no significant gender differences observed in the CNS in any of these measurements. At the termination of these experiments, the entire CNS was removed, including the spinal cord. In some studies, the CNS was divided into five regions identified as cerebrum, cerebellum, mid-brain, brain stem, and spinal cord. All experimental protocols were approved by the Institutional Animal Care and Research Advisory Committee.

Measurement of tissue cholesterol concentrations

Animals were exsanguinated from the inferior vena cava, and the CNS was removed and saponified. The cholesterol was extracted and measured by gas-liquid chromotography using stigmastanol as an internal standard (36, 42). The values were either expressed as mg of cholesterol/g wet weight of tissue (mg/g) or converted to mg of cholesterol in a specific tissue normalized to 1 kg body weight (mg/kg). The mean rate of expansion of the pool of cholesterol in the CNS at 7 weeks of age was determined by measuring the size of this pool per kg body weight in groups of mice exactly 6 and 8 weeks of age. These data were then en-

tered into a linear regression program in order to calculate the average daily accretion rate at 7 weeks expressed as mg of cholesterol accumulated/day/kg (mg/day/kg).

Measurement of cholesterol synthesis in vivo

Each mouse was injected with 50 mCi of ^3H_2O intraperitoneally. One hour later, the animals were anesthetized and exsanguinated. The tissues were removed and saponified, and digitonin-precipitable sterols were isolated as described previously. The rates of cholesterol synthesis in each tissue were determined and expressed as the nmol of ^3H_2O incorporated into sterols/h/g of tissue (nmol/h/g) (2, 36). Because the $^3H/C$ incorporation ratio was known, these rates could be converted to absolute rates of cholesterol synthesis and could also be expressed as milligrams of cholesterol synthesized in a particular organ or whole animal/day/kg body weight (mg/day/kg) (16, 43).

Measurement of mRNA expression in tissues

After exsanguination of the mice, aliquots of tissue were immediately removed and frozen in liquid nitrogen. Total RNA was prepared from tissues using the RNA Stat60 reagent (Tel-Test, Friendswood, TX). In one experiment, 5 μg of poly(A)$^+$ RNA was isolated and subjected to electrophoresis, and Northern blotting was performed as described previously (37). The amount of radioactivity in each signal was analyzed by PhosphorImaging (Molecular Dynamics). In another study, CYP46A1 mRNA was determined by an RNase protection assay using GAPDH as the internal control. Total RNA (40 μg) was hybridized with ^{32}P-labeled riboprobes. Following RNase digestion, the mRNA-protected ^{32}P-labeled probes were separated by electrophoresis, and the radioactivity in each band was quantified using a PhosphorImaging system (44). In a third series of experiments, levels of CYP46A1, ATP binding cassette transporter A1 (ABCA1), SR-BI, sterol regulatory element binding protein-1c (SREBP-1c), and SREBP-2 mRNAs were determined by real-time polymerase chain reactions using an Applied Biosystems PRISM 7900HT machine.

Immunoblot assay of CYP46A1 protein expression

Immunoblots of the CYP46A1 protein were carried out as described (34). The separated proteins were electroblotted to polyvinylidene difluoride membranes and incubated with T-623 antiserum (1:1,000 to 1:2,000 dilution of unpurified serum). A donkey anti-rabbit horseradish peroxidase-conjugated antibody (Amersham Pharmacia) was used as a secondary antibody. Visualization was via enhanced chemiluminescence kits (Amersham Pharmacia).

Calculations

All data are presented as means ± SEM. The differences between control and genetically modified animals were tested for statistical significance ($P < 0.05$) by an unpaired, two-tailed Student's *t*-test. In the figures and table, an asterisk indicates a value that was significantly different at this level from its appropriate control value.

RESULTS

Age and gender effects on brain cholesterol metabolism

Because previous studies in the mouse have shown significant age and gender effects on several aspects of sterol metabolism (41, 45), initial measurements were made in the brains of male and female mice of different ages. As shown in **Fig. 1A**, the mean concentration of cholesterol

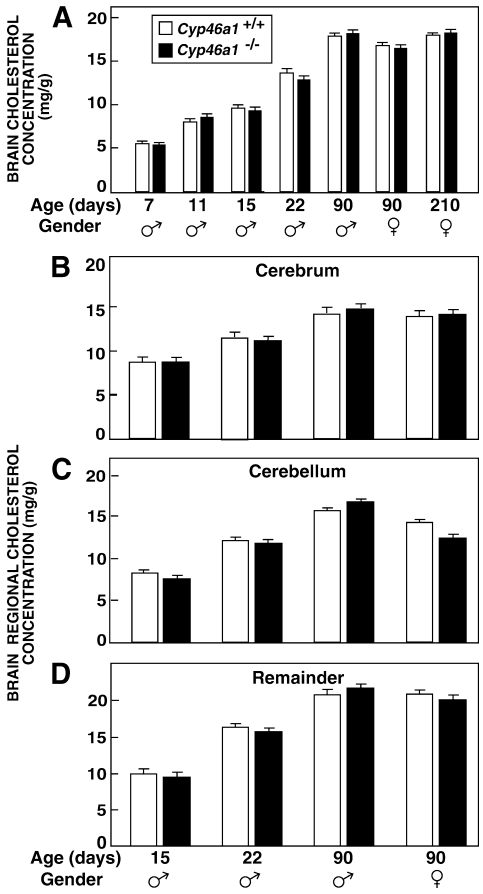

Fig. 1. Cholesterol concentration in the whole brain (A) and different regions of the brain (B, C, D) in *Cyp46a1*$^{+/+}$ and *Cyp46a1*$^{-/-}$ mice of various ages. Either male or female mice, with ages varying from 7 to 210 days, were used in these experiments. The animals were weaned onto the low-cholesterol rodent diet at 3 weeks of age. The whole brain or specific regions were removed and saponified. The cholesterol concentration was measured by gas liquid chromatography (GLC) and expressed as mg/g wet weight. Means ± SEM are shown for four to eight animals in each group.

in the whole brains of *Cyp46a1*$^{+/+}$ and *Cyp46a1*$^{-/-}$ animals increased 3-fold, from about 6 mg/g to 18 mg/g between 7 and 90 days of age. There was little further increase of these values in animals at 210 days of age. In contrast to these effects of age, there was no consistent difference in the cholesterol concentration in male and female mice or in *Cyp46a1*$^{+/+}$ and *Cyp46a1*$^{-/-}$ animals. Similar findings were observed in different regions of the CNS, as shown in Fig. 1B–D. In general, the mean concentration of cholesterol increased in every region of the brain up to 90 days of age, but there were no significant differences between these values in the control mice and those lacking cholesterol 24-hydroxylase activity or in male and female animals. Similar studies in the CNS of a large number of 7-week-old animals also revealed no differences in sterol metabolism in the brains of male and female mice. On the basis of these initial experiments, all subsequent studies were carried out in young adult mice 7 weeks of age, and all experimental groups contained equal, or nearly equal, numbers of male and female animals.

Brain cholesterol metabolism in *Cyp46a1*$^{-/-}$, *Cyp27a1*$^{-/-}$, and *Cyp7a1*$^{-/-}$ mice

Previous studies have fully characterized whole-animal cholesterol balance in mice lacking the enzymes that hydroxylate cholesterol at the 24, 27, and 7α positions (35–37). The tissue expression of the genes for these three enzymes in control animals was very different, as illustrated in **Fig. 2**. mRNA for CYP7A1 was found only in the liver while CYP46A1 was expressed principally in the brain and, to a much lesser degree, the eye and testis. In contrast, while CYP27A1 was found predominantly in the liver, significant expression was also apparent in other organs, including adipose tissue, intestine, kidney, lung, muscle, and uterus. Brain expressed essentially no mRNA for this enzyme even though sterol 27-hydroxylase activity has been described in the CNS (30, 32).

Studies were next undertaken to determine if alterations in sterol metabolism could be detected in mice lacking any one of these sterol hydroxylases. As shown in **Fig. 3A**, the mean concentration of cholesterol in the whole brain was virtually identical in the control, *Cyp46a1*$^{-/-}$, *Cyp27a1*$^{-/-}$, and *Cyp7a1*$^{-/-}$ animals and equaled about 15 mg/g. In contrast, the rate of cholesterol synthesis was suppressed about 35% in the animals lacking 24-hydroxylase activity, while there was no alteration in the rate of sterol synthesis in either the *Cyp27a1*$^{-/-}$ or *Cyp7a1*$^{-/-}$ animals. Thus, the 27- and 7α-hydroxylases that were primarily expressed in the liver profoundly affected whole-animal sterol balance (36, 37), but apparently had no physiological function in the CNS.

The distribution of CYP46A1 in different regions of the CNS is shown in **Fig. 4**. The mRNA for this gene was most abundant in the cerebrum and least abundant in the spinal cord. CYP46A1 protein levels also were relatively low in the spinal cord and appeared most prevelant in the cerebellum and mid-brain. The concentration of cholesterol and the rates of sterol synthesis in these same regions were next measured in 7-week-old animals. As seen in **Fig. 5A**, the concentration of cholesterol in the cerebrum and cerebellum averaged about 13 mg/g. These values were higher in the more myelinated regions of the mid-brain and brain stem, and reached nearly 35 mg/g in the spinal

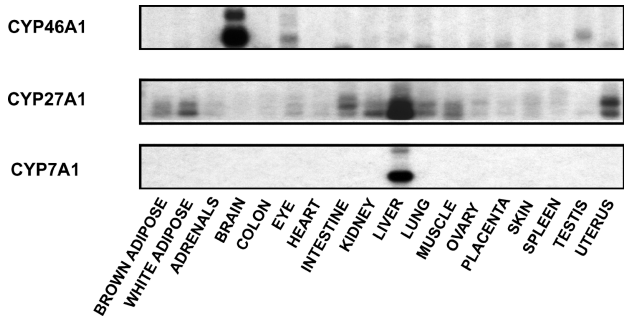

Fig. 2. Expression of CYP46A1, CYP27A1, and CYP7A1 mRNA in the organs of wild-type mice. Five micrograms of poly(A)$^+$ RNA prepared from the major organs was subjected to electrophoresis and Northern blot analysis using the ^{32}P-labeled cDNA probes indicated. The results were analyzed using a PhosphorImager.

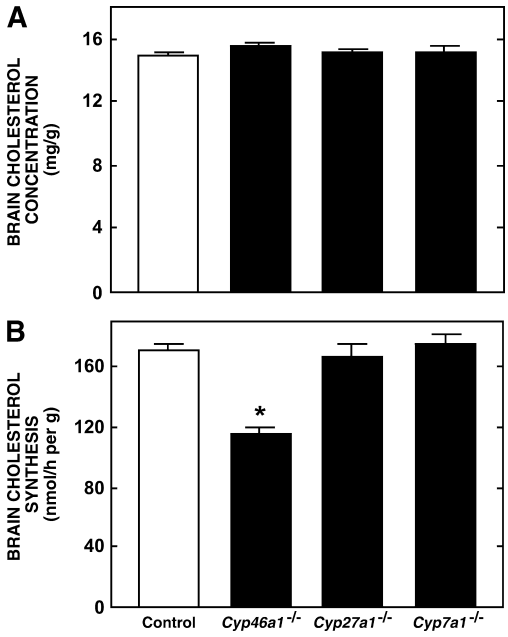

Fig. 3. Brain cholesterol concentration (A) and synthesis (B) in control, *Cyp46a1*$^{-/-}$, *Cyp27a1*$^{-/-}$, and *Cyp7a1*$^{-/-}$ mice. All animals used in this experiment were 7 weeks of age, and included nearly equal numbers of males and females. The whole brain was removed and saponified. The cholesterol concentration was measured by GLC. Sterol synthesis was determined in vivo. Means ± SEM are shown for 6 to 8 animals in each group. The asterisk (*) identifies the value that was significantly different from that in the control mice.

cord. There were no differences, however, in the concentration of cholesterol in any of these regions in wild-type versus *Cyp46a1*$^{-/-}$ animals. In contrast, cholesterol synthesis was significantly suppressed in all of these regions of the CNS except the spinal cord, as shown in Fig. 5B. As the 24-hydroxylase has been localized to neurons (34, 35), this finding is consistent with the hypothesis that this enzyme is more abundant in the neuron-rich areas of the ce-

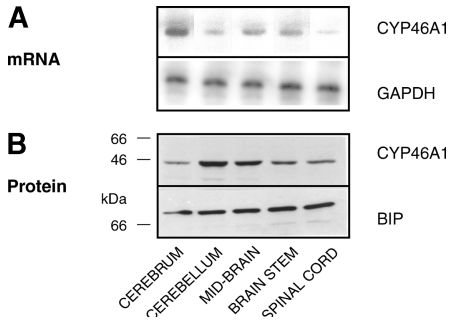

Fig. 4. Expression of CYP46A1 mRNA (A) and its product, cholesterol 24-hydroxylase (B), in five regions of the brain in wild-type mice. Forty micrograms of total RNA was prepared and hybridized with ^{32}P-labeled probes. The radioactivity was analyzed by PhosphorImaging using the signal from the glyceraldehyde phosphate dehydrogenase mRNA as an internal control. Immunoblots of the CYP46A1 protein were carried out in these same studies using the immunoglobulin heavy chain binding protein (BIP) as an internal control.

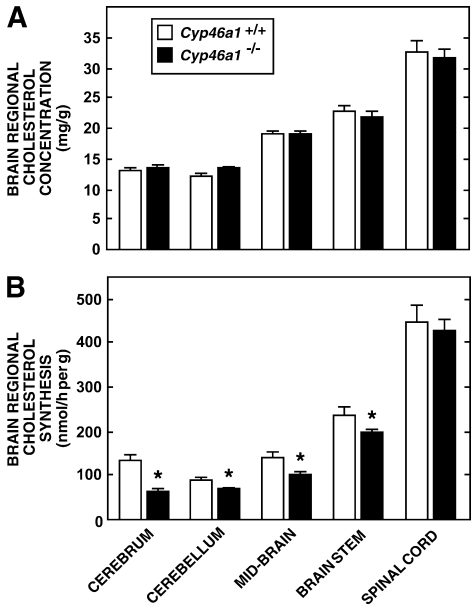

Fig. 5. Cholesterol concentration (A) and synthesis (B) in five regions of the brain in control and *Cyp46a1*$^{-/-}$ mice. All animals used in this experiment were 7 weeks of age and included nearly equal numbers of males and females. The brain was removed and dissected into five regions. The cholesterol concentration was measured by GLC, and sterol synthesis was determined in vivo. Means ± SEM are shown for six to nine animals in each group. The asterisk (*) identifies the values in the *Cyp46a1*$^{-/-}$ group that were significantly different from those in the wild-type mice.

rebrum, cerebellum, and mid-brain (Fig. 4) and is active in exporting cholesterol from these regions. Thus, loss of this activity would necessarily lead to a decrease of cholesterol synthesis in these areas, but such decreases would not be seen in the spinal cord, where CYP46A1 is less abundant and, presumably, less active.

Net cholesterol movement out of the CNS in *Cyp46a1*$^{+/+}$ and *Cyp46a1*$^{-/-}$ mice

With recognition that the 24-hydroxylase appears to play a role in the movement of sterol from the CNS, studies were next undertaken to quantitate this CYP46A1-dependent flux. Unfortunately, the pool of cholesterol in the brain of the 7-week-old mice was still expanding slightly because both brain weight and cholesterol concentration continued to increase at this age (Fig. 1). In 12 and 11 control animals, respectively, killed at 6 and 8 weeks of age, the cholesterol pool in the CNS increased from 321 ± 4 mg/kg to 332 ± 5 mg/kg. Thus, at 7 weeks of age, the mean rate of cholesterol accumulation in the developing brain equaled about 0.8 mg/day/kg (**Table 1**, column A). Based on ^3H_2O incorporation into sterols in 10 mice at 7 weeks of age, the rate of cholesterol synthesis in the whole brain was calculated to equal 2.2 mg/day/kg (Table 1, column B). As only 0.8 mg/day/kg of this newly synthesized sterol was used for brain growth, 1.4 mg/day/kg must have been moved from the CNS into the blood for excretion (Table 1, column C). The pool of sterol in the brains of these 7-week-old animals equaled 326 ± 3 mg/kg, so this rate of excretion accounted for the turnover of only 0.4% of the total pool in the brain each day (Table 1, column C). These values can be compared with those for the whole animal that excreted into the feces 179 mg of sterol/day/kg, a rate that would account for the turnover of 8.1% of the whole-animal pool of cholesterol each day (Table 1, column D).

Similar measurements were next carried out in *Cyp46a1*$^{-/-}$ animals, and are also shown in Table 1. Between 6 and 8 weeks of age, the rate of brain growth and the increase in cholesterol concentration in these knockout animals were indistinguishable from those found in the control mice. Consequently, the calculated rate of sterol accumulation in the 7-week-old animals also equaled 0.8 mg/day/kg (Table 1, column A). The rate of cholesterol synthesis in these animals, however, was determined to be 40% lower than the rate found in control animals, and was calculated to equal 1.3 mg/day/kg (Table 1, column B). Because the same amount of newly synthesized cholesterol was used to support brain growth (0.8 mg/day/kg), only 0.5 mg/day/kg was moved out of the CNS (Table 1, column C). Thus, deletion of the 24-hydroxylase activity reduced the rate of cholesterol movement out of the CNS by about 64%, from 1.4 to 0.5 mg/day/kg, and the turnover of the sterol pool in the brain was reduced from 0.4 to 0.2% per day. In contrast, there was no detectable difference in the rate of whole-animal cholesterol turnover in the *Cyp46a1*$^{-/-}$ mice compared with controls (Table 1, column D).

Effect of loss of 24-hydroxylase activity in the presence of ongoing neurodegeneration

Mice homozygous for a mutation in the gene encoding the NPC1 protein have a well-described syndrome marked by progressive neurodegeneration and death about 11 weeks after birth (40). The NPC1 protein is involved in the movement of unesterified cholesterol from the lysosomal compartment to the metabolically active pools of sterol in virtually every type of cell in the body. Thus, when NPC1 is impaired, any cholesterol entering these cells through the clathrin-coated pit pathway becomes irreversibly sequestered within the late endosome/lysosome compartment (46). As a consequence, the concentration of unesterified cholesterol increases in all tissues of the body outside of the CNS (28). Within the brain, however, this metabolic defect leads to the progressive death of some neurons and glial cells, and significant demyelination over the 11-week lifespan of the mutant mice (29, 47, 48).

The defect in sterol metabolism in the CNS was clearly apparent when net cholesterol flux out of the brain was measured in 7-week-old *Npc1*$^{-/-}$ animals (Table 1). The pool of sterol in the CNS actually decreased between 6 and 8 weeks of age, so that at 7 weeks the mean rate of change in this pool equaled −0.5 mg/day/kg, compared with the gain of 0.8 mg/day/kg found in the control mice (Table 1, column A). The rate of cholesterol synthesis in these animals was only marginally reduced (1.8 mg/day/kg) (Table 1, column B) however, so that net sterol excretion from the CNS increased to 2.3 mg/day/kg (Table 1,

TABLE 1. Net cholesterol flux out of the central nervous system and whole animal in control, *Cyp46a1*$^{-/-}$, *Npc1*$^{-/-}$, and *Npc1*$^{-/-}$/*Cyp46a1*$^{-/-}$ mice at 7 weeks of age

Animal Genotype	A: Net Expansion of the Cholesterol Pool in the CNS	B: Cholesterol Synthesis in the CNS	C: Net Sterol Excretion from the CNS	D: Net Sterol Excretion from the Whole Animal
		mg/day/kg (%/day)		
Wild-Type	0.8 ± 0.05	2.2 ± 0.10	1.4 (0.4)	179 ± 8 (8.1)
Cyp46a1$^{-/-}$	0.8 ± 0.03	1.3 ± 0.20	0.5 (0.2)	177 ± 13 (8.1)
Npc1$^{-/-}$	−0.5 ± 0.10	1.8 ± 0.08	2.3 (0.7)	152 ± 16 (2.8)
Npc1$^{-/-}$/*Cyp46a1*$^{-/-}$	−0.5 ± 0.04	1.3 ± 0.09	1.8 (0.5)	—

CNS, central nervous system. Groups of mice were killed at 6 and 8 weeks of age and the total pool of cholesterol in the CNS was measured. The average daily change in this pool was then calculated and is shown in column A. Cholesterol synthesis was measured in 7-week-old mice of each genotype and these values are presented in column B. Column C shows the calculated rate of cholesterol movement out of the CNS, while the data in column D give the rates of sterol movement out of the whole animal into the feces (35, 40). The percentage of the pool of cholesterol in the CNS and whole animal turned over each day is shown by the numbers in parentheses. Fecal sterol excretion was not determined in the *Npc1*$^{-/-}$/*Cyp46a1*$^{-/-}$ mice.

column C). Thus, in the face of ongoing neurodegeneration and demyelination, cholesterol export from the CNS was nearly doubled compared with control animals. In contrast, net sterol excretion from the whole animal remained essentially normal, while the whole-animal cholesterol pool increased nearly 2.5-fold at 7 weeks of age. As a consequence, the calculated percentage turnover rate for the whole animal (Table 1, column D) was markedly reduced.

Two experiments were done to evaluate the role of CYP46A1 in this accelerated cholesterol transport seen in the *Npc1*$^{-/-}$ mice. First, mRNA and protein levels for the cholesterol 24-hydroxylase were measured in *Npc1*$^{-/-}$ animals and were both found to be essentially unchanged compared with the control mice. Thus, the increase in sterol export seen in the mutant animals was apparently not associated with an increase in 24-hydroxylase activity. This finding was further explored in a second study, in which the *Npc1*$^{-/-}$ animals were bred with the *Cyp46a1*$^{-/-}$ mice to produce double homozygous *Npc1*$^{-/-}$/*Cyp46a1*$^{-/-}$ animals. Phenotypically, these mice were indistinguishable from the *Npc1*$^{-/-}$ animals. Both groups began to manifest gross neurological symptoms at about 7 to 8 weeks of age, and nine *Npc1*$^{-/-}$/*Cyp46a1*$^{-/-}$ mice were followed until death; all succumbed within the same time frame (79 ± 3 days) seen in the *Npc1*$^{-/-}$ animals.

The biochemical parameters of cholesterol metabolism in the *Npc1*$^{-/-}$/*Cyp46a1*$^{-/-}$ mice are shown in **Fig. 6**. The concentration of cholesterol in the *Npc1*$^{-/-}$ animals typically was minimally reduced in the gray matter of the cerebrum and cerebellum but was reduced to a greater degree in the more myelinated mid-brain compared with either control or *Cyp46a1*$^{-/-}$ animals (Fig. 6A, C, E). No additional changes in cholesterol concentration were observed in the *Npc1*$^{-/-}$/*Cyp46a1*$^{-/-}$ mice. Cholesterol synthesis was significantly higher in the cerebrum, cerebellum, and mid-brain of the *Npc1*$^{-/-}$ mice compared with the *Cyp46a1*$^{-/-}$ animals (Fig. 6B, D, F); however, these rates of synthesis were reduced to the same level found in the *Cyp46a1*$^{-/-}$ mice when the 24-hydroxylase gene was also deleted from the *Npc1*$^{-/-}$ animals.

These features of sterol metabolism also could be identified when net cholesterol flux across the CNS of the *Npc1*$^{-/-}$/*Cyp46a1*$^{-/-}$ animals was measured. Net cholesterol loss from the CNS equaled −0.5 mg/day/kg and was the same as found in the *Npc1*$^{-/-}$ animals (Table 1, column A). Cholesterol synthesis was decreased to 1.3 mg/

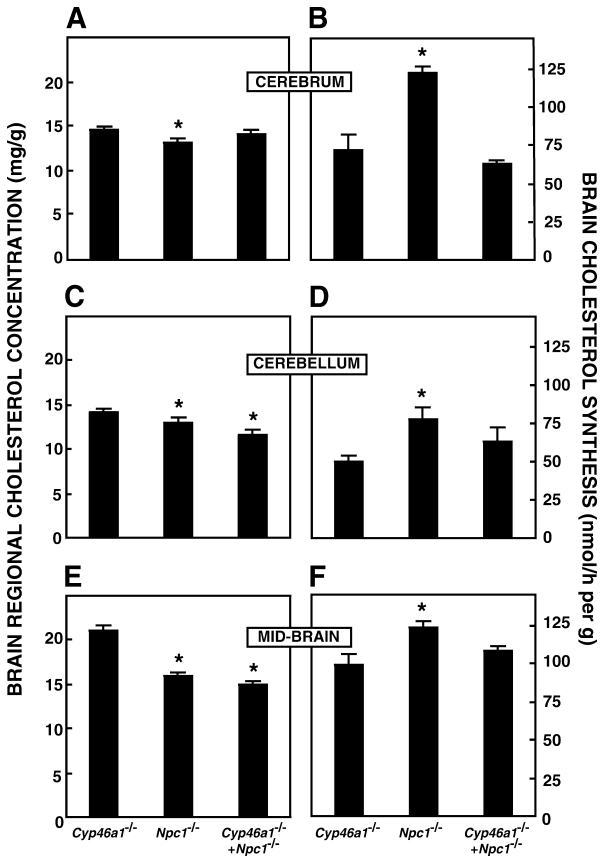

Fig. 6. Cholesterol concentration (A, C, E) and synthesis (B, D, F) in three regions of the central nervous system (CNS) in *Cyp46a1*$^{-/-}$, *Npc1*$^{-/-}$, and *Cyp46a1*$^{-/-}$/*Npc1*$^{-/-}$ mice. All animals in this experiment were 7 weeks of age, and each group contained nearly equal numbers of males and females. Means ± SEM are shown for six animals in each group. The asterisk (*) identifies the values that were significantly different from those in the *Cyp46a1*$^{-/-}$ mice.

day/kg and was the same as seen in the *Cyp46a1*$^{-/-}$ mice (Table 1, column B). As a result, the rate of cholesterol excretion from the CNS was reduced from the value of 2.3 mg/day/kg seen in the *Npc1*$^{-/-}$ mice to 1.8 mg/day/kg in the *Npc1*$^{-/-}$/*Cyp46a1*$^{-/-}$ (Table 1, column C).

Thus, these latter studies allowed for two important conclusions. First, partial suppression of cholesterol excretion from the brain by deletion of 24-hydroxylase activity did not alter the neurological phenotype of the NPC1 mice. The neurological symptoms, loss of brain cholesterol, and time of death remained unchanged. Second, while the CYP46A1-dependent component of sterol excretion from the CNS equaled about 0.9 mg/day/kg in the normal mouse, this component was apparently reduced to only 0.5 mg/day/kg in the animals with neurodegeneration. This reduction may have reflected the smaller pool of neurons known to be present in these 7-week-old *Npc1*$^{-/-}$ animals (47). However, it was not apparent from these studies what other biochemical pathway accounted for the CYP46A1-independent excretion of cholesterol from the CNS that was increased 3-fold in these same animals. While mRNA levels for apoE were significantly elevated 60% in the whole brains of these *Npc1*$^{-/-}$ mice compared with wild-type animals (1.6 ± 0.09), there were no similar significant differences found in the mRNA levels of ABCA1 (1.3 ± 0.11), scavenger receptor class B, type I (SR-BI) (1.0 ± 0.04), SREBP-1c (1.2 ± 0.04), or SREBP-2 (0.9 ± 0.03) mice.

DISCUSSION

These studies were undertaken to quantitate the rate of cholesterol excretion from the brain of the mouse, and they provide new information indicating that at least two separate pathways are involved in this excretory process. In normal animals, net sterol movement out of the CNS equaled 1.4 mg/day/kg body weight, but in *Npc1*$^{-/-}$ mice with ongoing neurodegeneration and demyelination, this flux was increased to 2.3 mg/day/kg (Table 1). In normal animals, 64% of this output (0.9 mg/day/kg) involved the formation of 24(*S*)-hydroxycholesterol. However, in the face of ongoing neurodegeneration, with loss of major groups of large neurons, this CYP46A1-dependent component of cholesterol export was diminished to only 0.5 mg/day/kg while the alternative CYP46A1-independent export pathway increased more than 3-fold from 0.5 mg/day/kg to 1.8 mg/day/kg.

The general features of sterol turnover in the whole animal are now fairly well understood. Most cholesterol is synthesized in peripheral organs and then transported through the plasma to the liver, where it is partially converted to hydroxylated sterols. Both these newly formed bile acids and the remaining cholesterol are then secreted across the canalicular membrane of the liver, and, ultimately, are excreted from the body as fecal acidic and neutral sterols. This overall flow of cholesterol through the body involves the participation of a number of plasma membrane sterol transport proteins, including, at a minimum, adenosine triphosphate binding cassette A1, SR-BI, ABCB4, ABCB11, and the two half-transporters, ABCG5 and ABCG8 (4, 49). As summarized in **Fig. 7A**, in mice that have a very high metabolic rate [~170 kcal/day/kg (50)], this turnover of membrane sterol is rapid and equals 179 mg/day/kg. Approximately half of this sterol is excreted as cholesterol itself (76 mg/day/kg), while the remainder comes out as acidic sterols (103 mg/day/kg) (35). Because the pool of cholesterol in these animals equals 2,197 mg/kg, this rate of excretion represents the turnover of 8.1% of the whole animal pool each day. In humans, with a lower metabolic rate (~25 kcal/day/kg), the turnover of membrane cholesterol is much slower and equals only 15 mg/day/kg (0.7% of the whole body pool each day). Nevertheless, this turnover is still accomplished by excreting into the feces nearly equal amounts of neutral sterols and bile acids (4).

In contrast to the whole mouse, these studies demonstrated that turnover of cholesterol in the brain equaled only 1.4 mg/day/kg (Fig. 7B). Because the pool of sterol in the CNS of these 7-week-old animals equaled 326 mg/kg, this rate of excretion represented an apparent turnover of only 0.4% of this pool each day. These experiments further delineated the role of cholesterol hydroxylation in this turnover process. Whereas both 24-hydroxylase and 27-hydroxylase activities have been reported in the brain (30–32), it was clear that only CYP46A1 was significantly expressed in the CNS of these mice, while CYP27A1 and CYP7A1 were most abundantly expressed in the liver (Fig. 2). Furthermore, deletion of 24-hydroxylase activity significantly altered cholesterol metabolism in the brain, while deletion of either 27-hydroxylase or 7α-hydroxylase activity had no effect on sterol metabolism in the CNS (Fig. 3). Thus, CYP46A1 plays a major role in CNS cholesterol metabolism, but has no demonstrable effect on whole-animal sterol turnover in the mouse (Fig. 7AB) (35). In contrast, changes in the CYP27A1 or CYP7A1 activity profoundly affect whole-mouse sterol balance but have no demonstrable effect on cholesterol metabolism in the brain (36, 37).

The finding in the current study that CYP46A1 accounts for the majority of cholesterol excreted from the CNS in the normal mouse is consistent with an earlier observation in the rat that the rate of 24(*S*)-hydroxycholesterol movement out of the CNS also accounts for a major portion of the sterol newly synthesized in the brain (31). Similarly, these observations are consistent with recent findings in the human that there is net movement of 24(*S*)-hydroxycholesterol from the brain into the cerebrospinal fluid and plasma, whereas most, if not all, 27-hydroxycholesterol found in the cerebrospinal fluid comes from the circulation, and not from the brain (33, 51).

Two lines of evidence support the possibility that the CYP46A1-dependent component of cholesterol export from the CNS reflects primarily sterol turnover in major neurons. First, previous work using in situ mRNA hybridization and immunohistochemistry revealed that *Cyp46A1* is expressed exclusively in neurons of the mouse brain and particularly in a subset of nerve cells in the cerebral

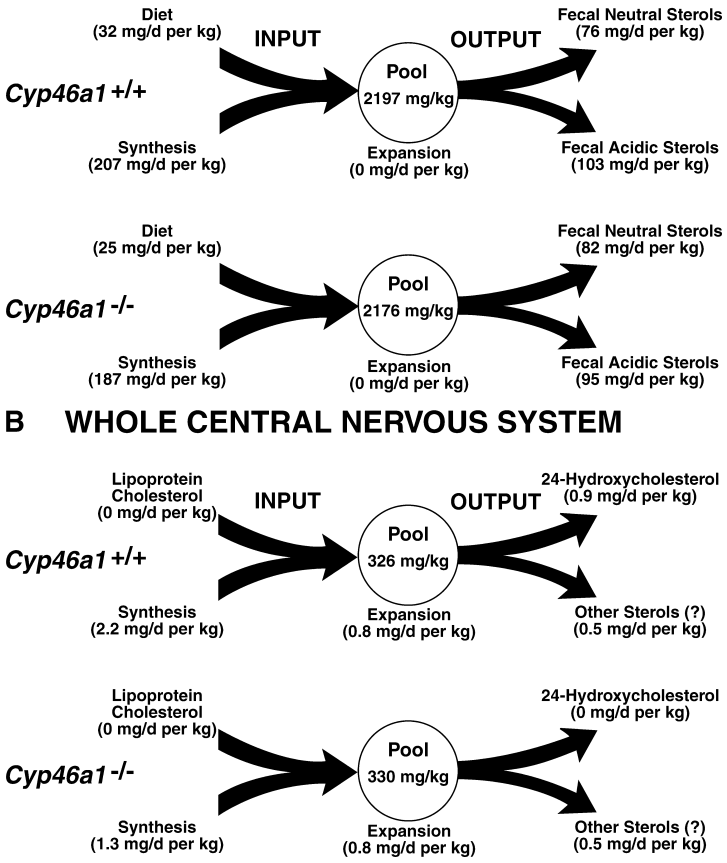

Fig. 7. Comparison of cholesterol balance across the whole mouse (A) and whole central nervous system (B) of wild-type and *Cyp46a1*$^{-/-}$ animals. A: Input of cholesterol into the whole animal from the diet and de novo synthesis and output as fecal neutral and acidic sterols (35). B: Similar data for the CNS of these animals derived from the present study. In this formulation, it was assumed that the input of cholesterol into the CNS came exclusively from synthesis and that the rate of output as "other sterols" was the same in the *Cyp46a1*$^{+/+}$ animals as in the *Cyp46a1*$^{-/-}$ mice.

cortex, hippocampus, dentate gyrus, and thalamus, and in large, metabolically active neurons like the Purkinje cells of the cerebellum (34). Second, in the present experiments the CYP46A1-dependent component of cholesterol excretion was found to be decreased by nearly half in the *Npc1*$^{-/-}$ mice (Table 1). These same animals have been shown to have lost many neurons in the CNS, including the majority of the Purkinje cells in the cerebellum (47, 48). Thus, taken together, these data support the conclusion that the rate of 24(*S*)-hydroxycholesterol movement out of the brain reflects primarily cholesterol turnover in a metabolically active subset of neurons within the CNS.

While the total pool of cholesterol in the CNS of these normal mice equaled 326 mg/kg (Fig. 7B), in the rodent, 69% of this sterol is localized to myelin (225 mg/kg) while the remainder is in cellular elements (101 mg/kg) (52). Furthermore, in most vertebrates <10% of the cells in the CNS are neurons, so the pool of cholesterol in the plasma membranes of these nerve cells must equal <10 mg/kg (53). If the excretion of 0.9 mg/day/kg of 24(*S*)-hydroxycholesterol reflects primarily sterol moving across the membranes of these neurons, then the rate of turnover must be very high, equaling >9% each day of the pool of cholesterol present in these cells. Such a rapid rate of turnover would be consistent with the fact that the basal metabolic rate is much higher in the CNS than in the animal as a whole. In the human, for example, the organ-specific metabolic rate of the brain (230 kcal/day/kg) is 9-fold higher than the average metabolic rate of the whole individual (25 kcal/day/kg) (54). Thus, contrary to earlier conclusions, these figures indicate that cholesterol turnover in neurons may be as rapid as or even more rapid than sterol turnover in most other types of cells in the body.

It is more difficult to judge the source for the sterol excreted by the CYP46A1-independent pathway. While this process is relatively small in the normal animal (0.5 mg/day/kg), it increases more than 3-fold in the face of neurodegeneration and demyelination (1.8 mg/day/kg) (Table 1). If, in the normal animal, this component of sterol excretion primarily reflected turnover in glial cells, then the movement of 0.5 mg/day/kg out of the pool of membrane cholesterol in these cells (91–100 mg/kg) would equal a turnover rate of about 0.5% of the pool each day, a rate that is very much lower than that calculated for the pool of cholesterol present in neurons, but similar to the rate of turnover in other cells of the body. In the *Npc1*$^{-/-}$ animals, there is not only loss of neurons, but also loss of a selected population of glial cells, i.e., oligodendrocytes, as well as myelin (47, 48). Thus, the 3-fold increase in the CYP46A1-independent excretion of sterol in these animals must reflect the export of cholesterol that comes from several sources. Conceivably, this flux reflects the enhanced activity of microglia, the CNS equivalent of macrophages, which are known to be activated to clear cellular debris following nerve cell damage and loss of myelin.

In any event, at least two sterols, 24(*S*)-hydroxycholesterol and, possibly, cholesterol itself or another metabolite are secreted by these various cells into the aqueous environment of the CNS and must then be moved across the blood-brain barrier into the circulation for disposition. Little is known of the nature of this (these) transport mechanism(s). As is true of all biological membranes, the log of the permeability coefficient describing the rate of passive movement of a particular molecule across the blood-brain barrier varies directly with the log of the coefficient describing the partitioning of this same molecule between a bulk lipid phase and water (55–57). By increasing the potential for hydrogen bonding in the bulk water phase, the addition of a hydroxyl group to the sterol molecule would reduce the passive permeability coefficient for that molecule while at the same time disproportionately increasing its maximal solubility in the bulk water phase. Because the maximal unidirectional flux of such a molecule is dictated by the product of its passive permeability coefficient and its maximal solubility, hydroxylation of a sterol molecule invariably increases the rate of maximal passive flux across biological membranes (58–60). Thus, the formation of 24(*S*)-hydroxycholesterol, which has a relatively high concentration in the brain of the normal mouse [~200 ng/mg protein (35)], might provide a passive mechanism for promoting net sterol movement from the brain into the blood. On the other hand, the liver, which transports relatively large amounts of both cholesterol and hydroxylated sterols across the canalicular membrane, uses specific membrane transport proteins such as ABCB4, ABCB11, and ABCG5/8 to promote the net transport of these two kinds of molecules. Which of these two types of transport mechanisms actually functions to move sterols out of the CNS remains to be elucidated.

The authors thank Brian Jefferson, Elizabeth Moore, Amanda Fletcher, Daphne Head, Stephen Ostermann, and Monti Schneiderman for their excellent technical assistance, and Merikay Presley for expert manuscript preparation. The authors also thank Dr. Joyce Repa for performing the Northern blot analyses of the various hydroxylases (Fig. 2), and Dr. Jay Horton for quantitating the mRNA levels for various proteins in the brain. This work was supported by National Institutes of Health research Grants R37 HL-09610 (J.M.D.) and P50 HL-20948 (D.W.R.), and by grants from the Moss Heart Fund (J.M.D.), the Keck Foundation (D.W.R.), and the Robert A. Welch Foundation (grant I-0971) (D.W.R.).

REFERENCES

1. Spady, D. K., and J. M. Dietschy. 1983. Sterol synthesis in vivo in 18 tissues of the squirrel monkey, guinea pig, rabbit, hamster and rat. *J. Lipid Res.* **24:** 303–315.
2. Osono, Y., L. A. Woollett, J. Herz, and J. M. Dietschy. 1995. Role of the low density lipoprotein receptor in the flux of cholesterol through the plasma and across the tissues of the mouse. *J. Clin. Invest.* **95:** 1124–1132.
3. Xie, C., S. D. Turley, and J. M. Dietschy. 1999. Cholesterol accumulation in tissues of the Niemann-Pick type C mouse is determined by the rate of lipoprotein-cholesterol uptake through the coated-pit pathway in each organ. *Proc. Natl. Acad. Sci. USA.* **96:** 11992–11997.
4. Dietschy, J. M., and S. D. Turley. 2002. Control of cholesterol turnover in the mouse. *J. Biol. Chem.* **277:** 3801–3804.
5. Simons, K., and E. Ikonen. 2000. How cells handle cholesterol. *Science.* **290:** 1721–1726.
6. Schütz, G. J., G. Kada, V. P. Pastushenko, and H. Schindler. 2000. Properties of lipid microdomains in a muscle cell membrane visualized by single molecule microscopy. *EMBO J.* **19:** 892–901.
7. Smart, E. J., G. A. Graf, M. A. McNiven, W. C. Sessa, J. A. Engelman, P. E. Scherer, T. Okamoto, and M. P. Lisanti. 1999. Caveolins, liquid-ordered domains, and signal transduction. *Mol. Cell. Biol.* **19:** 7289–7304.
8. Fassbender, K., M. Simons, C. Bergmann, M. Stoick, D. Lütjohann, P. Keller, H. Runz, S. Kühl, T. Bertsch, K. von Bergmann, M. Hennerich, K. Beyreuther, and T. Hartmann. 2001. Simvastatin strongly reduces levels of Alzheimer's disease b-amyloid peptides A*b*42 and A*b*40 *in vitro* and *in vivo*. *Proc. Natl. Acad. Sci. USA.* **98:** 8856–8861.
9. Sowa, G., M. Pypaert, and W. C. Sessa. 2001. Distinction between signaling mechanisms in lipid rafts vs. caveolae. *Proc. Natl. Acad. Sci. USA.* **98:** 14072–14077.
10. Hao, M., S. Mukherjee, and F. R. Maxfield. 2001. Cholesterol depletion induces large scale domain segregation in living cell membranes. *Proc. Natl. Acad. Sci. USA.* **98:** 13072–13077.
11. Ros-Baró, A., C. López-Iglesias, S. Peiró, D. Bellido, M. Palacín, A. Zorzano, and M. Camps. 2001. Lipid rafts are required for GLUT4 internalization in adipose cells. *Proc. Natl. Acad. Sci. USA.* **98:** 12050–12055.
12. Cook, R. P. 1958. Distribution of sterols in organisms and in tissues. *In* Cholesterol. Chemistry, Biochemistry, and Pathology. R. P. Cook, editor. Academic Press, Inc., New York. 145–180.
13. Dietschy, J. M., and S. D. Turley. 2001. Cholesterol metabolism in the brain. *Curr. Opin. Lipidol.* **12:** 105–112.
14. Wilson, J. D. 1970. The measurement of the exchangeable pools of cholesterol in the baboon. *J. Clin. Invest.* **49:** 655–665.
15. Björkhem, I., D. Lütjohann, U. Diczfalusy, L. Ståhle, G. Ahlborg, and J. Wahren. 1998. Cholesterol homeostasis in human brain: turnover of 24S-hydroxycholesterol and evidence for a cerebral origin of most of this oxysterol in the circulation. *J. Lipid Res.* **39:** 1594–1600.
16. Jeske, D. J., and J. M. Dietschy. 1980. Regulation of rates of cholesterol synthesis in vivo in the liver and carcass of the rat measured using [^{3}H]water. *J. Lipid Res.* **21:** 364–376.
17. Edmond, J., R. A. Korsak, J. W. Morrow, G. Torok-Both, and D. H. Catlin. 1991. Dietary cholesterol and the origin of cholesterol in the brain of developing rats. *J. Nutr.* **121:** 1323–1330.
18. Jurevics, H., and P. Morell. 1995. Cholesterol for synthesis of myelin is made locally, not imported into brain. *J. Neurochem.* **64:** 895–901.
19. Jurevics, H. A., F. Z. Kidwai, and P. Morell. 1997. Sources of cholesterol during development of the rat fetus and fetal organs. *J. Lipid Res.* **38:** 723–733.
20. Turley, S. D., D. K. Burns, and J. M. Dietschy. 1998. Preferential utilization of newly synthesized cholesterol for brain growth in neonatal lambs. *Am. J. Physiol.* **274:** E1099–E1105.
21. Turley, S. D., and J. M. Dietschy. 1997. Regional variation in cholesterol synthesis and low density lipoprotein transport in the brain of the fetus, newborn and adult animal. *Nutr. Metab. Cardiovasc. Dis.* **7:** 195–201.
22. Turley, S. D., D. K. Burns, C. R. Rosenfeld, and J. M. Dietschy. 1996. Brain does not utilize low density lipoprotein-cholesterol during fetal and neonatal development in the sheep. *J. Lipid Res.* **37:** 1953–1961.
23. Dietschy, J. M., T. Kita, K. E. Suckling, J. L. Goldstein, and M. S. Brown. 1983. Cholesterol synthesis in vivo and in vitro in the WHHL rabbit, an animal with defective low density lipoprotein receptors. *J. Lipid Res.* **24:** 469–480.
24. Pitas, R. E., J. K. Boyles, S. H. Lee, D. Hui, and K. H. Weisgraber. 1987. Lipoproteins and their receptors in the central nervous system. Characterization of the lipoproteins in cerebrospinal fluid and identification of apolipoprotein B, E(LDL) receptors in the brain. *J. Biol. Chem.* **262:** 14352–14360.
25. Borghini, I., F. Barja, D. Pometta, and R. W. James. 1995. Characterization of subpopulations of lipoprotein particles isolated from human cerebrospinal fluid. *Biochim. Biophys. Acta.* **1255:** 192–200.
26. Herz, J., and H. H. Bock. 2002. Lipoprotein receptors in the nervous system. *Annu. Rev. Biochem.* **71:** 405–434.
27. Mauch, D. H., K. Nägler, S. Schumacher, C. Göritz, E-C. Müller, A. Otto, and F. W. Pfrieger. 2001. CNS synaptogenesis promoted by glia-derived cholesterol. *Science.* **294:** 1354–1357.
28. Xie, C., S. D. Turley, P. G. Pentchev, and J. M. Dietschy. 1999. Cho-

lesterol balance and metabolism in mice with loss of function of Niemann-Pick C protein. *Am. J. Physiol.* **276:** E336–E344.
29. Xie, C., D. K. Burns, S. D. Turley, and J. M. Dietschy. 2000. Cholesterol is sequestered in the brains of mice with Niemann-Pick Type C disease but turnover is increased. *J. Neuropathol. Exp. Neurol.* **59:** 1106–1117.
30. Pedersen, J. I., H. Oftebro, and I. Björkhem. 1989. Reconstitution of C_{27}-steroid 26-hydroxylase activity from bovine brain mitochondria. *Biochem. Int.* **18:** 615–622.
31. Björkhem, I., D. Lütjohann, O. Breuer, A. Sakinis, and Å. Wennmalm. 1997. Importance of a novel oxidative mechanism for elimination of brain cholesterol. *J. Biol. Chem.* **272:** 30178–30184.
32. Zhang, J., Y. Akwa, M. El-Etr, E-E. Baulieu, and J. Sjövall. 1997. Metabolism of 27-, 25- and 24-hydroxycholesterol in rat glial cells and neurons. *Biochem. J.* **322:** 175–184.
33. Lütjohann, D., O. Breuer, G. Ahlborg, I. Nennesmo, Å. Sidén, U. Diczfalusy, and I. Björkhem. 1996. Cholesterol homeostasis in human brain: evidence for an age-dependent flux of 24S-hydroxycholesterol from the brain into the circulation. *Proc. Natl. Acad. Sci. USA.* **93:** 9799–9804.
34. Lund, E. G., J. M. Guileyardo, and D. W. Russell. 1999. cDNA cloning of cholesterol 24-hydroxylase, a mediator of cholesterol homeostasis in the brain. *Proc. Natl. Acad. Sci. USA.* **96:** 7238–7243.
35. Lund, E. G., C. Xie, T. Kotti, S. D. Turley, J. M. Dietschy, and D. W. Russell. 2003. Knockout of the cholesterol 24-hydroxylase gene in mice reveals a brain-specific mechanism of cholesterol turnover. *J. Biol. Chem.* **278:** 22980–22988.
36. Schwarz, M., D. W. Russell, J. M. Dietschy, and S. D. Turley. 1998. Marked reduction in bile acid synthesis in cholesterol 7a-hydroxylase-deficient mice does not lead to diminished tissue cholesterol turnover or to hypercholesterolemia. *J. Lipid Res.* **39:** 1833–1843.
37. Repa, J. J., E. G. Lund, J. D. Horton, E. Leitersdorf, D. W. Russell, J. M. Dietschy, and S. D. Turley. 2000. Disruption of the sterol 27-hydroxylase gene in mice results in hepatomegaly and hypertriglyceridemia. Reversal by cholic acid feeding. *J. Biol. Chem.* **275:** 39685–39692.
38. Ishibashi, S., M. Schwarz, P. K. Frykman, J. Herz, and D. W. Russell. 1996. Disruption of cholesterol 7a-hydroxylase gene in mice: I. Postnatal lethality reversed by bile acid and vitamin supplementation. *J. Biol. Chem.* **271:** 18017–18023.
39. Rosen, H., A. Reshef, N. Maeda, A. Lippoldt, S. Shpizen, L. Triger, G. Eggertsen, I. Björkhem, and E. Leitersdorf. 1998. Markedly reduced bile acid synthesis but maintained levels of cholesterol and vitamin D metabolites in mice with disrupted sterol 27-hydroxylase gene. *J. Biol. Chem.* **273:** 14805–14812.
40. Loftus, S. K., J. A. Morris, E. D. Carstea, J. Z. Gu, C. Cummings, A. Brown, J. Ellison, K. Ohno, M. A. Rosenfeld, D. A. Tagle, P. G. Pentchev, and W. J. Pavan. 1997. Murine model of Niemann-Pick C disease: mutation in a cholesterol homeostasis gene. *Science.* **277:** 232–235.
41. Xie, C., S. D. Turley, and J. M. Dietschy. 2000. Centripetal cholesterol flow from the extrahepatic organs through the liver is normal in mice with mutated Niemann-Pick Type C protein (NPC1). *J. Lipid Res.* **41:** 1278–1289.
42. Turley, S. D., M. W. Herndon, and J. M. Dietschy. 1994. Reevaluation and application of the dual-isotope plasma ratio method for the measurement of intestinal cholesterol absorption in the hamster. *J. Lipid Res.* **35:** 328–339.
43. Dietschy, J. M., and D. K. Spady. 1984. Measurement of rates of cholesterol synthesis using tritiated water. *J. Lipid Res.* **25:** 1469–1476.
44. Masliah, E., M. Mallory, I. Veinbergs, A. Miller, and W. Samuel. 1996. Alterations in apolipoprotein E expression during aging and neurodegeneration. *Prog. Neurobiol.* **50:** 493–503.
45. Schwarz, M., D. W. Russell, J. M. Dietschy, and S. D. Turley. 2001. Alternate pathways of bile acid synthesis in the cholesterol 7a-hydroxylase knockout mouse are not upregulated by either cholesterol or cholestyramine feeding. *J. Lipid Res.* **42:** 1594–1603.
46. Blanchette-Mackie, E. J. 2000. Intracellular cholesterol trafficking: role of the NPC1 protein. *Biochim. Biophys. Acta.* **1486:** 171–183.
47. German, D. C., E. M. Quintero, C. L. Liang, B. Ng, S. Punia, C. Xie, and J. M. Dietschy. 2001. Selective neurodegeneration, without neurofibrillary tangles, in a mouse model of Niemann-Pick C disease. *J. Comp. Neurol.* **433:** 415–425.
48. German, D. C., E. M. Quintero, C-L. Liang, C. Xie, and J. M. Dietschy. 2001. Degeneration of neurons and glia in the Niemann-Pick C mouse is unrelated to the low-density lipoprotein receptor. *Neuroscience.* **105:** 999–1005.
49. Yu, L., R. E. Hammer, J. Li-Hawkins, K. von Bergmann, D. Lutjohann, J. C. Cohen, and H. H. Hobbs. 2002. Disruption of *Abcg5* and *Abcg8* in mice reveals their crucial role in biliary cholesterol secretion. *Proc. Natl. Acad. Sci. USA.* **99:** 16237–16242.
50. Kleiber, M. 1961. Body size and metabolic rate. *In* The Fire of Life. An Introduction to Animal Energetics. M. Kleiber, editor. John Wiley & Sons, Inc., New York. 177–216.
51. Leoni, V., T. Masterman, P. Patel, S. Meaney, U. Diczfalusy, and I. Björkhem. 2003. Side chain oxidized oxysterols in cerebrospinal fluid and integrity of blood-brain and blood-cerebrospinal fluid barriers. *J. Lipid Res.* **44:** 793–799.
52. Davison, A. N. 1965. Brain sterol metabolism. *Adv. Lipid Res.* **3:** 171–196.
53. Kandel, E. R. 2000. Principles of Neural Science. Elsevier, New York.
54. Aiello, L. C., and P. Wheeler. 1995. The expensive-tissue hypothesis. The brain and the digestive system in human and primate evolution. *Curr. Anthropol.* **36:** 199–221.
55. Cornford, E. M., L. D. Braun, W. H. Oldendorf, and M. A. Hill. 1982. Comparison of lipid-mediated blood-brain-barrier penetrability in neonates and adults. *Am. J. Physiol.* **243:** C161–C168.
56. Laterra, J., R. Keep, A. L. Betz, and G. W. Goldstein. 1999. Blood-brain-cerebrospinal fluid barriers. *In* Basic Neurochemistry. Molecular, Cellular and Medical Aspects. G. J. Siegel, B. W. Agranoff, R. W. Albers, S. K. Fisher, and M. D. Uhler, editors. Lippincott-Raven, Philadelphia. 671–689.
57. Diamond, J. M., and E. M. Wright. 1969. Biological membranes: the physical basis of ion and nonelectrolyte selectivity. *Annu. Rev. Physiol.* **31:** 581–646.
58. Schiff, E. R., N. C. Small, and J. M. Dietschy. 1972. Characterization of the kinetics of the passive and active transport mechanisms for bile acid absorption in the small intestine and colon of the rat. *J. Clin. Invest.* **51:** 1351–1362.
59. Dietschy, J. M. 1978. The uptake of lipids into the intestinal mucosa. *In* Physiology of Membrane Disorders. T. E. Andreoli, J. F. Hoffman, and D. D. Fanestil, editors. Plenum Publishing Corporation, New York. 577–592.
60. Dietschy, J. M. 1979. Determinants of absorption from the gastrointestinal tract. *In* Research Monograph 2. Alcohol & Nutrition. T. K. Li, S. Schenker, and L. Lumeng, editors. US Department of Health, Education and Welfare, Washington, DC. 67–102.