7α-hydroxy-3-oxo-4-cholestenoic acid in cerebrospinal fluid reflects the integrity of the blood-brain barrier

Ahmed Saeed, 1,*† Federico Floris, 1,* Ulla Andersson, *, Irina Pikuleva, ‡ Anita Lövgren-Sandblom, *, Maria Bjerke, ** Martin Paucar, †† Anders Wallin, ** Per Svenningsson, †† and Ingemar Björkhem ††*‡

Division of Clinical Chemistry, Department of Laboratory Medicine, * and Department of Neuroscience, ††Karolinska Institute, Stockholm, Sweden; Department of Biochemistry, †Faculty of Medicine, University of Khartoum, Khartoum, Sudan; Departments of Ophthalmology and Visual Sciences, §Case Western Reserve University, Cleveland, Ohio; and Sahlgrenska Academy, ‡‡University of Gothenburg, Gothenburg, Sweden

Abstract There is a continuous flux of the oxysterol 27-hydroxycholesterol (27-OHC) from the circulation across the blood-brain barrier (BBB) into the brain. The major metabolite of 27-OHC in the brain is 7α-hydroxy-3-oxo-4-cholestenoic acid (7-HOCA). We confirm a recent report describing the presence of this metabolite in cerebrospinal fluid (CSF) at a relatively high concentration. A simple and accurate method was developed for assay of 7-HOCA in CSF based on isotope dilution-mass spectrometry and use of 2H4-labeled internal standard. The concentration of this metabolite was found to be markedly increased in CSF from patients with a dysfunctional BBB. There was a high correlation between the levels of 7-HOCA in CSF and the CSF/serum albumin ratio. The concentration of 7-HOCA in CSF was not significantly affected by neurodegeneration. Our findings suggest that 7-HOCA could be used as a diagnostic marker for conditions with a dysfunctional BBB.—Saeed, A., F. Floris, U. Andersson, I. Pikuleva, A. Lövgren-Sandblom, M. Bjerke, M. Paucar, A. Wallin, P. Svenningsson, and I. Björkhem. 7α-hydroxy-3-oxo-4-cholestenoic acid in cerebrospinal fluid reflects the integrity of the blood-brain barrier. J. Lipid Res. 2014. 55: 313–318.

Supplementary key words brain cholesterol homeostasis • 27-hydroxycholesterol • CYP7B1

7α-Hydroxy-3-oxo-4-cholestenoic acid (7-HOCA) is a metabolite of cholesterol that is formed extrahepatically (1, 2), present in the circulation (3–5), and taken up by the liver (5). Thus there is a continuous flux of this steroid to the liver where it is further oxidized into bile acids. In 1992, Nagata et al. (4) reported an accumulation of this compound in chronic subdural hematomas which contained markedly higher levels than in the circulation. No significant levels of 7-HOCA were detected in cerebrospinal fluid (CSF) under normal conditions. In patients with subarachnoid hemorrhage, however, the concentration was also increased in the CSF (6). The findings by Nagata et al. (4) suggest that there is a local production of 7-HOCA in the brain.

27-Hydroxycholesterol (27-OHC) is a precursor to 7-HOCA, and it was shown by Heverin et al. (7) that there is a continuous uptake of about 5 mg/24 h of 27-OHC from the circulation by the human brain. In the brain there is a very efficient metabolism of 27-OHC, and 7-HOCA was shown to be the major end metabolite (2). This conversion involves participation of the enzymes oxysterol 7α-hydroxylase (CYP7B1), sterol 27-hydroxylase (CYP27A1), and 3β-hydroxydelta-5-steroid dehydrogenase. Presence of CYP7B1 was found to be restricted to neuronal cells only (2). Based on measurements of arteriovenous concentration differences in catheterization experiments, it was shown that there is a continuous flux of 7-HOCA from the human brain into the circulation (7). The magnitude of this flux is about 2 mg/24 h.

In the study by Nagata et al. (4), no significant levels of 7-HOCA were detected in CSF under the conditions employed. The detection limit of their method was not defined, however. Using combined liquid chromatography-mass spectrometry, Ogundare et al. (8) recently reported the presence of 7-HOCA in the CSF.

Abbreviations: BBB, blood-brain barrier; CSF, cerebrospinal fluid; CYP7B1, oxysterol 7α-hydroxylase; CYP27A1, sterol 27-hydroxylase; 7-HOCA, 7α-hydroxy-3-oxo-4-cholestenoic acid; 27-OHC, 27-hydroxycholesterol.

1A. Saeed and F. Floris contributed equally to this work.
2To whom correspondence should be addressed. e-mail: ingemar.bjorkhem@karolinska.se
3According to the recommendation by The Lipid Map, the term 7α-hydroxy-3-oxocholesterol-4-en-26-oic acid should be used rather than 7α-hydroxy-3-oxo-4-cholestenoic acid. Here we prefer to use 7α-hydroxy-3-oxo-4-cholestenoic acid in order to fit with our previous publications.
4According to a recent paper, the preferred nomenclature for 27-hydroxylation and 27-hydroxycholesterol should be (25R)26-hydroxylation and (25R)26-hydroxycholesterol, respectively (18). Here we prefer to use 27-hydroxycholesterol to fit with our previous publications.
In the present work, we have developed a simple accurate method for assay of 7-HOCA based on isotope dilution mass spectrometry and use of d4-labeled 7-HOCA as internal standard. We have confirmed the finding by Ogundare et al. (8), that there are high levels of this steroid acid in the CSF. We found that patients with a dysfunctional blood-brain barrier (BBB) have markedly elevated levels of this oxysterol in CSF and discuss the possibility that this may be used diagnostically.

MATERIALS AND METHODS

Patients

Headache controls. This group consisted of eight subjects (four women and four men, age range 30–49 years, median age 44 years) referred to the neurological clinic because of headache, who were investigated with a CT-scan of the brain, plasma analysis, and a lumbar puncture. All these patients had a normal CSF/serum albumin ratio (<10) (9), and therefore were used as a control group.

Alzheimer’s disease group. This group consisted of 11 subjects (4 females and 7 males, age range 52–72 years, median age 64 years). The patients were diagnosed as having Alzheimer’s disease based on the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria (10). With one exception, all these patients had a normal CSF/serum albumin ratio.

Patients with vascular dementia. This group consisted of 13 subjects (5 females and 8 males, age range 59–79 years, median age 68 years). The diagnostic criteria used were those of Erkinjuntti et al. (11). Three of the patients had a slightly increased CSF/serum albumin ratio (11–13, normally <10).

Patients with BBB dysfunction. This group consisted of 22 subjects (12 females and 10 males, age range 23–77 years, median age 55 years) who had a defective BBB function defined as an increased CSF/serum albumin ratio (14–160, normally <10). Diagnoses in this group included Guillain-Barré’s disease (n = 5), meningitis (n = 9), encephalitis (n = 2), polyneuropathy (n = 1), pareses (n = 2), and miscellaneous (n = 5).

Synthesis of d4-7-HOCA

The synthetic procedure is depicted in Fig. 1. The starting material was 25,26,26,27,27,27-d7-labeled 7α-hydroxycholesterol obtained from Avanti Polar Lipids. d7-7α-Hydroxycholesterol (1 mg) was oxidized with cholesterol oxidase (Cellulomonas species, Sigma C-5421) under the conditions recommended by Sigma. The reaction mixture was extracted with Folch solution (chloroform/methanol, 2:1, v/v). The yield of d7-7α-hydroxy-4-cholesten-3-one was about 800 μg as quantitated by GC-MS. This material was used directly without purification (remaining d7-7α-hydroxycholesterol will not interfere with later quantitation). d7-7α-Hydroxy-4-cholesten-3-one (16 μg) was further oxidized by a reconstituted human CYP27A1 system consisting of CYP27A1, adrenodoxin, and adrenodoxin reductase (2). The material was extracted from the acidified reaction mixture with diethyl ether and the ether phase was washed with water until neutral. The yield in this reaction was about 12 μg of d4-7-HOCA (75%). It should be noted that the oxidation of the steroid side-chain will remove three atoms of deuterium from one of the two methyl groups. The material had a mass spectrum as trimethylsilyl (TMS) ether that differed from the mass spectrum of the unlabeled reference compound (3, 4) with 4 mass units.

Synthesis and quantitation of unlabeled 7-HOCA

Unlabeled 7-HOCA was synthesized as above from unlabeled 7α-hydroxy-4-cholesten-3-one. The material was quantitated by two methods: spectrophotometry utilizing the strong absorption band at 241 mλ with 4-cholesten-3-one as calibrator and combined gas chromatography-mass spectrometry (GC-MS) (as methyl ester TMS ether) using total ion current and comparison with 4-cholesten-3-one as calibrators. The material had a mass spectrum as TMS derivative identical to that published previously (3, 4).

Assay of 7-HOCA

d4-7-HOCA (60 ng) dissolved in 10 μl ethanol, 1 ml CSF, and 5 ml ether were added to a separation funnel. After addition of 20 μl of 0.1 M HCl, extraction was performed and the water phase was removed and saved for the subsequent extraction. The extraction was repeated, and the ether phases were pooled and washed with water until neutral. The ether was evaporated under nitrogen. Then the material was converted into methyl ester with TMS-diazomethane (Aldrich) by adding 100 μl of methanol, 400 μl of toluene, and 40 μl of hexane solution containing 2 M of TMS-diazomethane directly to a glass tube. The mixture was vortexed for 10 s and agitated for 5 min. The solvent and reagents were removed under a stream of nitrogen.

After methylation, the material was converted into TMS ether. TMS reagent (pyridine/hexamethyldisilazane/chlorotrimethylsilane, 3:2:1, v/v/v) was added to the dried extracts and the sealed tube was treated at 60°C for 30 min. The solvent and reagents were removed under a stream of nitrogen until complete dryness. The residue was then dissolved in 20 μl of hexane and transferred to a glass vial, suitable for GC/MS injection.

![Fig. 1. Synthesis of d4-labeled 7-HOCA.](image-url)
GC-MS was performed on a Hewlett Packard 6890 Plus series gas chromatograph equipped with an HP-5MS capillary column (30 m × 0.25 mm, 0.25 μm phase thickness). The chromatograph was connected to an HP 5963 mass selective detector and to CTC Analytics Pal system automatic injector. The oven temperature program was as follows: 180°C for 1 min, 20°C/min to 250°C, and then 4°C/min to 300°C where the temperature was kept for 8 min. Helium was used as a carrier gas. The gas chromatograph was operated in the constant flow mode, with the flow rate set to 0.8 ml He/min. The injector was operated in the splitless mode and was kept at 270°C, and the detector transfer line was kept at 280°C. The samples were analyzed with GC-MS using single ion monitoring, following the ion m/z 426 (for the derivative of 7-HOCA) and the ion m/z 430 (for d4-7-HOCA).

Replicas of samples (n = 5) were analyzed by GC-MS. For the recovery experiment, four replicas of the same sample, with the addition of 20 ng of 7-HOCA, were also analyzed by GC-MS.

Ethical aspects
All patients gave their informed consent to this study. All the investigations of the patients and the analyses of their serum and CSF were approved by the ethic committees of the respective institutions.

RESULTS

Assay
Different conditions were tested for extraction of 7-HOCA by diethyl ether. Increased acidification of the CSF increased the recovery of the steroid up to a certain level. At a high degree of acidification, there was an increased decomposition of 7-HOCA into 3-oxo-cholesta-4,6-dienoic acid. Addition of 20 μl of 0.1 M HCl to 1 ml of CSF was found to give the best recovery of intact 7-HOCA under the conditions employed.

Under the standard conditions employed, the limit of detection was about 0.5 ng/ml and the limit of quantitation about 1 ng/ml. It was possible, however, to reduce the limit of detection by increasing the volume of CSF in relation to the internal standard.

The mass spectrum of the methyl ester TMS derivative of unlabeled and d4-labeled 7-HOCA were in accordance with previously published spectra of the unlabeled compound (3, 4) and showed prominent peaks at m/z 426 and m/z 430, respectively, corresponding to loss of a trimethylsilanol group from the molecular ion.

The standard curve obtained in the analyses of standard mixtures of unlabeled and d4-labeled 7-HOCA was linear with the equation Y (relative response) = 1.03 × 7-HOCA level + 0.01 and r² = 0.999.

Figure 2 shows a typical ion chromatogram obtained in the analysis of a sample of CSF to which d4-7-HOCA had been added. It should be mentioned that in addition to the selected ion monitoring, full scan chromatograms of the methylated and trimethylsilylated serum extract were also generated. Such analyses did not reveal any heterogeneity of the chromatographic peaks.

Replicate analysis of the same pool of CSF (a pool obtained by mixing CSF from about 10 anonymized patient samples) gave a coefficient of variation of 5%. The level of the steroid acid in this pool was 13.9 ± 0.7 ng/ml. Analyses of four replicates of this pool after addition of 20 ng/ml of the steroid acid increased the measured level to 34.6 ± 2.0 ng. The recovery of the added 7-HOCA was thus 104%. The difference between the expected and found levels after the addition was 2%.

Analyses of CSF from controls and patients
Figure 3 shows the results of analyses of CSF from the control group (subjects affected by headache with normal findings on CT-scan of the brain and serum and CSF analyses). The levels of 7-HOCA were 15 ± 5 ng/ml (mean ± SD). The upper normal level (mean + 2 SD) was 21 ng/ml.

We also analyzed CSF from two populations of patients with neurodegeneration: 1) patients with established Alzheimer’s disease according to the diagnostic criteria described in (10); and 2) patients with vascular dementia according to the criteria described in (11). The levels of 7-HOCA in these two populations were both similar to those of the controls, indicating that neurodegeneration does not significantly affect the level of 7-HOCA in CSF. The levels of 7-HOCA in patients with Alzheimer’s disease were found to be 13 ± 4 ng/ml, whereas the corresponding levels in patients with vascular dementia were found to be 14 ± 7 ng/ml.

Figure 3 also shows the results of analyses of CSF from 22 patients with a BBB defect, defined as increased CSF/serum albumin ratio. All but three of these patients had increased levels of 7-HOCA in CSF (range 7–392 ng/ml). There were no trends in the levels of 7-HOCA between the different diagnoses. Thus the levels of 7-HOCA in patients with encephalitis and meningitis varied from 33 to 392 ng/ml.
DISCUSSION

In the previous assay of 7-HOCA in plasma or serum (1–5), two chromatographic steps had to be included in the assay prior to quantitation by combined GC-MS of the methyl ester TMS derivative. $^{14}$C-labeled 3-oxocholanolic acid was used as a recovery marker in the method described by Axelsson and Sjövall (3). It should be emphasized that CSF contains fewer lipids than plasma. Thus, it is not possible to use the present simple method for analysis of 7-HOCA in plasma without including a least one purification step prior to combined GC-MS. The levels of 7-HOCA in plasma have been reported to be about 130 ng/ml (4), about 9-fold higher than the normal levels in CSF found in the present work.

In the method reported by Ogundare et al. (8), the oxysterols in CSF are first isolated by reversed phase C18 chromatography, converted into a hydrazine derivative, and subjected to one additional C18 column chromatography before liquid chromatography-mass spectrometry. The increased level of 7-HOCA in CSF of patients with a BBB defect is in accord with our previous observation that the flux of the precursor, 27-OHC, into CSF is increased under such conditions (14). As expected, there was a significant correlation between 7-HOCA and 27-OHC in CSF from the few patients in which both these steroids could be measured.

The CSF/serum albumin ratio reflects the blood-CSF barrier, but is generally used as a marker for the integrity of the BBB. The possibility must be considered that the high levels of 7-HOCA in CSF in patients with an increased CSF/serum albumin ratio is due to a direct flux of this acid from the circulation into the CSF. Given the net uptake of 27-OHC by the brain from the circulation (7), the very high capacity to convert this steroid into 7-HOCA (2), and the net

The internal standard used was $^{3}$H$_2$-24-hydroxycholesterol. This procedure is considerably more laborious then the present method. From an accuracy point of view, the most obvious advantage of the present method in relation to the method used by Ogundare et al. (8) is the use of an ideal internal standard. It should be emphasized that 7-HOCA is very labile, in particular to alkaline. Thus the 7α-hydroxyl group is easily lost by transaxial elimination together with the 6β-hydrogen to form 3-oxo-cholesta-4,6-dienoic acid. The methyl ester of the latter compound has a mass spectrum containing the ion at m/z 426, and in most analyses we could see at least traces of this compound. In some analyses the amount of 3-oxo-cholesta-4,6-dienoic acid was up to 30% that of 7-HOCA. The ratio between the unlabeled and the $^{3}$H$_2$-labeled compound was, however, always about the same in 7-HOCA as in 3-oxo-cholesta-4,6-dienoic acid. Thus it was evident that most of the 3-oxo-cholesta-4,6-dienoic acid appearing in the chromatogram had been formed from 7-HOCA during extraction and/or work up with the same degree of dehydration of the analyte as of the internal standard. In the absence of the present ideal internal standard with the same chemical identity as the authentic compound, falsely low levels of 7-HOCA can be expected in the assay.

In the present work, we have utilized combined GC-MS. It is likely that combined liquid chromatography-mass spectrometry can be used as well with the same extraction procedure as here.

Similarly to Ogundare et al. (8), we found relatively high levels of 7-HOCA in the CSF. The levels obtained with the present assay, about 15 ng/ml, are however about twice those reported by Ogundare et al. (8) (about 7 ng/ml). Part of the explanation for this could be our use of an ideal internal standard that compensates for degradation of the compound during extraction and workup.

It should be emphasized that the level of 7-HOCA found in CSF in the present work and in the work by Ogundare et al. (8) is higher than that of any other oxysterol hitherto quantitated in this fluid. The levels of 24S-hydroxycholesterol and 27-OHC are present at considerably lower concentrations (12–14). The upper normal limits for 24S-hydroxycholesterol and 27-OHC in CSF have been reported to be 3.0 and 1.5 ng/ml, respectively (13).

Fig. 3. Levels of 7-HOCA in patients with headache (controls) (n = 8), Alzheimer’s disease (n = 11), vascular dementia (n = 13), and BBB defects (n = 22). For details, see Materials and Methods.
Due to the low number of patients, it is not possible to calculate diagnostic sensitivity and specificity from the present study. Three of the patients with BBB defects, according to the CSF/serum albumin ratio, had levels of 7-HOCA within normal limits. 7-HOCA may thus have a lower sensitivity for detection of BBB defects than the CSF/albumin ratio. One of the control subjects had a level of this acid from the brain into the circulation (2), most of the acid present in CSF is likely to originate from the brain rather than from the circulation. While the flux of 7-HOCA across the BBB is about 2 mg/24 h (2), the flux of this steroid from CSF into the circulation can be calculated to be less than 10 μg/24 h. The different fluxes are summarized in Fig. 5.

Due to the low number of patients, it is not possible to calculate diagnostic sensitivity and specificity from the present study. Three of the patients with BBB defects, according to the CSF/serum albumin ratio, had levels of 7-HOCA within normal limits. 7-HOCA may thus have a lower sensitivity for detection of BBB defects than the CSF/albumin ratio. One of the control subjects had a level of this acid from the brain into the circulation (2), most of the acid present in CSF is likely to originate from the brain rather than from the circulation. While the flux of 7-HOCA across the BBB is about 2 mg/24 h (2), the flux of this steroid from CSF into the circulation can be calculated to be less than 10 μg/24 h. The different fluxes are summarized in Fig. 5.
of 7-HOCA in CSF slightly above the upper normal level in spite of a normal CSF/albumin ratio. There is, however, no golden standard, and further work is needed to evaluate whether assay of 7-HOCA has merits in relation to the CSF/albumin ratio.

Neurodegenerative conditions like Alzheimer’s disease are associated with loss of neuronal cells. Because CYP7B1 is located in these cells (2), and because this enzyme activity is critical for the formation of 7-HOCA, neurodegenerative diseases would be expected to be associated with reduced levels of this steroid in CSF. The patients with neurodegeneration studied here, however, had normal levels of 7-HOCA in CSF. Neurodegeneration is sometimes associated with a BBB defect (15), and a long-standing BBB defect may lead to neurodegeneration (16). This may be part of the explanation for the relatively low degree of correlation between 7-HOCA in CSF and the CSF/serum albumin ratio in a minority of the patients studied here. There is a marked difference in molecular weight and polarity between 7-HOCA and albumin, and it seems likely that different types of damage to the BBB may have somewhat different effects on the permeability toward different compounds.

To summarize, the levels of 7-HOCA in CSF seem to reflect the integrity of the BBB and may be used diagnostically as an alternative to the CSF/serum albumin ratio.

The authors gratefully acknowledge the skillful technical assistance of Inger Moberg.

REFERENCES

1. Shoda, J., A. Toll, M. Axelson, F. Pieper, K. Wikvall, and J. Sjövall. 1993. Formation of 7 alpha- and 7 beta-hydroxylated bile acid precursors from 27-hydroxycholesterol in human liver microsomes and mitochondria. Hepatology. 17: 395–403.
2. Meaney, S., M. Heverin, U. Panzenboeck, L. Ekström, M. Axelson, U. Andersson, U. Diczfalusy, I. Pikuleva, J. Wahren, W. Sattler, et al. 2007. Novel route for elimination of brain oxysterols across the blood-brain barrier: conversion into 7alpha-hydroxy-3-oxo-4-cholestenoic acid. J. Lipid Res. 48: 944–951.
3. Axelson, M., B. Mörk, and J. Sjövall. 1988. Occurrence of 3β-hydroxy-5-cholestenoic acid, 3β,7-dihydroxy-5-cholestenoic acid, and 7α-hydroxy-3-oxo-4-cholestenoic acid as normal constituents of human blood. J. Lipid Res. 29: 629–641.
4. Nagata, K., K. Takakura, T. Asano, Y. Seyama, H. Hirota, N. Shigematsu, I. Shima, T. Kasama, and T. Shimizu. 1992. Identification of 7α-hydroxy-3-oxo-4-cholestenoic acid in chronic subdural hematoma. Biochem. Biophys. Acta. 1126: 229–236.
5. 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.
6. Nagata, K., Y. Seyama, and T. Shimizu. 1995. Changes in the level of 7α-hydroxy-3-oxo-4-cholestenoic acid in cerebrospinal fluid after subarachnoid hemorrhage. Neuro. Med. Chin. (Tokyo). 35: 294–297.
7. Heverin, M., S. Meaney, D. Lütjohann, U. Diczfalusy, J. Wahren, and I. Björkhem. 2005. Crossing the barrier: net flux of 27-hydroxycholesterol into the human brain. J. Lipid Res. 46: 1047–1052.
8. Ogundare, M., S. Theofilopoulos, A. Lockhart, L. J. Hall, E. Arenas, J. Sjövall, A. G. Brenton, Y. Wang, and W. J. Griffiths. 2010. Cerebrospinal fluid steroidomics: are bioactive bile acids present in brain? J. Biol. Chem. 285: 4666–4679.
9. Nilsson-Ehle, P., M. Berggren-Söderlund, and E. Theodorsson. 2012. Laurells Klinisk Kemi i praktisk medicin, Studentlitteratur pp 562–567.
10. McKhann, G., D. Drachman, M. Folstein, R. Katzman, D. Price, and E. M. Stadlan. 1984. Clinical diagnosis of Alzheimer’s disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer’s Disease. Neurology. 34: 939–944.
11. Erkinjuntti, T., D. Inzitari, L. Pantoni, A. Wallin, P. Scheltens, K. Rockwood, G. C. Roman, H. Chui, and D. W. Desmond. 2000. Research criteria for subcortical vascular dementia in clinical trials. J. Neural Transm. Suppl. 59: 25–30.
12. Leoni, V., T. Masterman, F. S. Mousavi, B. Wretlind, L. O. Wahlund, U. Diczfalusy, J. Hillert, and I. Björkhem. 2004. Diagnostic use of cerebral and extracerebral oxysterols. Clin. Chem. Lab. Med. 42: 186–191.
13. Björkhem, I., A. Lövgren-Sandblom, V. Leoni, S. Meaney, L. Brodin, L. Salveson, K. Winge, S. Falhagen, and P. Svenningsson. 2013. Oxysterols and Parkinson’s disease: evidence that 24S-hydroxycholesterol in cerebrospinal fluid correlates with the disease. Neurosci. Lett. 555: 102–105.
14. Leoni, V., T. Masterman, P. Patel, S. Meaney, U. Diczfalusy, and I. Björkhem. 2003. Side chain oxidized oxysterols in cerebrospinal fluid and the integrity of blood-brain and blood-cerebrospinal fluid barriers. J. Lipid Res. 44: 793–799.
15. Blennow, K., A. Wallin, P. Fredman, I. Karlsson, C. G. Gottfries, and L. Svennerholm. 1990. Blood brain barrier disturbances in patients with Alzheimer’s disease is related to vascular factors. Acta Neurol. Scand. 81: 323–326.
16. Zlokovic, B. V. 2008. The blood-brain barrier in health and chronic neurodegenerative disorders. Neuron. 57: 178–201.
17. Björkhem, I. 2006. Crossing the barrier: oxysterols as cholesterol transporters and metabolic modulators in the brain. J. Intern. Med. 260: 493–508.
18. Fakheri, R. J., and N. B. Javitt. 2012. 27-Hydroxycholesterol, does it exist? On the nomenclature and stereochemistry of 26-hydroxylated steroids. Steroids. 77: 575–577.