Changes in the levels of cerebral and extracerebral sterols in the brain of patients with Alzheimer's disease

Maura Heverin,* Nenad Bogdanovic,† Dieter Lütjohann,§ Thomas Bayer, Irina Pikuleva,†† Lionel Bretillon,[1,*] Ulf Diczfalusy,* Bengt Winblad,† and Ingemar Björkhem[2,*]**

Divisions of Clinical Chemistry* and Geriatrics,† Karolinska Institutet, Huddinge University Hospital, Stockholm, Sweden; Department of Clinical Pharmacology,§ University of Bonn, Bonn, Germany; Department of Psychiatry,** Division of Neurobiology, University of the Saarland, Homburg/Saar, Germany; and Department of Pharmacology and Toxicology,†† The University of Texas Medical Branch, Galveston, TX

Abstract **24S-hydroxycholesterol is a side-chain oxidized oxysterol formed in the brain that is continuously crossing the blood-brain barrier to reach the circulation. There may be an opposite flux of 27-hydroxycholesterol, which is formed to a lower extent in the brain than in most other organs. Here we measured cholesterol, lathosterol, 24S- and 27-hydroxycholesterol, and plant sterols in four different brain areas of deceased Alzheimer's disease (AD) patients and controls. 24S-hydroxycholesterol was decreased and 27-hydroxycholesterol increased in all the brain samples from the AD patients. The difference was statistically significant in four of the eight comparisons. The ratio of 27-hydroxycholesterol to 24S-hydroxycholesterol was significantly increased in all brain areas of the AD patients and also in the brains of aged mice expressing the Swedish Alzheimer mutation APP751. Cholesterol 24S-hydroxylase and 27-hydroxylase protein was not significantly different between AD patients and controls. A high correlation was observed between the levels of 24S-hydroxycholesterol and lathosterol in the frontal cortex of the AD patients but not in the controls. Most probably the high levels of 27-hydroxycholesterol are due to increased influx of this steroid over the blood-brain barrier and the lower levels of 24S-hydroxycholesterol to decreased production. The high correlation between lathosterol and 24-hydroxycholesterol is consistent with a close coupling between synthesis and metabolism of cholesterol in the frontal cortex of the AD brain.**—Heverin, M., N. Bogdanovic, D. Lütjohann, T. Bayer, I. Pikuleva, L. Bretillon, U. Diczfalusy, B. Winblad, and I. Björkhem. **Changes in the levels of cerebral and extracerebral sterols in the brain of patients with Alzheimer's disease.** ***J. Lipid Res.*** **2004.** 45: **186–193.**

Supplementary key words cerebral cholesterol • cholesterol 24S-hydroxylase • sterol 27-hydroxylase • oxysterols

Transport and turnover of cholesterol in the brain seems to be of importance for development of Alzheimer's disease (AD) (1). Apolipoprotein E (apoE) is involved in transport of cholesterol in the brain, and there is a strong association between the apoE4 allele and AD (2). Cholesterol loading or depletion affects deposition of amyloid-β protein both in vitro (3, 4) and in vivo (5). Clinical studies with HMG-CoA reductase inhibitors suggest that reduction of cholesterol synthesis may have a preventive effect on development of AD (6–8).

Under normal conditions, with an intact blood-brain barrier, there is little or no exchange of cholesterol over the blood-brain barrier (9, 10). We have defined a mechanism by which 6–7 mg of cholesterol is eliminated daily from the human brain in the form of a side-chain oxidized oxysterol, 24S-hydroxycholesterol (11–13), which is able to pass the intact blood-brain barrier. The enzyme responsible for the conversion of cholesterol into 24S-hydroxycholesterol is the cytochrome P450 species cholesterol 24S-hydroxylase (CYP46), which has been reported to be almost exclusively expressed in the neuronal cells in the normal human brain (14).

The neurodegeneration occurring in AD would be expected to eventually lead to loss of 24S-hydroxylase activity, with a resulting decrease in the flux of 24S-hydroxycholesterol from the brain into the circulation. In accordance with this, we found that patients with advanced AD had reduced plasma levels of 24S-hydroxycholesterol (15). A population of AD patients with less-advanced disease was, however, reported to have slightly increased levels of 24S-hydroxycholesterol, possibly due to an ongoing active neuronal destruction with increased liberation of cholesterol and 24S-hydroxycholesterol (16). Very recently, we studied the amount and distribution of CYP46 in autopsy brain material from AD and control patients

Manuscript received 22 July 2003 and in revised form 18 September 2003.

Published, JLR Papers in Press, October 1, 2003.
DOI 10.1194/jlr.M300320-JLR200

[1] Present address of L. Bretillon: Lipid Nutrition Unit, National Institute for Research on Agronomy, Dijon, France.

[2] To whom correspondence should be addressed.
e-mail: Ingemar.Bjorkhem@hs.se

(17). Neuronal cells from the frontal cortex of AD patients contained less CYP46 than did those of the controls. Surprisingly, a positive staining could be seen in the glial cells in AD brain but not in the controls. This indicates that in the normal state, the flux of 24S-hydroxycholesterol from the brain into the circulation is likely to be derived exclusively from the neurons, but possibly from both neurons and glial cells in patients with AD.

In a recent work (18), 24S-hydroxycholesterol was measured in the brains of transgenic mice carrying the Swedish mutation APP23, which causes typical AD-related pathological changes. The levels of this oxysterol were not significantly different between the transgenic mice and the controls. Because cholesterol 24S-hydroxylase has a broader organ distribution in mouse than in man, and is expressed to a considerable extent also in the liver, it is difficult to draw conclusions that are valid for humans. Interestingly, the levels of plant sterols were significantly increased in the brain of APP23 transgenic animals at the age of 12 and 18 months (18). Because these sterols are exclusively of dietary origin, the possibility must be considered that the APP23 transgenic mice had developed a defect in the blood-brain barrier. It is well established that patients with advanced Alzheimer's disease may have such defects (19, 20).

27-Hydroxycholesterol is another side-chain oxidized oxysterol, formed by sterol 27-hydroxylase (CYP27A1). In contrast to 24S-hydroxycholesterol, it is formed in most cells, and there is a constant flux of this oxysterol from extrahepatic tissues to the liver (21). There is, however, no net flux of 27-hydroxycholesterol from the brain, and the levels of 27-hydroxycholesterol in this organ are about 10-fold lower than those of 24S-hydroxycholesterol (11). In similarity with 24S-hydroxycholesterol, 27-hydroxycholesterol is likely to pass the blood-brain barrier, and an interchange between 27-hydroxycholesterol in the circulation and in the brain is likely to occur. Very recently, we showed that most of the 27-hydroxycholesterol present in human cerebrospinal fluid is of vascular origin (22).

In view of the contrasting information obtained from in vivo studies in humans and the studies with the transgenic mouse model, we considered it to be of interest to measure the levels of the oxysterols 24S- and 27-hydroxycholesterol in autopsy materials from patients with Alzheimer's disease and from controls. In addition to oxysterols, the levels of cholesterol, lathosterol, and plant sterols were also measured. For reasons of comparison, we also assayed 27-hydroxycholesterol in the brains of APP23 transgenic mice. Attempts were made to measure cholesterol 24S-hydroxylase and sterol 27-hydroxylase protein in the brain material in order to investigate possible correlations between the protein expression and steroid levels. There may be a close coupling between cholesterol synthesis and cholesterol elimination by the CYP46 mechanism in the brain (23). In view of this, it was of interest to see if a correlation could be found between the levels of lathosterol (a marker for cholesterol synthesis) and levels of 24S-hydroxycholesterol (a marker for cholesterol elimination) in the two groups of patients.

MATERIALS AND METHODS

Antibodies and reagents

The rabbit antipeptide antibodies toward human CYP46 were a kind gift from Prof. D. W. Russell (Department of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas, TX) (14). Polyclonal rabbit antibodies toward the whole sterol 27-hydroxylase protein were used in the Western blotting of CYP27A1. All organic solvents used were of gas chromatography or high performance liquid chromatography grade.

Patients

Formalin-fixed autopsy tissue from the frontal cortex, occipital cortex, basal ganglia, and pons was obtained from 15 controls (age 61–88 years, mean age 73 years, male-to-female ratio 9:6) and 15 AD patients (age 66–92 years, mean age 82 years, male-to-female ratio 5:10). The control samples were obtained from assumed-healthy subjects who had died as a result of road traffic accidents. The AD patients fulfilled the clinical criteria for "probable" AD (24) and the neuropathological criteria for "definite" AD (25). The AD patients died as a result of the disease. These samples were collected for lipid extraction and sterol analysis only. Although all brain samples from all patients were analyzed, the great differences in age and female-to-male ratio necessitated the selection of a subset of subjects from each group. Eight of the AD patients and eight of the controls were paired together to form a gender- and age-matched group, with a female-to-male ratio of 5:3 and a mean age of 78 years (range 63–86 years) for the controls and 79 years (range 66–89 years) for the AD patients. The maximum difference of age in the pairs was 3 years.

In addition to the above materials, frozen brain samples from the same four brain regions were obtained from five additional AD patients and from five age- and gender-matched controls for Western blot analysis. Part of this material was also used for analyses of the sterols.

Experiments with APP23 mice

The experiments with transgenic mice expressing the Swedish mutation APP751 under the control of the murine Thy1 promoter (APP23) and wild-type littermate control mice have been described in detail previously (18). In short, groups of five wild-type and five APP transgenic mice were killed at the age of 3, 6, 9, and 12 months and 10 wild-type and 11 APP transgenic mice at 18 months. Brain homogenates were prepared for sterol extraction essentially as previously described. In the previous publication, results of measurements of cholesterol, cholesterol precursors, and 24S-hydroxycholesterol have been reported. The complementary analysis made here was the assay of 27-hydroxycholesterol by isotope dilution-mass spectrometry as described below.

Ethical aspects

Permission to create and utilize the brain bank at Huddinge University Hospital was obtained from the local ethical committee of the hospital. The animal experiments were performed in accordance with the German animal protection laws.

Assay of cholesterol, lathosterol, plant sterols, and oxysterols

Small pieces of the brains (~400 mg) were shock frozen in liquid nitrogen for 10 min and pulverized in a microdismembrator. The solid powder was weighed, and the lipid components were extracted with chloroform-methanol (2:1; v/v) by stirring overnight at room temperature under argon gas. The volume of this solution was adjusted to 10 ml, and aliquots were taken for analysis of cholesterol and oxysterols. Cholesterol and lathosterol

were assayed by isotope dilution-mass spectrometry with the use of deuterium-labeled cholesterol and lathosterol, respectively, as internal standard (26, 27). The plant sterols campesterol and sitosterol were assayed in a similar fashion using trideuterated 24-hydroxycholesterol as internal standard and selected-ion monitoring at *m/z* 472 and *m/z* 486 for campesterol and sitosterol, respectively. Oxysterols were also analyzed by isotope dilution-mass spectrometry with the use of an individual deuterium-labeled oxysterol in each case as previously described (28).

Determination of the water content of the brain samples

Formalin-fixed tissue (~200 mg) from the four brain regions of five AD patients and five control patients was patted dry and weighed. The samples were then incubated overnight at 60°C, and the dehydrated samples weighed again. The difference in weight before and after drying was determined to be the water content of the brain samples.

Western blotting

For immunoblotting, frozen brain tissue from each brain area of the controls and AD patients was homogenized in 0.32 M sucrose medium and centrifuged to produce crude organelle fractions.

The nuclear and microsomal fractions, corresponding to 2 and 4 μg of total protein, respectively, were diluted in sample buffer and run on 7.5% polyacrylamide gel. The samples were transferred to Hybond® nitrocellulose membranes (Amersham Pharmacia Biotech, Little Chalfont, UK) and blocked with 5% Biorad blocking agent in Tris-buffered saline containing 0.1% Tween (TBS-T). The membranes were washed with TBS-T and incubated with primary antibody to CYP46 or CYP27A1 overnight, followed by washing with TBS-T and incubation with peroxidase-conjugated goat anti-rabbit IgG (Sigma) for 4–5 h. The protein was visualized with an enhanced chemiluminescence kit (Amersham) according to the manufacturer's instructions, and films were exposed to the membranes for a few minutes. Band intensity was estimated using a Pharmacia Imagemaster system.

RESULTS

Levels of cholesterol, lathosterol, side-chain oxidized oxysterols, and plant sterols in the AD and control brains

No significant age- or gender-related effects on the different parameters were found within the relatively heterogeneous groups of 15 controls and 15 AD patients. In view of the relatively small size of the groups, such differences cannot be excluded. Because of this, and in order to utilize paired *t*-tests, we defined eight gender- and age-matched pairs from the 30 subjects.

In the following section, we show the detailed results obtained from the two smaller homogeneous groups (8 + 8 subjects, **Fig. 1A**) but also describe the results obtained with the two larger, more heterogeneous groups.

As shown in Fig. 1A, the cholesterol content was slightly higher in the AD patients in three of the four regions. In the basal ganglia, this difference was statistically significant ($P < 0.05$). In the larger, more heterogeneous group, there were no consistent differences between the two groups in any brain area (results not shown).

Similarly to cholesterol, lathosterol levels were slightly higher in basal ganglia from AD patients than in the corresponding controls (Fig. 1B). The situation was similar in the larger groups (results not shown). The ratio of lathosterol to cholesterol, believed to be a marker for cholesterol synthesis, was, however, not significantly different between controls and AD patients in either of the patient groups (results not shown).

The levels of 24S-hydroxycholesterol were consistently lower in all areas of the brain of the AD patients compared with controls (Fig. 1C). Statistical significance was reached only in the case of occipital cortex. In the complete group, however, this difference was statistically different in all the areas (results not shown). The situation was similar with the cholesterol-related levels of 24S-hydroxycholesterol. Although the levels were decreased in all brain areas of the AD patients, the difference only reached statistical significance in the basal ganglia (Fig. 1D).

The levels of 27-hydroxycholesterol were significantly increased in all the different brain areas of the AD patients, with the exception of the pons (Fig. 1E). In the larger groups, the magnitude of the increase was greater in all areas, including the pons (results not shown).

The ratio of 27-hydroxycholesterol to cholesterol was higher in all brain areas of the AD patients (Fig. 1F), but this difference was not statistically significant. In contrast, this difference was found to be statistically significant in all areas of the brain in the larger groups.

The ratio of 27-hydroxycholesterol to 24S-hydroxycholesterol was found to be significantly increased in all areas of the AD brains, in both the smaller (Fig. 1G) and the larger groups.

The plant sterols campesterol and sitosterol were found to be slightly increased in all brain areas except the pons (Figs. 1H, I). This difference reached statistical significance in the case of sitosterol in frontal cortex and basal ganglia. The situation was similar in the larger groups. The same pattern was observed also in the ratios of sitosterol to cholesterol and campesterol to cholesterol.

Correlation analyses

Attempts were made to uncover correlations between the different parameters measured. The highest correlations were observed between the levels of 24S-hydroxycholesterol and 27-hydroxycholesterol in the frontal and occipital cortex of the AD patients ($r^2 = 0.9$ in both cases) (**Fig. 2**). This correlation was not evident in controls ($r^2 = 0.2$ and 0.6, respectively). Good correlations were also found between lathosterol and 24S-hydroxycholesterol in the frontal and occipital cortex of the AD patients (**Fig. 3**). Significant correlations were absent in the corresponding control patients ($r^2 = 0.2$ in both cases). The correlations between 24S-hydroxycholesterol and lathosterol, as well as between 24S-hydroxycholesterol and 27-hydroxycholesterol, were not due to the cholesterol levels, because the correlations were retained after correction for differences in cholesterol concentrations.

Water content of the brain samples

In theory, part of the differences observed in absolute concentrations of the oxysterols and cholesterol between

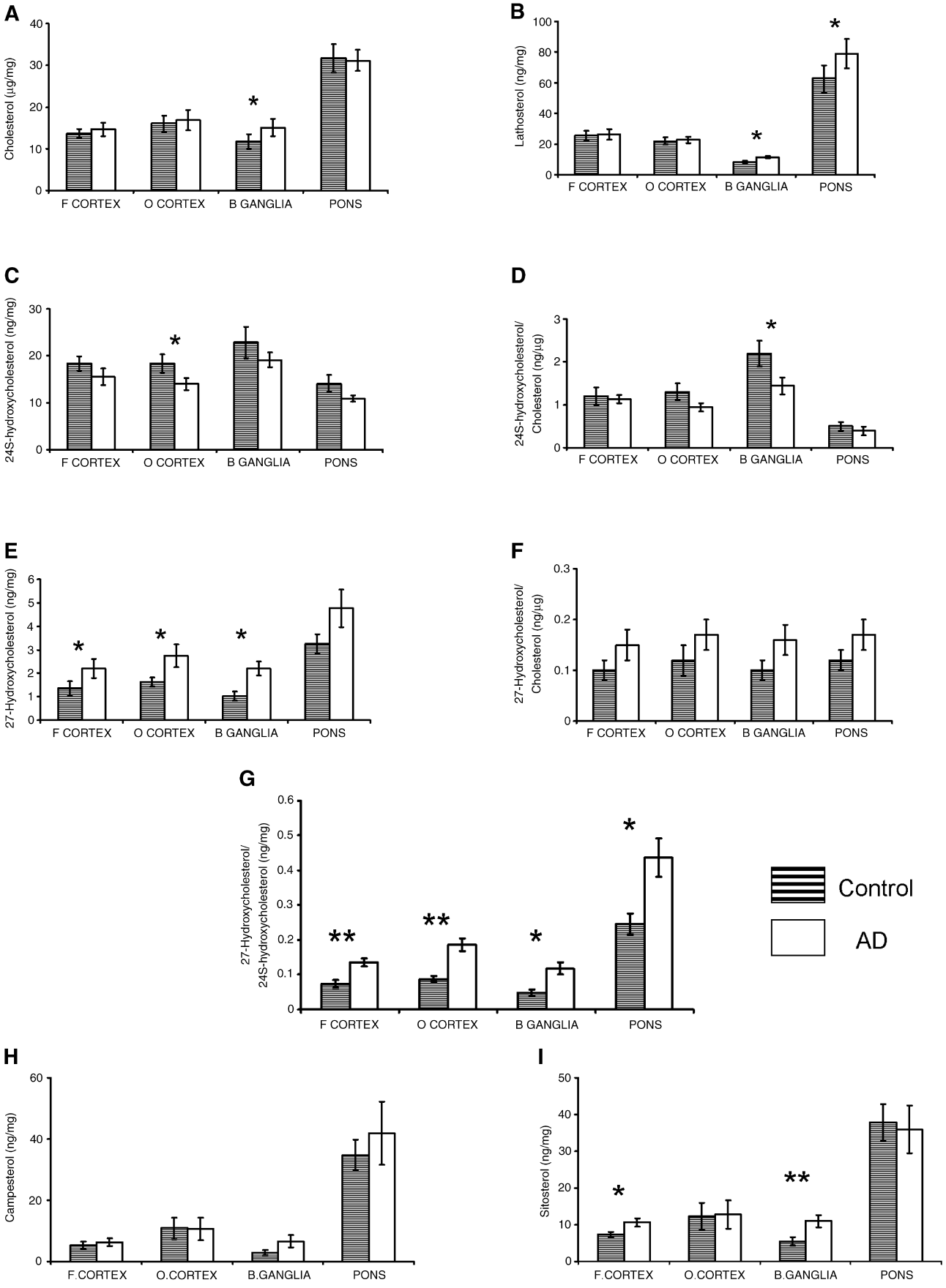

Fig. 1. Levels of cholesterol (A), lathosterol (B), 24S-hydroxycholesterol (C), ratio of 24S-hydroxycholesterol to cholesterol (D), levels of 27-hydroxycholesterol (E), ratio of 27-hydroxycholesterol to cholesterol (F), ratio of 27-hydroxycholesterol to 24S-hydroxycholesterol (G), and levels of campesterol (H) and sitosterol (I) in the four different brain areas from the age- and gender-matched eight controls (striped bars) and the eight AD patients (open bars). * $P < 0.05$; ** $P < 0.01$. Error bars indicate means ± SEM.

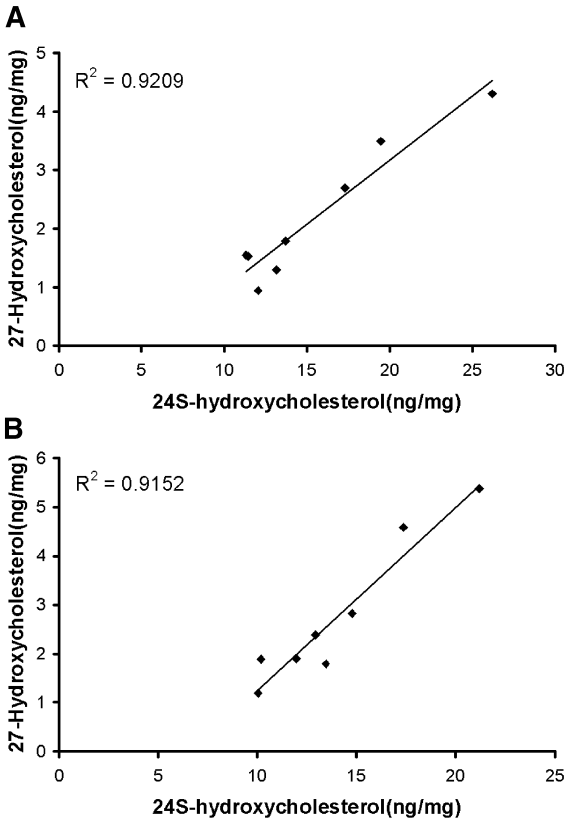

Fig. 2. Correlation between 24S-hydroxycholesterol and 27-hydroxycholesterol in frontal cortex (A) and occipital cortex (B) from the eight AD patients belonging to the age- and gender-matched group.

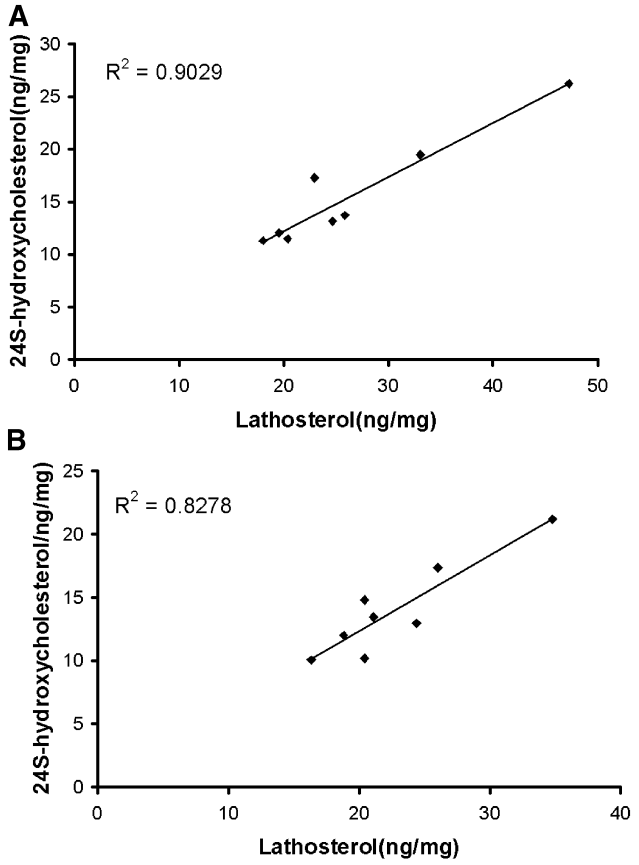

Fig. 3. Correlation between lathosterol and 24S-hydroxycholesterol in frontal cortex (A) and occipital cortex (B) from the eight AD patients belonging to the age- and gender-matched group.

the samples from the Alzheimer brains and the control brains may be due to differences in water content. To exclude this possibility, we measured the water content of samples from each area in age- and gender-matched samples from five control and five Alzheimer patients. The water content of the frontal cortex of the AD and control samples was 79 ± 1% and 79 ± 1%, respectively. The water content of the occipital cortex was 74 ± 1% and 75 ± 1% for AD and control samples, respectively. The water content of the basal ganglia was 77 ± 1% and 74 ± 6% for AD and control samples, respectively. The water content of the pons was 68 ± 2% and 68 ± 1% for AD and control samples, respectively (mean ± SEM). Thus, there was no significant difference between AD and control samples with respect to water content.

Measurements of cholesterol 24S-hydroxylase and sterol 27-hydroxylase protein in brain samples from AD patients and controls by Western blotting

The levels of the two proteins were assessed by Western blotting. Initially, crude homogenates of the brain samples were used. The results obtained in these measurements were less than satisfactory. Considerably higher quality analyses could be performed by measuring cholesterol 24S-hydroxylase in isolated microsomal fractions and sterol 27-hydroxylase in isolated mitochondrial fractions from the frozen brain samples. In accordance with previous studies (14, 29), only one band with the expected molecular mass of ~57 kDa was obtained in the analysis of CYP46, whereas two bands with molecular mass of ~57 kDa and 60 kDa, respectively, were obtained in the analyses of CYP27. In both cases, no significant differences between AD and controls were observed with respect to enzyme levels in the different areas of the brain. In the analysis of the content of CYP46 in the frontal cortex from the AD patients and from the controls, the response was 84 ± 19 and 99 ± 5 arbitrary units, respectively ($P > 0.05$). In the analysis of the content of the sterol 27-hydroxylase protein in the same area, the response was 25 ± 2 and 22 ± 4 arbitrary units, respectively ($P > 0.05$). Similar results were also obtained in the other brain areas.

The levels of 24S-hydroxycholesterol and 27-hydroxycholesterol were also measured in the specific brain samples used for immunoblotting. No significant correlations were found between these levels and the levels of the corresponding enzyme protein (results not shown).

Measurement of 27-hydroxycholesterol and 27:24 ratio in APP23 transgenic mice

Levels of cholesterol-related sterols in the brain of aging wild-type and APP transgenic mice were measured previously (18). The levels of 27-hydroxycholesterol were

not analyzed at that time. The results of the present measurements of 27-hydroxycholesterol in the brain of these mice are summarized in **Fig. 4**.

At the age of 12 and 18 months, respectively, the transgenic mice were found to have significantly higher brain levels of 27-hydroxycholesterol than the control mice (increase of 21% and 29%, respectively) ($P < 0.01$ and 0.001, respectively). The ratio of 27-hydroxycholesterol to 24S-hydroxycholesterol showed a pattern similar to that of the absolute levels of 27-hydroxycholesterol. The ratio of 27:24 was 0.14 ± 0.02 in the brain of APP transgenic mice compared with 0.11 ± 0.01 in the corresponding controls at the age of 12 months ($P = 0.001$). A similar difference was found at the age of 18 months (0.15 ± 0.02 vs. 0.12 ± 0.01, $P < 0.001$). A significant correlation was found between levels of 27-hydroxycholesterol and campesterol ($r^2 = 0.6$) as well as between 27-hydroxycholesterol and sitosterol ($r^2 = 0.8$).

DISCUSSION

Cholesterol is synthesized by neuronal cells and astrocytes, and brain cholesterol is predominantly found in myelin (10, 30). The oligodendrocyte seems to be the most active cell in the synthesis of the cholesterol that accumulates in myelin. The content of cholesterol in the brain of patients with AD has been reported in most studies to be unchanged (10). In the present work, slightly increased levels of cholesterol were found in most areas of the brain of AD patients in the small homogenous group but not in the larger heterogeneous group. The same pattern was observed with lathosterol. The ratio of lathosterol to cholesterol is believed to reflect cholesterol synthesis. This ratio did not differ between the two groups, however, suggesting that the rate of cholesterol synthesis had been about the same in the AD and in the control brains.

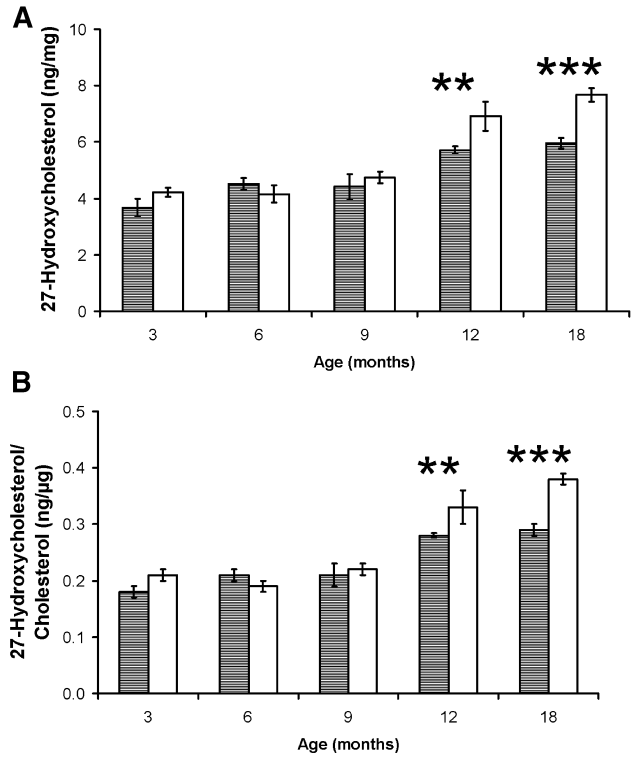

Fig. 4. The absolute (upper panel) and cholesterol-related (lower panel) brain levels of 27-hydroxycholesterol in wild-type (striped bars) and APP23 transgenic mice (open bars) of different ages. ** $P < 0.01$; *** $P < 0.001$. Error bars indicate means ± SEM.

The levels of 24S-hydroxycholesterol were lower in all areas of AD brains compared with controls. This decrease is likely to reflect the lower number of CYP46-containing neuronal cells in AD. It is evident that the abnormal expression of CYP46 in glial cells in AD brains observed in a previous study (17) is not able to functionally compensate for the loss of the neuronal enzyme. The results are consistent with our observation that patients with advanced AD have significantly reduced plasma levels of 24S-hydroxycholesterol as compared with healthy controls (15). The fact that we could not demonstrate a significant reduction of CYP46 enzyme protein in our study may be due to the less-accurate Western blotting technique used, which is not well suited to detect relatively small changes. There was a tendency, however, toward lower levels in the frontal cortex of the AD brains.

A significant finding of the present study was the relatively high increase in 27-hydroxycholesterol levels in all areas of the brain tested. In view of our recent demonstration that most of the 27-hydroxycholesterol present in human cerebrospinal fluid originates in the circulation (22), it seems likely that a considerable part of the 27-hydroxycholesterol present in the brain is derived from extracerebral sources. Sterol 27-hydroxylase is present in the brain, but only at very low levels. In the present work, we did not find evidence of a difference in the levels of the enzyme in the brain of AD patients and controls. A defective blood-brain barrier would be expected to permit an increased flux of 27-hydroxycholesterol from the circulation into the brain. Abnormal blood-brain barrier function has, in fact, been reported in at least some populations of AD patients (19, 20). We have recently shown that a disturbed blood-brain function in some other neurological diseases leads to increased flux of 27-hydroxycholesterol from the circulation into the cerebrospinal fluid (22). Consistent with our observations in patients with AD, increased levels of 27-hydroxycholesterol were found also in the APP23 mice at the age of 12 and 18 months. It is of interest that there may be alterations in the blood-brain barrier of mice expressing both APP and presenilin-1 (31).

If there is an increased flux of 27-hydroxycholesterol from the circulation into the brain as a consequence of a disturbed blood-brain barrier, there may also be an increased flux of other steroids. Slightly higher levels of plant sterols were found in most areas of the AD brain studied, and in the APP23 mice, a statistically significant accumulation of the plant sterols sitosterol and campesterol has been reported (18). In the transgenic mice, there was a clear correlation between the levels of the two

plant sterols and 27-hydroxycholesterol, supporting the contention that the increased flux of 27-hydroxycholesterol is related to a defect in the blood-brain barrier.

The "driving force" for the flux of 24S-hydroxycholesterol from the brain into the circulation is likely to be the concentration difference between the two compartments. The ratio of 24S-hydroxycholesterol to cholesterol is 20-fold higher in the brain than in the circulation, and a similar difference is present between the brain and most other organs (11). In contrast, the ratio of 27-hydroxycholesterol to cholesterol is of a similar magnitude in the circulation and the brain (~0.1 ng/μg), whereas this ratio is higher in almost all other tissues and organs in the body. In the lung, the ratio is about 70-fold higher than in the circulation and the brain (32). In view of this, some net flux of 27-hydroxycholesterol into the brain seems likely to occur. A prerequisite for such a passage is a transport over the blood-brain barrier. It is well established that both 24S- and 27-hydroxycholesterol are able to pass lipophilic membranes at a much higher rate than cholesterol (33). The rate of transport of 27-hydroxycholesterol across such membranes is, however, significantly higher than that of 24S-hydroxycholesterol. This may be the explanation for our finding here that the ratio of 27-hydroxycholesterol to cholesterol was almost identical in all the brain areas studied (Fig. 1F). Considerably greater differences in the relative concentrations of 24S-hydroxycholesterol were found in the different brain areas (Fig. 1D).

Both 24S-hydroxycholesterol and 27-hydroxycholesterol are ligands for the nuclear receptor LXRβ, which is present at relatively high levels in the brain. The ligand binding efficiency is, however, higher with 24S-hydroxycholesterol than with 27-hydroxycholesterol, and the activation efficiency toward LXRβ is almost 10-fold higher (34). It is noteworthy that the expression patterns of cholesterol 24S-hydroxylase and LXRβ are remarkably similar (35). In view of the role of liver X receptors as regulators of cholesterol and phospholipid export proteins (35), the possibility must be considered that a change in the ratio of 27- to 24S-hydroxycholesterol may reduce the activation of the receptor, with consequences for lipid homeostasis in the brain. It was recently reported that a knockout of the liver X receptors causes age-related neurodegeneration, with lipid deposits, proliferation of astrocytes, loss of neurons, and disorganized myelin sheaths (36).

Theoretically, the most important factors regulating the levels of cholesterol in the brain should be the rate of de novo synthesis and the rate of metabolism by the CYP46-mediated mechanism. Very recently, it was reported that a disruption of the CYP46 gene causes a marked reduction of cholesterol synthesis in mouse brain (37). Under steady-state conditions, a relatively constant relation would be expected between a marker for cholesterol synthesis (lathosterol) and a marker for cholesterol metabolism (24S-hydroxycholesterol). A high correlation was found between levels of these two steroids in the frontal and occipital cortex of the brains from the AD patients. A similar correlation has also been observed in APP23 transgenic mice (18). A high correlation between 24S-hydroxycholesterol and 27-hydroxycholesterol was also found in these two areas of the brains from the AD patients.

This is consistent with a relation between increased metabolism of cholesterol in the brain and a decreased blood-brain barrier function in AD. That the above correlations were most significant in the frontal cortex is consistent with a previous finding that this area of the brain seems to be more sensitive to changes in overall cholesterol homeostasis than most other areas of the brain, at least in mice (38). There was a distinct difference between the findings in the AD brains and the control brains, possibly as a consequence of the ongoing degeneration in the former.

To summarize, we have shown that the ratio of two oxysterols, one of cerebral and one of mainly extracerebral origin, is markedly changed in all brain areas of patients with Alzheimer's disease, as well as in brains of transgenic mice carrying the Swedish Alzheimer mutation. The results confirm and extend previous studies demonstrating disturbed cholesterol turnover in this disease.

This work was supported by grants from the Swedish Lions Foundation for Age-Related Diseases, the Swedish Medical Research Council, the Swedish Heart-Lung Foundation, the Foundation for Geriatric Diseases at Karolinska Institutet, the Alzheimer Foundation, Gunvor och Josef Aners Stiftelsen, Demensförbundet, the Bundesministerium für Bildung, Forschung und Technologie (01EC9402), and the National Institutes of Health (Grant no. GM-62882).

REFERENCES

1. Puglielli, L., R. E. Tanzi, and D. M. Kovacs. 2003. Alzheimer's disease: the cholesterol connection. *Nat. Neurosci.* **6:** 345–351.
2. Strittmatter, W. J., A. M. Saunders, D. Schmechel, M. Pericak Vance, J. Enghild, S. Salvesen, and A. D. Roses. 1993. Apolipoprotein E: high-avidity binding to β-amyloid and increased frequency of Type 4 allele in late-onset familial Alzheimer disease. *Proc. Natl. Acad. Sci. USA.* **90:** 1977–1981.
3. Simons, M., P. Keller, B. De Strooper, K. Beyreuther, C. G. Dotti, and K. Simons. 1998. Cholesterol depletion inhibits the generation of β-amyloid in hippocampal regions. *Proc. Natl. Acad. Sci. USA.* **95:** 6460–6464.
4. Howland, D. S., S. P. Trusko, M. J. Savage, A. G. Reaume, D. M. Lang, J. D. Hirsch, N. Maeda, R. Siman, B. D. Greenberg, R. W. Scott, and D. G. Flood. 1998. Modulation of secreted β-amyloid precursor protein and amyloid β-peptide by cholesterol. *J. Biol. Chem.* **273:** 16576–16582.
5. Refolo, L. M., M. A. Pappolla, B. Malester, J. LaFrancois, T. Bryant-Thomas, R. Wang, G. S. Tint, K. Sambamurti, and K. Duff. 2000. Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. *Neurobiol. Dis.* **7:** 321–331.
6. Wolozin, B., W. Kellman, and P. Ruosseau. 2000. Decreased prevalence of Alzheimer disease associated with HMG CoA reductase inhibitors. *Arch. Neurol.* **57:** 1439–1443.
7. Jick, H., G. L. Zornberg, S. Jick, S. Seshadri, and A. Drachman. 2000. Statins and the risk of dementia. *Lancet.* **356:** 1627–1631.
8. Buxbaum, J. D., E. I. Cullen, and L. T. Friedhoff. 2002. Pharmacological concentrations of the HMG CoA reductase inhibitor lovastatin decrease the formation of the Alzheimer beta-amyloid peptide in vitro and in patients. *Front. Biosci.* **7:** 50–59.
9. Dietschy, J. M., and S. D. Turley. 2001. Cholesterol metabolism in the brain. *Curr. Opin. Lipidol.* **12:** 105–112.
10. Snipes, G. J., and U. Suter. 1997. Cholesterol and myelin. *In* Subcellular Biochemistry. Vol. 28. R. Bittman, editor. Plenum Press, New York. 173–204.

11. 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.
12. Björkhem, I., D. Lütjohann, O. Breuer, A. Sakinis, and A. Wennmalm. 1997. Importance of a novel oxidative mechanism for elimination of brain cholesterol. Turnover of cholesterol and 24(S)-hydroxycholesterol in rat brain as measured with 18O2 techniques in vivo and in vitro. *J. Biol. Chem.* **272:** 30178–30184.
13. Björkhem, I., D. Lütjohann, U. Diczfalusy, L. Ståhle, G. Ahlborg, and J. Wahren. 1998. Cholesterol homeostasis in the 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.
14. 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.* **16:** 7238–7243.
15. Bretillon, L., Å. Sidén, L-O. Wahlund, D. Lütjohann, L. Minthon, M. Crisby, J. Hillert, C-G. Groth, U. Diczfalusy, and I. Björkhem. 2000. Plasma levels of 24S-hydroxycholesterol in patients with neurological diseases. *Neurosci. Lett.* **293:** 87–90.
16. Lütjohann, D., A. Papassotiropoulos, I. Björkhem, S. Locatelli, M. Bagli, M. L. Rao, K. von Bergmann, and R. Heun. 2000. 24S-hydroxycholesterol (cerebrosterol) and Alzheimer's disease. *J. Lipid Res.* **41:** 195–198.
17. Bogdanovic, N., L. Bretillon, E. Lund, U. Diczfalusy, L. Lannfelt, B. Winblad, D. Russell, and I. Björkhem. 2001. On the turnover of brain cholesterol in patients with Alzheimer's disease. Abnormal induction of the cholesterol-catabolic enzyme CYP46 in glial cells. *Neurosci. Lett.* **314:** 45–48.
18. Lütjohann, D., A. Brzezinka, E. Barth, D. Abramowski, M. Staufenbiel, K. von Bergmann, K. Beyreuther, G. Multhaup, and T. A. Bayer. 2002. Profile of cholesterol-related sterols in aged amyloid precursor protein transgenic mouse brain. *J. Lipid Res.* **43:** 1078–1085.
19. Blennow, K., A. Wallin, P. Fredman, I. Karlsson, C. G. Gottfries, and L. Svennerholm. 1990. Blood brain barrier disturbance in patients with Alzheimer's disease is related to vascular factors. *Acta Neurol. Scand.* **81:** 323–326.
20. McKhann, G., D. Drachman, M. Folstein, R. Katzman, D. Price, and E. M. Stadlan. 1984. Clinical diagnosis of Alzheimer disease. *Neurology.* **34:** 939–944.
21. Lund, E., O. Andersson, J. Zhang, A. Babiker, G. Ahlborg, U. Diczfalusy, K. Einarsson, J. Sjövall, and I. Björkhem. 1996. Importance of a novel oxidative mechanism for elimination of intracellular cholesterol in humans. *Arterioscler. Thromb. Vasc. Biol.* **16:** 208–212.
22. Leoni, M., T. Masterman, P. Patel, S. Meaney, U. Diczfalusy, and I. Björkhem. 2003. Side-chain oxidized oxysterols in cerebrospinal fluid and integrity of the blood-brain barrier. *J. Lipid Res.* **44:** 793–799.
23. Björkhem, I., U. Diczfalusy, and D. Lütjohann. 1999. Removal of cholesterol from extrahepatic sources by oxidative mechanisms. *Curr. Opin. Lipidol.* **10:** 161–165.
24. Mirra, S. S., A. Heyman, D. McKeel, S. M. Sumi, B. J. Crain, L. M. Brownlee, F. S. Vogel, J. P. Hughes, G. van Belle, and L. Berg. 1991. The consortium to establish a register for Alzheimer's disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology.* **41:** 479–486.
25. Bogdanovic, N., and J. H. Morris. 1995. Diagnostic criteria for Alzheimer's disease in multicentre brain banking. *In* Neuropathological Diagnostic Criteria for Brain Banking. F. F. Cruz-Sanchez, R. Ravid, and M. L. Cuzner, editors. Biomedical and Health Research Series. IOS Press, Amsterdam, The Netherlands. 20–29.
26. Björkhem, I., R. Blomstrand, and L. Svensson. 1974. Determinations of cholesterol by mass fragmentography. *Clin. Chim. Acta.* **54:** 185–193.
27. Lund, E., L. Sisfontes, E. Reihner, and I. Björkhem. 1989. Determination of serum levels of unesterified lathosterol by isotope dilution-mass spectrometry. *Scand. J. Clin. Lab. Invest.* **49:** 165–171.
28. Dzeletovic, S., O. Breuer, E. G. Lund, and U. Diczfalusy. 1995. Determination of cholesterol oxidation products in human plasma by isotope dilution-mass spectrometry. *Anal. Biochem.* **225:** 73–80.
29. Cali, J. J., C. L. Hsieh, U. Francke, and D. W. Russell. 1991. Mutations in the bile acid biosynthetic enzyme sterol 27-hydroxylase underlie cerebrotendinous xanthomatosis. *J. Biol. Chem.* **266:** 7779–7783.
30. Wood, W. G., F. Schroeder, N. A. Adulov, S. V. Chochina, and U. Igbavboda. 1999. Recent advances in cholesterol dynamics: transport, domains and Alzheimer disease. *Lipids.* **34:** 225–234.
31. Poduslo, J. F., G. L. Curran, T. M. Wengenack, B. Malester, and K. Duff. 2001. Permeability of proteins at the blood-brain barrier in the normal adult mouse and double transgenic mouse of Alzheimer's disease. *Neurobiol. Dis.* **8:** 555–567.
32. Babiker, A., O. Andersson, D. Lindblom, J. van der Linden, B. Wiklund, D. Lütjohann, U. Diczfalusy, and I. Björkhem. 1999. Elimination of cholesterol as cholestenoic acid in human lung by sterol 27-hydroxylase: evidence that most of this steroid in the circulation is of pulmonary origin. *J. Lipid Res.* **40:** 1417–1425.
33. Meaney, S., K. Bodin, U. Diczfalusy, and I. Björkhem. 2003. On the rate of translocation in vitro and kinetics in vivo of the major oxysterols in human circulation: critical importance of the position of the oxygen function. *J. Lipid Res.* **44:** 2130–2135.
34. Lehmann, J. M., S. A. Kliewer, L. B. Moore, T. A. Smith-Oliver, B. B. Oliver, J-L. Su, S. S. Sundseth, D. A. Winegar, D. E. Blanchard, T. A. Spencer, and T. B. Willson. 1997. Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway. *J. Biol. Chem.* **272:** 3137–3140.
35. Whitney, K. D., M. A. Watson, J. L. Collins, W. G. Benson, T. M. Stone, M. J. Numerick, T. K. Tippin, J. G. Wilson, D. A. Winegar, and S. A. Kliewer. 2002. Regulation of cholesterol homeostasis by the liver X receptors in the central nervous system. *Mol. Endocrinol.* **16:** 1378–1385.
36. Wang, L., G. Schuster, K. Hultenby, Q. Zhang, S. Andersson, and J-Å. Gustafsson. 2002. Liver X receptors in the central nervous system: from lipid homeostasis to neuronal degeneration. *Proc. Natl. Acad. Sci. USA.* **99:** 13878–13883.
37. 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.
38. Simmonds, M. A. 2002. The emerging neurobiology of cholesterol. *Neurobiol. Lipids.* **1:** 1–4.