Crossing the barrier: net flux of 27-hydroxycholesterol into the human brain

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Abstract **Side chain oxidized oxysterols have a unique ability to traverse lipophilic membranes. We tested the hypothesis that there is a net flux of 27-hydroxycholesterol from the circulation into the brain using plasma samples collected from the internal jugular vein and an artery of healthy male volunteers. Two independent studies were performed, one in which total levels of 27-hydroxycholesterol were measured and one in which the free fraction of 27-hydroxycholesterol was measured. In the majority of subjects studied, the level of 27-hydroxycholesterol was higher in the artery than in the vein, and uptake from the circulation was calculated to be about 5 mg/24 h. The distribution of 27-hydroxycholesterol in human brain was found to be consistent with an extracerebral origin, with a concentration gradient from the white to the gray matter—a situation opposite that of 24S-hydroxycholesterol, which is exclusively formed in brain. In view of the fact that the blood–brain barrier is impermeable to cholesterol and that 27-hydroxycholesterol is a potent regulator of several cholesterol-sensitive genes, the flux of 27-hydroxycholesterol into the brain may be an important link between intra- and extracerebral cholesterol homeostasis.**—Heverin, M., S. Meaney, D. Lütjohann, U. Diczfalusy, J. Wahren, and I. Björkhem. **Crossing the barrier: net flux of 27-hydroxycholesterol into the human brain.** ***J. Lipid Res.*** **2005.** 46: **1047–1052.**

Supplementary key words blood–brain barrier • brain cholesterol • oxysterol • 24S-hydroxycholesterol

In contrast to cholesterol itself, derivatives with a polar group in the side chain have a unique ability to traverse lipophilic membranes (1, 2). For example, introduction of a hydroxyl group into the steroid side chain markedly facilitates the mobility of the steroid, and conversions of cholesterol into 24S-hydroxycholesterol and 27-hydroxycholesterol are mechanisms by which cholesterol is eliminated from the brain and from a number of extrahepatic organs and tissues, respectively. Attempts have been made in our laboratory to evaluate the quantitative importance of these excretory mechanisms (3–6). By measuring the arterio–venous concentration differences of oxysterols across different organs, we have shown that the flux of 27-oxygenated metabolites from extrahepatic organs to the liver corresponds to ~25 mg/24 h, and the flux of 24S-hydroxycholesterol from the brain to the liver to ~7 mg/24 h. The latter mechanism seems to be the quantitatively most important for elimination of cholesterol from the brain in both man and experimental animals (3, 7–9). We have shown that most of the flux of 24S-hydroxycholesterol from the brain represents a direct transport over the blood–brain barrier, whereas only a minor part is eliminated through cerebrospinal fluid.

Cholesterol 24-hydroxylase (CYP46A1), the enzyme responsible for formation of 24S-hydroxycholesterol, is almost exclusively located in the brain (9), and as a consequence, almost all 24S-hydroxycholesterol present in the human circulation originates from this organ (3). On the other hand, the enzyme responsible for formation of 27-hydroxycholesterol, sterol 27-hydroxylase (CYP27A1), is present in most organs and tissues. Although there is some expression of CYP27A1 in the brain, the levels of the enzymatic product, 27-hydroxycholesterol, in brain tissues are low, only 10–20% those of 24S-hydroxycholesterol (4). Recently, evidence has been presented that there may be some passage of 27-hydroxycholesterol from the circulation into the brain. In a metabolic experiment in which deuterium-labeled cholesterol was infused into a healthy volunteer, deuterium-labeled 27-hydroxycholesterol could be detected in the cerebrospinal fluid (10). In accordance with the demonstrated inability of circulating cholesterol to pass the blood–brain barrier, no detectable deuterium enrichment of cerebrospinal fluid cholesterol or 24S-hydroxycholesterol was observed. Moreover a significant correlation was found between the levels of 27-hydroxycholesterol

Manuscript received 20 January 2005 and in revised form 9 February 2005.

Published, JLR Papers in Press, March 1, 2005.
DOI 10.1194/jlr.M500024-JLR200

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in cerebrospinal fluid and in the circulation of a population of human subjects (11).

In the present work, we tested the hypothesis that there is a significant net flux of 27-hydroxycholesterol from the circulation into the brain by measuring the concentration of this oxysterol in the internal jugular vein and in the brachial artery of healthy volunteers. If there is a net uptake of this oxysterol in the brain, higher levels would be expected in an artery than in the internal jugular vein. For reasons of validation and comparison, respectively, we also measured the levels of 24S-hydroxycholesterol and 7α-hydroxycholesterol. According to our previous work (3, 4), the levels of 24S-hydroxycholesterol would be expected to be higher in the vein than in the artery, whereas 7α-hydroxycholesterol would be expected to be present at the same concentration in both the vein and the artery. As with steroid hormones, a possible uptake of oxysterols from the circulation into the brain would be expected to occur with the steroid in the free form. However, most oxysterols in the circulation are esterified.

In view of these concerns, we made one initial study on 12 healthy volunteers, in which the total concentrations (free and lipoidal esters) of 24S-hydroxycholesterol and 27-hydroxycholesterol were measured, and a later independent study, in which the free forms of all three oxysterols were measured in eight other volunteers. Because the brain represents the donor and acceptor compartments for 24S- and 27-hydroxycholesterol, respectively, we also examined the possible existence of steroid gradients within the brain.

MATERIALS AND METHODS

Materials

Hexadeuterium-labeled cholesterol used in the in vivo experiments on rats was the same as that used in a previous study (12). Deuterium-labeled internal standards for the quantitation of the different steroids, as well as $^2H_4$-labeled 27-hydroxycholesterol for the injection experiment were synthesized as described previously (13).

Autopsy materials from brain were obtained from a male subject who died at the age of 80 from auricular fibrillation and cardiac insufficiency and from a male subject who died at the age of 75 due to occipital infarction. This material was the same as that used in a previous study from our laboratory (3). Four different areas of each brain were analyzed: cerebellum, temporal lobe, frontal lobe, and occipital lobe.

Studies on healthy volunteers

In Study 1, blood samples for determination of the levels of 24S-hydroxycholesterol and 27-hydroxycholesterol were available from 12 healthy male subjects previously studied in connection with brain cholesterol homeostasis (3). In that investigation, only levels of 24S-hydroxycholesterol were reported. The investigation was performed in a fasting state. The blood samples were taken from catheters inserted percutaneously. A thin Teflon catheter was introduced into the brachial artery and a Cournand catheter no. 7 was introduced into a peripheral vein, with the tip positioned in the internal jugular vein at the level of the orbita. Samples were taken simultaneously from the vein and from the artery.

Study 2 was performed as above, but with eight healthy male volunteers aged 21–35 years. Permission to carry out the two sets of experiments was obtained from the ethical committee of the local hospital.

Intravenous injection of [$^2H_4$]27-hydroxycholesterol in the rat

Two male rats (outbred Sprague-Dawley; 150 g) were lightly anesthetised with a solution of Hypnorm (Janssen Pharmaceuticals; Oxford, UK) in saline (1:10; v/v). Immediately before injection, 20 μg of [$^2H_4$]27-hydroxycholesterol dissolved in ethanol was mixed with albumin and physiological saline, and the mixture was injected into the tail vein. The animal was allowed to recover for ~15 min before sacrifice and organ collection as described below. The experiment was approved by the local animal ethics committee.

Animal feeding experiment

Two male rats (outbred Sprague-Dawley; 150 g) were fed a diet of powdered rat chow (Lactamin R36; Vadstena, Sweden) supplemented with 0.3% [$^2H_6$]cholesterol and 2% peanut oil prepared as described previously (12) for 5 and 10 days, respectively. The animals were allowed free access to the chow and water over the course of the experiment. The experiment was approved by the local animal ethics committee.

Organ collection and lipid extraction

Animals were stunned by brief exposure to CO_2 and sacrificed by decapitation. The brain was freed from the skull, rinsed with saline, and transferred to ice-cold saline before being extracted as previously described (12). Briefly, tissue was snap frozen in liquid nitrogen, mechanically pulverized, and extracted with a 40-fold excess of chloroform-methanol (2:1; v/v). Lipid extracts were treated as previously described (13), with the exception that no internal standards were added.

Oxysterol measurements

The oxysterols 24S-hydroxycholesterol, 27-hydroxycholesterol, and 7α-hydroxycholesterol were analyzed by isotope dilution–mass spectrometry using deuterium-labeled oxysterols as internal standards, as previously described (13). The *m/z* of the ions monitored were as follows: 24S-hydroxycholesterol, 413.4; [$^2H_3$]24S-hydroxycholesterol, 416.4; 27-hydroxycholesterol, 456.4; [$^2H_6$]27-hydroxycholesterol, 462.4; 7α-hydroxycholesterol, 456.4; and [$^2H_6$]7α-hydroxycholesterol, 462.4.

Statistics

Data are expressed as mean ± SEM. The significance of differences between the mean arterio–venous concentration difference of each group was tested by two-tailed paired Student's *t*-test. *P* values of <0.05 were regarded as significant. Calculation of percent extraction of individual oxysterols was as follows:

$$E = \frac{(C_a - C_v)}{C_a} \times 100$$

where C_a is the mean concentration in the artery, C_v is the mean concentration in the vein, and E is the net percent extraction. Negative values were considered to represent a net output of sterol. Daily flux of oxysterols across the blood–brain barrier were calculated according to:

$$E = (C_a - C_v) \times CPF$$

where CPF is the mean daily cerebral plasma flow. For the purpose of calculations, CPF was set at 650 l/day.

RESULTS

Concentration of total oxysterols in internal jugular vein and brachial artery of 12 healthy male volunteers (Study 1)

As previously reported (3, 4), the levels of total 24S-hydroxycholesterol were significantly higher in the internal jugular vein than in the brachial artery, 79 ± 7 ng/ml and 68 ± 5 ng/ml, respectively ($P < 0.01$). Among the 12 healthy volunteers, 11 had higher levels of the oxysterol in the vein than in the artery and one had the same level in the artery as in the vein (**Fig. 1**). The net extraction of 24S-hydroxycholesterol was calculated to be −14.7 ± 3.6% ($P < 0.002$), which is equivalent to a net output from the brain to the circulation.

In contrast to the situation with 24S-hydroxycholesterol, the levels of 27-hydroxycholesterol were significantly higher in the artery than in the vein, 167 ± 14 ng/ml and 159 ± 13 ng/ml, respectively ($P = 0.03$). Among the 12 subjects, 10 had higher levels of the oxysterol in the artery than in the vein, whereas the opposite was observed in one subject, and one had the same level in the artery as in the vein. The net extraction of 27-hydroxycholesterol was calculated to be 4.6 ± 2.1% ($P = 0.05$), which is equivalent to a net input into the brain.

Concentration of free oxysterols in internal jugular vein and brachial artery of eight healthy male volunteers (Study 2)

Because the uptake of 27-hydroxycholesterol by the brain is likely to occur in the free form, the fractional extraction of the free steroid would be expected to be considerably greater than that of total 27-hydroxycholesterol. As shown in Fig. 1, the level of free 27-hydroxycholesterol was higher in the artery than in the vein in seven of the eight subjects studied (Fig. 1). The mean concentration in the artery was 25.1 ± 1.9 ng/ml as compared with 21.9 ± 1.4 ng/ml in the vein ($P = 0.06$). The net extraction of free 27-hydroxycholesterol was calculated to be 11.8 ± 4.3% ($P < 0.03$). The level of free 24S-hydroxycholesterol was higher in the vein than in the artery in all eight subjects. As expected, the arterio–venous concentration difference of 24S-hydroxycholesterol (7.3 ± 1.5 ng/ml) was significant ($P < 0.01$), and the net extraction of this steroid was calculated to be −25.7 ± 4.9% ($P < 0.001$). The levels of free 7α-hydroxycholesterol were also measured. The arterio–venous difference (−8.4 ± 9.9 ng/ml) was not significant ($P = 0.43$).

Assuming a flux of 450 ml plasma per minute through the brain (4, 3), the above arterio–venous differences correspond to a total daily flux of about 7 mg 24S-hydroxycholesterol from the brain into the circulation and an uptake of about 5 mg of 27-hydroxycholesterol from the circulation into the brain (calculations based on the figures for free and lipoidal sterols presented in Fig. 1).

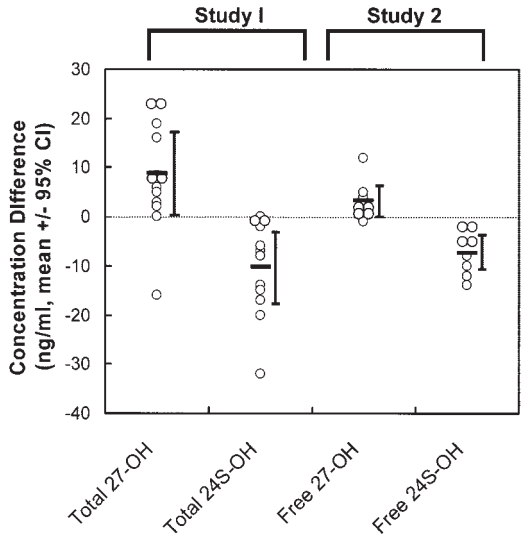

Fig. 1. Arterio–venous concentration difference of free or total 24S-hydroxycholesterol and 27-hydroxycholesterol in the healthy volunteers studied (Studies 1 and 2). Individual data points are represented by open circles, and the mean of each population is represented by a solid line. To the right of each population, 95% confidence intervals are shown. Data on total 24S-hydroxycholesterol in Study 1 were obtained from a previous publication (3) and is reproduced with permission from the publishers.

Entry of extracerebral 27-hydroxycholesterol into the rat brain

Two complementary strategies were employed to evaluate the possible flux of 27-hydoxycholesterol into the brain in either acute or chronic situations. In both cases, a small but significant passage of deuterated 27-hydroxycholesterol from the circulation into the brain was observed. First, injection of a bolus dose of [$^2H_4$]27-hydroxycholesterol (equivalent to a 500-fold excess of the estimated total 27-hydroxycholesterol present in the rat circulation) led to a deuterium enrichment of brain 27-hydroxycholesterol of 3.2% and 9.6%, respectively, in two separate experiments. Similar results were obtained following the feeding of two rats with hexadeuterated cholesterol. In this case, a deuterium enrichment of brain 27-hydroxycholesterol of 4.3% and 5.6%, respectively, was observed, with a corresponding enrichment of brain cholesterol and brain 24S-hydroxycholesterol of less than 1%.

Ratio of 27-hydroxycholesterol to cholesterol in gray and white matter

As shown in **Table 1**, the ratio of 27-hydroxycholesterol to cholesterol was significantly higher in the white matter

TABLE 1. Distribution of cholesterol-related levels of 27-hydroxycholesterol and 24S-hydroxycholesterol in the white and grey matter of human brain (autopsy material from two male subjects)

	27-hydroxycholesterol: cholesterol		24S-hydroxycholesterol: cholesterol	
	White Matter	Grey Matter	White Matter	Grey Matter
	ng/μg			
Subject 1	0.29 ± 0.02	0.20 ± 0.01[a]	0.42 ± 0.05	1.75 ± 0.12[b]
Subject 2	0.30 ± 0.02	0.20 ± 0.02[a]	0.40 ± 0.04	1.63 ± 0.18[b]

Results are given as mean ± SEM.
[a] $P < 0.001$ (paired *t*-test).
[b] $P < 0.01$ (paired *t*-test).

than in the gray matter of four different brain regions of autopsy materials from two subjects. In marked contrast to this, the corresponding ratio of 24S-hydroxycholesterol to cholesterol was 4-fold higher in the gray matter than in the white matter.

DISCUSSION

The results presented here from two independent studies on healthy volunteers are consistent with a net uptake of 27-hydroxycholesterol from the circulation into the brain. Because there was a separate analytical focus in each study—total steroids in Study 1 and free steroids in Study 2—it was not possible to combine the data sets.

The observed net extraction of total 27-hydroxycholesterol was low, only about 5%, and subject to great interindividual variations. Because the brain does not take up cholesterol and oxysterol-containing lipoproteins, it is reasonable to assume that it is the free steroid that is crossing the blood–brain barrier (an assumption that is in line with the current mechanistic models of sterol transfer between lipophilic compartments). In accordance with these assumptions, the net extraction of free 27-hydroxycholesterol, 12 ± 4%, was considerably higher than above. The differences in the measured extractions are likely to be a consequence of the very high activity of plasma lecithin-cholesterol acyltransferase toward side chain oxidized oxysterols (14). It should be pointed out that venous 27-hydroxycholesterol may consist of the secretion of both absorbed and internally synthesized steroid. In view of this, and of a possible secretion of metabolites of 27-hydroxycholesterol, the apparent extraction may underestimate the rate of entry of this steroid into the brain.

The net flux of 27-hydroxycholesterol from the circulation into the human brain demonstrated here is consistent with two previous findings: *a*) a significant flux of deuterium-labeled 27-hydroxycholesterol from the circulation into cerebrospinal fluid in a metabolic experiment performed on a healthy volunteer (10); and *b*) a significant correlation between levels of 27-hydroxycholesterol in circulation and cerebrospinal fluid of both control subjects and patients with different neurological diseases (11). In addition to this, we show here that a flux of 27-hydroxycholesterol from the circulation into the brain is also possible in experimental animals.

The magnitude of the flux of 27-hydroxycholesterol from the circulation into the brain of rats was low, considerably lower than in humans. This may be due to the fact that the absolute plasma levels of 27-hydroxycholesterol are markedly lower in rat than in man, only a few ng/ml as compared with about 150 ng/ml, respectively. It may be mentioned that there are great variations in levels of 27-hydroxycholesterol in the animal kingdom, from only a few ng/ml to levels of up to 250 ng/ml or more (avian species) (I. Björkhem and T. Mörner, unpublished observations).

According to the data presented here, the apparent flux of 27-hydroxycholesterol into the human brain is of a magnitude similar to the flux of 24S-hydroxycholesterol

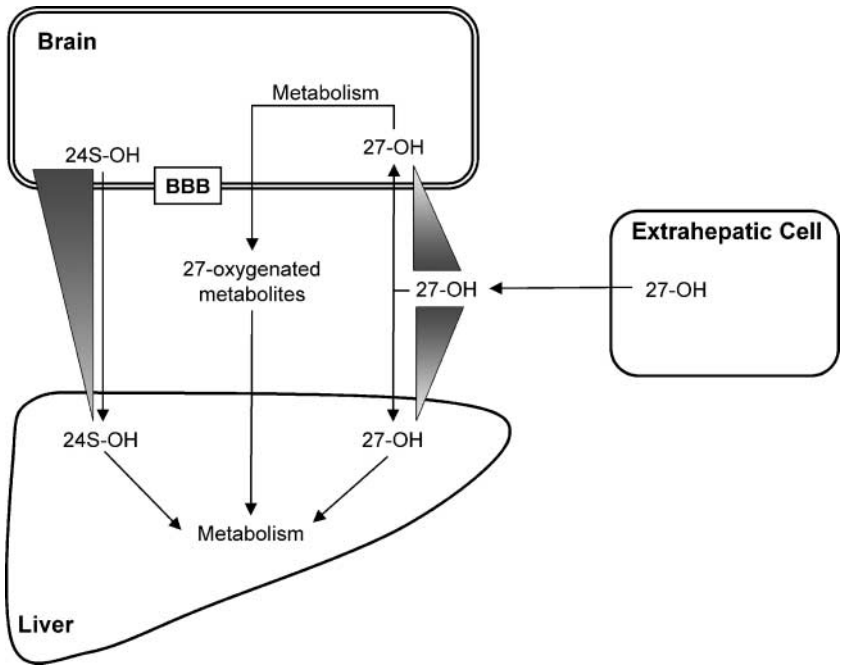

Fig. 2. Schematic view of concentration-dependent movements of 24S-hydroxycholesterol and 27-hydroxycholesterol. The concentration gradients are illustrated by the filled triangles, with darker shading equivalent to higher concentration. In this simplified view, the flux of 24S-hydroxycholesterol from the brain to the liver is facilitated by a corresponding hepatic metabolism (i.e., the metabolic sink). Similar gradients exist for the movement of 27-hydroxycholesterol from the periphery to the liver. The movement of 27-hydroxycholesterol from the circulation to the brain can also be explained by such a metabolic sink.

from the brain. There was, however, no significant correlation between the concentration differences of these steroids (results not shown). Furthermore, because patients with a genetic lack of CYP27A1 (cerebrotendinous xanthomatosis) have normal levels of 24S-hydroxycholesterol in the circulation (I. Björkhem, unpublished observation), it is unlikely that the efflux of 24S-hydroxycholesterol from the brain is dependent upon a corresponding uptake of 27-hydroxycholesterol.

It should be pointed out that although the two oxysterols have essentially identical physicochemical properties, the capacity of 27-hydroxycholesterol to pass lipophilic membranes appears to be somewhat greater than that of 24S-hydroxycholesterol (2). In addition, 24S-hydroxycholesterol and 27-hydroxycholesterol are potent suppressors of cholesterol synthesis and regulators of gene expression (15, 16). However, compared with 27-hydroxycholesterol, 24S-hydroxycholesterol is a more robust activator of the nuclear receptor LXRβ, which appears to have an important role in cholesterol homeostasis in the brain (17–20). Moreover, from a metabolic point of view, there is a marked difference between 24S-hydroxycholesterol and 27-hydroxycholesterol. Under in vitro conditions, there is little or no metabolism of 24S-hydroxycholesterol in various preparations from neurological tissues, whereas highly efficient systems exist for conversion of 27-hydroxycholesterol into more polar products. These include 7α,27-dihydroxycholesterol, 7α-hydroxy-3-oxo-4-cholestenoic acid, and perhaps also chenodeoxycholic acid (21–23). However, to what extent this occurs under in vivo conditions, and how the different metabolites may be removed from the brain, are unknown at present.

In the present work, we compared the oxysterol composition between the white and the gray matter. The concentration of 27-hydroxycholesterol was significantly lower in the cell-rich gray matter than in the white matter in the subjects studied. This was in marked contrast to the distribution of 24S-hydroxycholesterol, where the ratio of the oxysterol to the cholesterol was much higher in the gray matter than in the white matter (Table 1). It may be mentioned that according to a recent immunocytochemical study, only small amounts of CYP27A1 were observed in the white matter of normal brains, with a much more intensive staining in the gray matter (24). The difference between the distribution of the synthetic enzyme and the distribution of the oxysterol supports the contention that most of the 27-hydroxycholesterol in the brain originates from extracerebral sources. The lower levels in the gray matter may possibly reflect a dedicated metabolic activity in these regions of the brain.

An explanation for the low levels of 27-hydroxycholesterol in the brain, particularly in the gray matter, may be a high capacity of the brain to convert 27-hydroxycholesterol into more polar products, in effect creating a metabolic "sink." The first step in the metabolism of 27-hydroxycholesterol in the brain is likely to be catalyzed by the oxysterol 7α-hydroxylase (CYP7B1), which is present at surprisingly high levels in the brain (25). Because 7α-hydroxylated metabolites of oxysterols may be less cytotoxic and also less inhibitory in relation to cholesterol synthesis (26–28), the high levels of CYP7B1 in the brain may be regarded as a protective mechanism. These metabolites must, however, be removed from the brain. Attempts are now being made in our laboratory to define the terminal excretion products of 27-hydroxycholesterol from the human brain.

In previous studies that examined the arterio–venous concentration differences across the liver (3, 6), the apparent net extraction of total 27-hydroxycholesterol was found to be similar to that found here for the brain (3, 6). Importantly, the apparent net extraction of more polar metabolites increased in line with increasing activity of the steroid as a bile acid intermediate, with an apparent extraction of >40% of 7α-hydroxy-3-oxo-4-cholestenoic acid reaching the liver. This provides independent support for the importance of metabolic sinks in establishing and maintaining concentration gradients. A schematic view of possible metabolic sinks governing movement between some physiological compartments is shown in **Fig. 2**.

In conclusion, we have demonstrated a net uptake of 27-hydroxycholesterol by the brain, of the same magnitude as that in the liver. In view of the potent effects of 27-hydroxycholesterol on cholesterol metabolism, this flux is likely to be of importance for intracerebral cholesterol homeostasis.

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 Swedish Brain Foundation, and the Foundation "Gamla Tjänarinnor."

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