On the mechanism of cerebral accumulation of cholestanol in patients with cerebrotendinous xanthomatosis

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Abstract   The most serious consequence of sterol 27-hydroxylase deficiency in humans [cerebrotendinous xanthomatosis (CTX)] is the development of cholestanol-containing brain xanthomas. The cholestanol in the brain may be derived from the circulation or from 7α-hydroxylated intermediates in bile acid synthesis, present at 50- to 250-fold increased levels in plasma. Here, we demonstrate a transfer of 7α-hydroxy-4-cholesten-3-one across cultured porcine brain endothelial cells (a model for the blood-brain barrier) that is ~100-fold more efficient than the transfer of cholestanol. Furthermore, there was an efficient conversion of 7α-hydroxy-4-cholesten-3-one to cholestanol in cultured neuronal and glial cells as well as in monocyte-derived macrophages of human origin.▪▪ It is concluded that the continuous intracellular production of cholestanol from a bile acid precursor capable of rapidly passing biomembranes, including the blood-brain barrier, is likely to be of major importance for the accumulation of cholestanol in patients with CTX. Such a mechanism also fits well with the observation that treatment with chenodeoxycholic acid, which normalizes the level of the bile acid precursor, results in a reduction of cholestanol-containing xanthomas even in the brain.—Panzenboeck, U., U. Andersson, M. Hansson, W. Sattler, S. Meaney, and I. Björkhem. On the mechanism of cerebral accumulation of cholestanol in patients with cerebrotendinous xanthomatosis. *J. Lipid Res.* 2007. 48: 1167–1174.

Sterol 27-hydroxylase deficiency [cerebrotendinous xanthomatosis (CTX)] is a rare familial lipid storage disease characterized by the accumulation of cholesterol and cholestanol in most tissues, in particular in tendon and brain xanthomas (for review, see Ref. 1). The most serious symptoms (dementia, cerebellar ataxia, and spinal cord paresis) are caused by the brain xanthomas.

The link between this gene defect and the accumulation of cholestanol is not, however, immediately obvious. We have shown previously that the major part of the cholestanol present in the circulation of CTX patients is derived from 7α-hydroxylated intermediates in bile acid synthesis, such as 7α-hydroxycholesterol and 7α-hydroxy-4-cholesten-3-one (1, 2). These intermediates accumulate in the circulation of CTX patients as a result of the reduced synthesis of bile acids, which in turn leads to a reduced suppression of cholesterol 7α-hydroxylase, the rate-limiting enzyme in bile acid synthesis. In untreated CTX patients, the levels of these intermediates are increased by >100-fold (1, 3, 4). We have also shown that 7α-hydroxy-4-cholesten-3-one is metabolized to cholestanol under in vitro conditions, with cholesta-4,6-dien-3-one and 4-cholesten-3-one as intermediates (1) (**Fig. 1**).

An enduring mystery of CTX is the origin of the cholestanol present in the brain of patients. The simplest mechanism, direct blood-to-brain passage of cholestanol formed extracerebrally, is predicated on the ability of cholestanol to cross the blood-brain barrier. In support of such a mechanism, Buchmann and Clausen (5) showed that rabbits fed a diet enriched with cholestanol for 8 weeks had brain cholestanol approximately twice that of animals fed a control diet. Additionally, Byun et al. (6) showed that feeding mice with 1% cholestanol for 8 months led to a significant enrichment of this sterol in cerebellum. It is thus clear that cholestanol can to some extent pass the blood-brain barrier. An alternative pathway could be the passage of a circulating precursor such as 7α-hydroxycholesterol or 7α-hydroxy-4-cholesten-3-one into the brain, where it may be subsequently converted to cholestanol.

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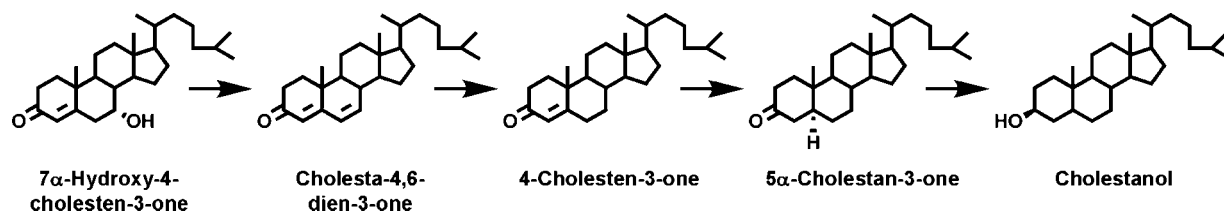 e-mail: ingemar.bjorkhem@karolinska.se

Fig. 1. Sequence of reactions in the conversion of 7α-hydroxy-4-cholesten-3-one to cholestanol.

We show here, using an established model of the blood-brain barrier, that the bile acid precursor 7α-hydroxy-4-cholesten-3-one is able to pass at a markedly higher rate than cholestanol. Moreover, we also show that 7α-hydroxy-4-cholesten-3-one is efficiently converted to cholestanol by human cell models of neurons, astrocytes, and microglia and also by human monocyte-derived macrophages. These results are discussed in relation to the mechanism underlying the accumulation of cholestanol.

MATERIALS AND METHODS

Labeled steroids

7β-^{3}H-labeled 7α-hydroxycholesterol and 7α-hydroxy-4-cholesten-3-one were prepared as described previously (7, 8), and both compounds had a specific radioactivity of 0.1×10^6 cpm/µg. ^{3}H-labeled 27-hydroxycholesterol was prepared as described previously (7) and had a specific radioactivity of 0.4×10^6 cpm/µg. [4-^{14}C]Cholesterol from Applied Biosystems was diluted with "cold" cholesterol to a specific radioactivity of 0.25×10^6 cpm/µg. [4-^{14}C]Cholestanol with the same specific radioactivity was obtained by hydrogenation of [4-^{14}C]cholesterol and purification by thin-layer chromatography as described previously (9). [26,26,26,27,27,27-^{2}H$_6$]Cholesterol was from Medical Isotopes (Concord, NH). ^{2}H$_6$-labeled 7α-hydroxy-4-cholesten-3-one was obtained from [^{2}H$_6$]cholesterol by conversion to [^{2}H$_6$]7α-hydroxycholesterol (10) followed by oxidation with cholesterol oxidase (11). The final product was purified by preparative thin-layer chromatography using toluene-ethyl acetate (3:7, v/v) as the moving phase.

Isolation of porcine brain microvascular endothelial cells

Porcine brains were obtained from freshly slaughtered pigs in the local slaughterhouse. After removal of the meninges and secretory areas, the gray and white matter of the cerebral cortex was minced and the endothelial cells were isolated by several enzymatic digestion and centrifugation steps as described (12). Clusters from one brain were seeded in M199 (containing 10% ox serum, 1% penicillin/streptomycin, and 1% gentamycin; medium A) on six 75 cm^2 calf skin collagen (60 µg/ml)-coated flasks. After 1 day in vitro, cells were washed with PBS and cultivated in medium B (medium A without gentamycin).

In vitro blood-brain barrier experiments

Cells were seeded on calf skin collagen-coated multiwell Transwell cell culture clusters (12 wells) at a density of 40,000 cells/cm^2 in medium B, with 0.5 ml of medium in the apical compartment and 1.5 ml in the basolateral compartment. After 3 days, induction of tight junctions was initiated via overnight incubation in DMEM/Ham's F12 supplemented with 150 nM hydrocortisone, 0.25% glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin. The tightness of the monolayer was ascertained by measuring the transendothelial electrical resistance (TEER) using an Endohm electrode (13).

For flux experiments, labeled steroids, dissolved in ethanol, were added to the apical compartment. Fatty acid-free BSA (2 mg/ml) or human serum (1%, v/v) was added as sterol acceptor to the apical and/or basolateral compartment in some experiments. At the indicated time points, 100 µl of the apical medium was removed to count the radioactivity and was replaced with fresh medium. At the end of the incubation period, TEERs were again measured to verify the integrity of the monolayer. Finally, to measure the cell-associated radioactivity, cells were washed with PBS, transferred to a new 12-well chamber, and lysed in 0.3 N NaOH by overnight shaking at 4°C.

In addition to TEER of the Transwell cultures used, [^{14}C] sucrose permeability was used as a second parameter to ascertain the tightness of the monolayers. Two hundred microliters of the medium in the apical compartment was removed and substituted with assay medium containing 2.5 µCi of [^{14}C]sucrose. Fifty microliter samples were taken in duplicate from the basolateral compartment every 20 min (over 2 h) and replaced with 100 µl of fresh medium. Permeability coefficients were calculated as described (12).

Incubation of 7α-hydroxy-4-cholesten-3-one with different cultured human brain cells

SH-SY5Y neuroblastoma cells were routinely cultured in DMEM with 10% (v/v) fetal calf serum, 100 U/ml , 100 U/ml penicillin, and 100 µg/ml streptomycin. CHME-3 microglia (SV-40 large T-antigen immortalized fetal microglia) and D-384 astrocytoma cells (a clonal cell line established from human astrocytoma) were cultured in high-glucose DMEM (4,500 mg/l glucose) supplemented with 10% fetal calf serum, 2 µM Glutamax™, 100 U/ml penicillin, and 100 µg/ml streptomycin. All cells were grown at 37°C in a humidified atmosphere containing 5% CO$_2$.

For metabolic transformation experiments, the medium was removed from the cells and replaced with serum-free medium containing 2 µg/ml unlabeled or ^{2}H-labeled 7α-hydroxy-4-cholesten-3-one dissolved in 45% 2-hydroxypropyl-β-cyclodextrin. Triplicate 100 mm dishes (each containing ∼10 × 10^6 cells) were incubated, and cells were harvested at 24, 48, and 72 h. Cells were washed twice with PBS containing a protease inhibitor cocktail (Complete EDTA free; Roche) using a cell scraper. Cell preparations were then centrifuged at 10,000 g for 2 min. After removal of the supernatant, the cell pellets were frozen at −20°C until required.

In some specific experiments, cells were incubated with [^{2}H$_6$] 7α-hydroxy-4-cholesten-3-one or [7β-^{3}H]7α-hydroxycholesterol under similar conditions to those described above. Medium was extracted and analyzed as described below or by radio-HPLC.

Incubation of 7α-hydroxy-4-cholesten-3-one with human monocyte-derived macrophages

Partially purified human buffy coats from the district blood bank were used as a source of monocytes, which were further purified using Ficoll Paque (Pharmacia)-based density gradient centrifugation. Briefly, 1 volume of buffy coat was mixed with 1.5 volumes of PBS, layered on 1 volume of Ficoll Paque, and centrifuged at 2,000 g for 30 min. The resulting supernatant (containing a mixture of monocytes and lymphocytes) was collected and washed three times in PBS at 600 g, seeded onto 60 mm dishes, and incubated in 37°C overnight. Nonadherent cells were removed by washing, and adherent monocytes were grown in Macrophage-SFM (Invitrogen, Carlsbad, CA) supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin. Medium was changed every 48 h.

Steroid analyses in cultured cells

The incubations were extracted with chloroform-methanol (2:1, v/v) and purified by liquid-liquid and solid-phase extraction. Steroids were analyzed by combined gas chromatography-mass spectrometry as trimethylsilyl ether derivatives. The molecular ion of $^2H_6$-labeled cholesterol (m/z 464) was used as a standard for the quantitation of both cholestanol (m/z 460) and 4-cholesten-3-one (m/z 384). In the experiment using [$^2H_6$]7α-hydroxy-4-cholesten-3-one, the ions corresponding to [$^2H_6$]cholestanol (m/z 466) and [$^2H_6$]4-cholesten-3-one (m/z 390) were monitored.

Statistical evaluations

Results are presented as means ± SEM or as indicated in the figure legends. Significance was determined using the Student's t-test.

RESULTS

Rapid transfer of 7α-hydroxy-4-cholesten-3-one across porcine brain microvascular endothelial cells

The quality and tightness of the Transwell cultures were ascertained by measuring the TEER during the transport experiments and by measuring the corresponding permeability coefficient for [^{14}C]sucrose (12). The electrical resistance varied between 250 and 800 Ω/cm^2, and the corresponding permeability coefficient for [^{14}C]sucrose was on average 1.2×10^{-6} cm/s.

As a first step in testing our hypothesis that 7α-hydroxy-4-cholesten-3-one is the source of intracerebral cholestanol, we investigated the capacity of this steroid to undergo apical-to-basolateral transport. Using a well-established in vitro model of the blood-brain barrier based on porcine cerebral microvascular endothelial cells, we could demonstrate an efficient time-dependent transfer of [^3H]7α-hydroxy-4-cholesten-3-one across the cell monolayer. This transfer, corresponding to 14 ± 1% of the total radioactivity present in each well, was significantly greater than that of Δ^5 oxysterols such as 7α-hydroxycholesterol (1.9 ± 0.4%) and 27-hydroxycholesterol (2.7 ± 0.1%) (**Fig. 2A**). Importantly, the low transfer of either cholestanol (0.09 ± 0.01%) or cholesterol (0.21 ± 0.02%) (Fig. 2B) indicates that the cell monolayer accurately reflects the in vivo sterol resistance of the blood-brain barrier. To gauge the retention of each steroid by the cells, the residual radioactivity was also measured. The percentage recovery of each steroid is shown in Fig. 2C. Finally the influence of the steroid concentration on the transfer of cholestanol, 27-hydroxycholesterol, and 7α-hydroxy-4-cholesten-3-one was investigated. As Fig. 2D shows, there was no significant effect of concentration on the fraction of the steroid transferred. Because of solubility problems, it was not possible to study the rate of transfer of the steroids across the cultured cells with higher concentrations of the oxysterols than those used here.

In addition to these experiments, we performed several experiments in which albumin was excluded from the medium or added to the basolateral side only. All of these experiments gave similar results with respect to the relative rate of transfer of 7α-hydroxy-4-cholesten-3-one, cholesterol, and cholestanol.

Conversion of 7α-hydroxy-4-cholesten-3-one to cholestanol by cultured neuronal and glial cells

We incubated $^2H_6$-labeled 7α-hydroxy-4-cholesten-3-one with neuroblastoma SH-SY5Y cells for 48 h under the conditions described in Materials and Methods. Gas chromatography-mass spectrometry of the trimethylsilyl ether derivative of an extract of the cells showed a significant conversion to $^2H_6$-labeled 4-cholesten-3-one and cholestanol (**Fig. 3**).

Based on these results, we then investigated the capacity of different cell systems to carry out the reactions depicted in Fig. 1. Incubation of SH-SY5Y neuroblastoma, D-384 astrocytoma, and CHME-3 microglia cells with 20 μg of 7α-hydroxy-4-cholesten-3-one led to a time-dependent formation of both 4-cholesten-3-one and cholestanol (**Fig. 4A, B**, respectively).

The most efficient conversion to cholestanol was found in the neuroblastoma cells, although there was a lag during the first 24 h. There was a similar lag also in the conversion to the precursor 4-cholesten-3-one. The lag may be attributable to the slow growth of the cells (see below). In astrocytoma cells, there was a relatively efficient conversion to 4-cholesten-3-one but only a low conversion to cholestanol. In the microglia cells, there was a time-dependent increase in both 4-cholesten-3-one and cholestanol during the first 48 h of incubation. Between 48 and 72 h of incubation, there was a decrease in the accumulation of 4-cholesten-3-one but an increase in the formation of cholestanol.

Because the cellular cholesterol pools expanded during the incubations, the accumulations of cholestanol and 4-cholesten-3-one shown in Fig. 4A, B are shown in relation to cholesterol. During the 72 h of incubation, the total cholesterol pool expanded by 51% in the neuroblastoma cells, by 124% in the astrocytoma cells, and by 164% in the microglia cells.

Possible conversion of 7α-hydroxycholesterol to cholestanol in brain cells

Plasma levels of 7α-hydroxycholesterol are markedly increased in patients with CTX (4). As 7α-hydroxycholesterol is the immediate precursor of 7α-hydroxy-4-cholesten-3-one, and because the enzyme involved in the conversion, HSD3B7, is present in most cells, the possibility was tested that 7α-hydroxycholesterol may be converted to cholestanol in brain cells. Incubation of cul-
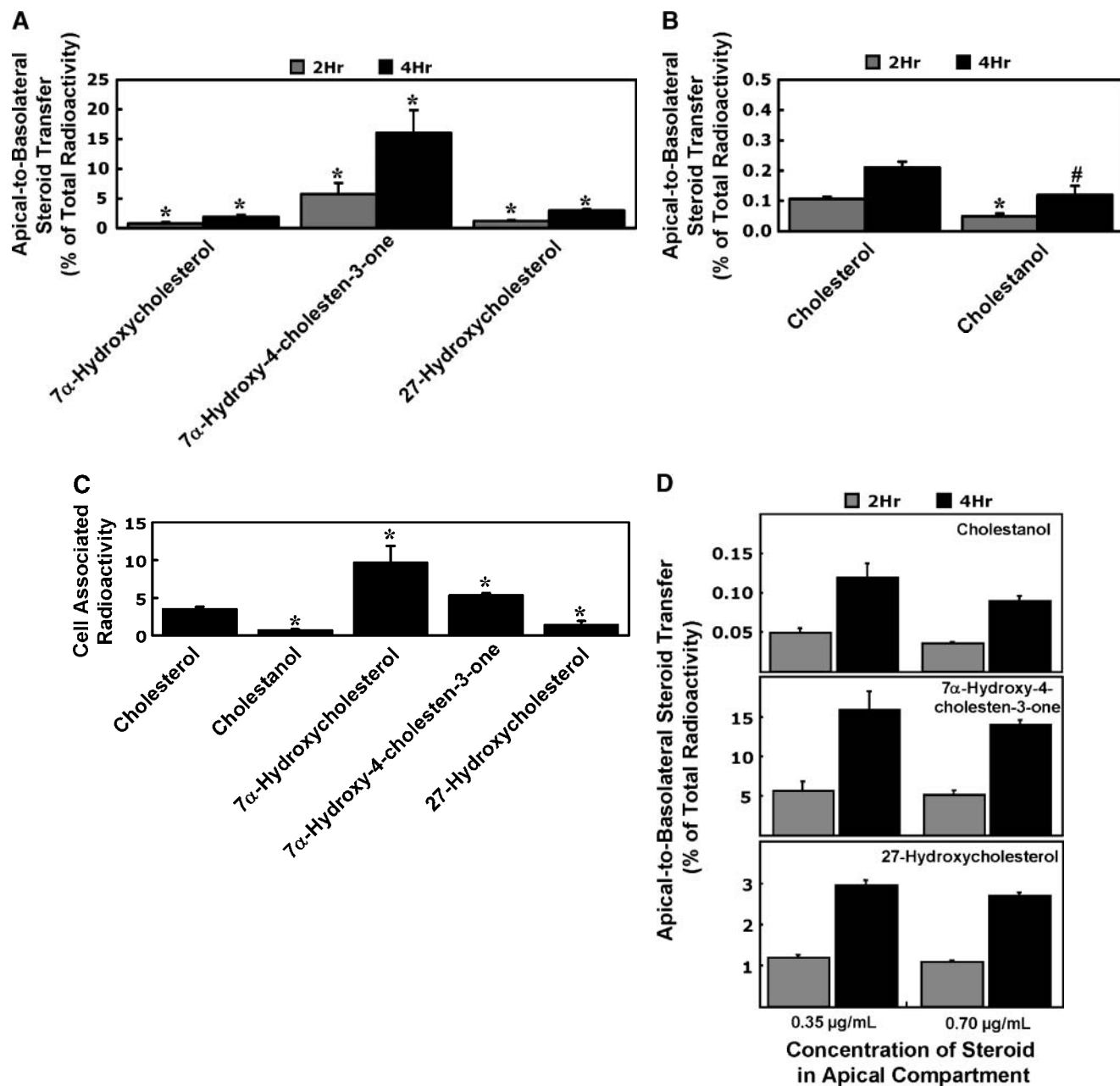
Fig. 2. Transfer of cholestanol precursors across porcine brain endothelial cells. A, B: Apical-to-basolateral transfer of 7α-hydroxy-4-cholesten-3-one, 7α-hydroxycholesterol, 27-hydroxycholesterol (A), cholestanol, and cholesterol (B). Note the difference in scales between the two panels. Cholesterol and 27-hydroxycholesterol were included as controls for sterol impermeability and permeability, respectively. C: Cell-associated radioactivity retained in the cells (4 h experiment). D: Effect of two steroid concentrations on the transfer. Means ± SD of three experiments are shown. For details, see Materials and Methods. *$P < 0.01$, #$P < 0.05$.

tured astrocytes and neuroblastoma cells with radioactive 7α-hydroxycholesterol resulted in a low conversion to 7α-hydroxy-4-cholesten-3-one but only traces of radioactive cholestanol (results not shown).

Conversion of 7α-hydroxy-4-cholesten-3-one to cholestanol in monocyte-derived macrophages

As shown in **Fig. 5**, there was a significant accumulation of cholestanol in monocyte-derived macrophages exposed to different concentrations of 7α-hydroxy-4-cholesten-3-one in

the culture medium during 4 days of culture. The production of cholestanol was dose-dependent up to a concentration of 4 μg/ml oxysterol. The accumulation of cholestanol in the experiments described above did not affect the concentration of cholesterol or its degree of esterification (results not shown). The amount of cholestanol accumulated in the cells after exposure to 4 μg/ml oxysterol corresponded to ~6% of the total steroid pool in the cells.

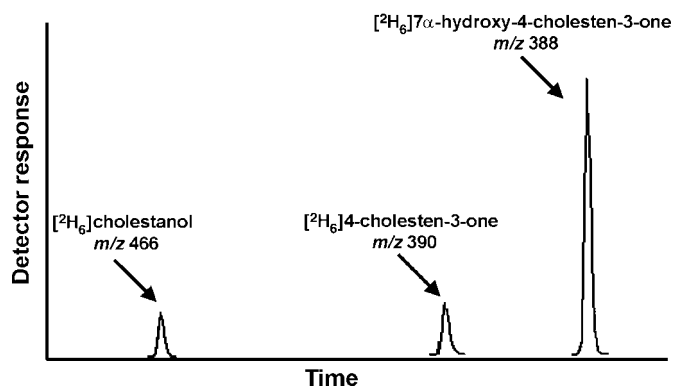When exposing the cells to a concentration of 8 μg/ml or higher of 7α-hydroxy-4-cholesten-3-one, a toxic effect

Fig. 3. Metabolism of $^2H_6$-labeled 7α-hydroxy-4-cholesten-3-one by SH-SY5Y neuroblastoma cells. Conversion of the putative precursor steroid to both 4-cholesten-3-one and cholestanol is clearly evident. For details, see Materials and Methods.

was seen, with cell death and reduction of cholestanol formation (Fig. 5A).

Effects of direct loading of monocyte-derived macrophages with cholestanol

Direct exposure of monocyte-derived macrophages to cholestanol at levels from 2 to 20 μg/ml caused a dose-dependent accumulation of this compound in the cells up to a level almost twice that of cholesterol. Figure 5B shows the results from a typical set of experiments. When increasing the concentration of cholestanol to 50 μg/ml, there was extensive cell death. Notably, the accumulation of cholestanol had very a small effect on the total level of cholesterol, consistent with the possibility that cholestanol is less efficient than cholesterol in suppressing cholesterol synthesis (see below).

DISCUSSION

Accumulation of cholestanol is one of the hallmarks of CTX, and it has been reported that cholestanol may account for up to one-third of the steroid fraction of the CTX cerebellum (14). However, the source of this cholestanol has not been obvious. The impermeability of the blood-brain barrier to cholesterol is well documented, and given the virtually identical physicochemical properties of cholesterol and its saturated analog (e.g., polarity, partition coefficient), cholestanol would be expected to behave in a similar manner with respect to the blood-brain barrier. In the rather extreme animal experiments described above, however, chronic exposure of the cerebral circulation to levels of cholestanol up to 100-fold greater than normal resulted in a cholestanol enrichment corresponding to 3% of the steroid pool (5, 6). In view of the modest enrichment with cholestanol in these experiments, the possibility must be considered that a significant part of the cholestanol present in the brain of CTX patients is derived from an alternative source. In view of our previous findings that plasma levels of 7α-hydroxy-
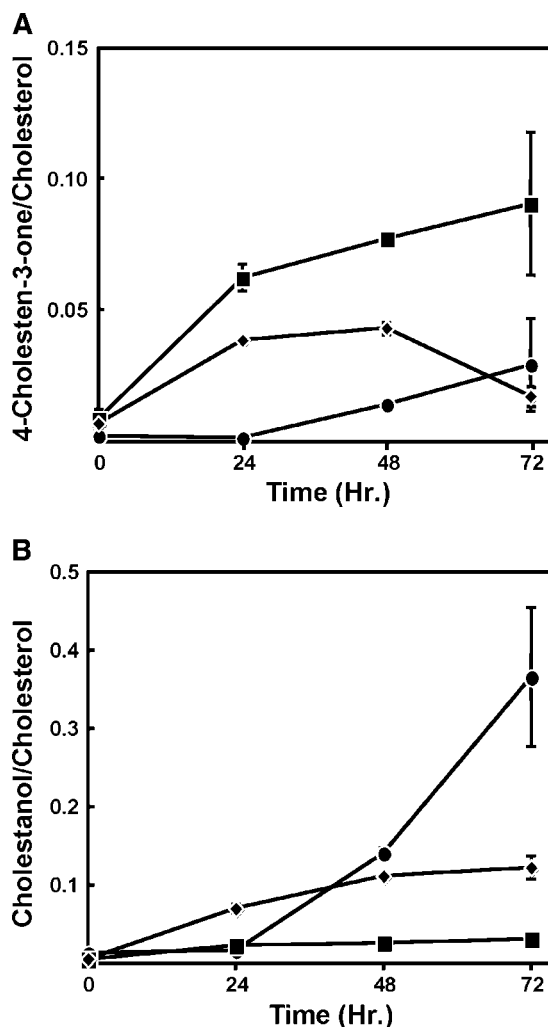

Fig. 4. Time-dependent conversion of 7α-hydroxy-4-cholesten-3-one to 4-cholesten-3-one (A) and cholestanol (B) in cultured SH-SY5Y neuroblastoma cells (closed circles), D-384 astrocytoma cells (closed squares), and CHME-3 microglia cells (closed diamonds). To correct for any expansion in the pool of cells (and thus cholesterol), cholestanol and 4-cholesten-3-one are expressed in relation to cholesterol. Means ± SEM are shown.

4-cholesten-3-one are increased by 50- to 250-fold in CTX (1, 3), we considered this steroid to be a likely candidate precursor.

As a first step, we examined the capacity of this steroid to pass a model system of the blood-brain barrier. The integrity of this model system was ascertained by three different observations: *1*) the high transendothelial resistance; *2*) the low permeability of sucrose; and *3*) the very low rate of passage of cholesterol, mimicking the situation in vivo (15). Apical-to-basolateral passage (which corresponds to transfer in the blood-to-brain direction) of 7α-hydroxy-4-cholesten-3-one was more than five times greater than that of 27-hydroxycholesterol, a polar oxysterol that is known to traverse the blood-brain barrier in vivo (16). Interestingly, the rate of transfer of 7α-hydroxycholesterol was almost 10 times lower than that of 7α-hydroxy-4-cholesten-3-one, suggesting that the 3-oxo-Δ^4 configuration is a
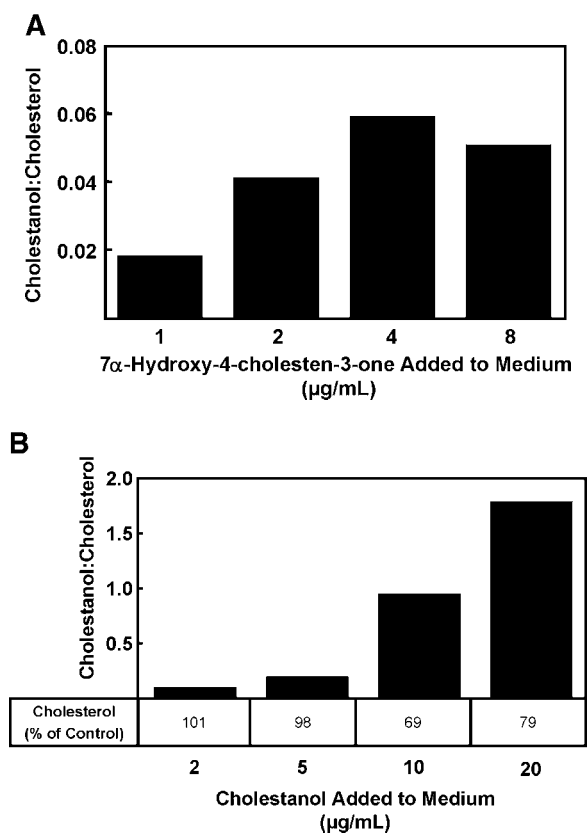
Fig. 5. Cholestanol accumulation in monocyte-derived macrophages. Exposure to different concentrations of either 7α-hydroxy-4-cholesten-3-one (A) or cholestanol (B) itself led to a concentration-dependent intracellular accumulation of cholestanol. The influence of cholestanol accumulation on cell cholesterol levels is shown at the bottom of panel B. Means of two experiments are shown.

structure allowing a high-efficiency transport across the blood-brain barrier.

The polarity of the different steroids tested here increases in the following order: cholestanol < cholesterol < monohydroxylated cholesterol. In connection with thin-layer chromatography, the order of polarity is the following: cholestanol < cholesterol < 24S-hydroxycholesterol and 27-hydroxycholesterol < 7α-hydroxy-4-cholesten-3-one < 7α-hydroxycholesterol. This order of polarities does not reflect the hydrophobic interactions occurring between a steroid and the fatty acid acyl chains in membrane phospholipids. The introduction of a hydroxyl group in the side chain of cholesterol leads to a local reordering of the membrane phospholipids (7). This leads to a much more rapid passage of side chain oxidized oxysterols than of cholesterol across phospholipid membranes. In a previous work, we measured the rate of transfer of different oxysterols from erythrocyte membranes to plasma lipoproteins, and the following order of transfer was found: cholesterol < 7α-hydroxycholesterol < 24S-hydroxycholesterol < 27-hydroxycholesterol (7). The difference between nuclear and side chain oxidized sterols was readily apparent. The rate of transfer of cholesterol

and monohydroxylated cholesterol species across the endothelial cells closely followed the pattern observed in the previous study with erythrocyte membranes. 7α-Hydroxy-4-cholesten-3-one was never tested in the previous studies, however, and the high rate of transfer of this compound is surprising and difficult to explain by its physicochemical properties or by a possible interaction with membrane phospholipids. In preliminary experiments, we found that the rate of transfer of 7α-hydroxy-4-cholesten-3-one from erythrocytes to plasma was much higher than the corresponding rate of transfer of 7α-hydroxycholesterol and similar to the rate of transfer of 27-hydroxycholesterol. Because of the very high rate of transfer, ~80% or greater for both of these steroids, it was not possible to discriminate between 27-hydroxycholesterol and 7α-hydroxycholesterol with this method. It is evident, however, than both of these steroids pass very efficiently from erythrocyte membranes to plasma and move across endothelial cells

Because of the solubility problems, it is not possible to draw firm conclusions from the results of the present experiments concerning whether or not an active transport of oxysterols, in particular 3-oxo-Δ^4-steroids, is involved in the transfer over brain endothelial cells. Experiments designed to study this possibility are now in progress.

As it has been reported previously that the dehydrated metabolite (i.e., cholesta-4,6-dien-3-one; Fig. 1) is converted to cholestanol in rat brain microsomes (17), we next investigated the possibility that 7α-hydroxy-4-cholesten-3-one may be converted to cholestanol in different cell systems. Using human-derived models of neurons, astrocytes, and microglia, we found a rather efficient metabolism of 7α-hydroxy-4-cholesten-3-one and were able to identify the intermediate in the proposed pathway (i.e., 4-cholesten-3-one) as well as cholestanol itself. It should be emphasized that the concentration of 7α-hydroxy-4-cholesten-3-one used in these experiments was similar to that present in the circulation of patients with CTX (see below). Because microglial cells can be regarded as central nervous system-resident macrophages, we also studied the possibility that human monocyte-derived macrophages are able to convert 7α-hydroxy-4-cholesten-3-one to cholestanol. The accumulation of cholestanol in the latter cells, however, was lower than that of the cells originating from the brain.

Plasma cholestanol levels in patients with CTX are increased up to 13–150 μg/ml (1), whereas the levels of 7α-hydroxy-4-cholesten-3-one are increased to between 2 and 8 μg/ml (3). According to the present work with cultured brain endothelial cells, the transfer of 7α-hydroxy-4-cholesten-3-one across the blood-brain barrier may be ~100-fold faster than that of cholestanol. Thus, it seems likely that a substantial part of the accumulation of cholestanol in patients with CTX is a consequence of the flux of 7α-hydroxy-4-cholesten-3-one across the blood-brain barrier.

According to a previous investigation by Salen, Tint, and Shefer (18), the blood-brain barrier may be markedly

defective in untreated patients with CTX. Under such conditions, significant increases in the direct transfer of both cholestanol and 7α-hydroxy-4-cholesten-3-one are likely to occur. However, blood-brain barrier defects are not a universal finding in CTX, and in studies of other CTX patients, the blood-brain barrier appeared to be intact (19, 20) or only slightly affected (20–22).

The sterol 27-hydroxylase appears to have a protective role in connection with the accumulation of cholesterol, converting cholesterol to products that are more easily eliminated from the cells (23). Because cholestanol is also a substrate for the sterol 27-hydroxylase (24), the lack of the enzyme in patients with CTX may further exacerbate the accumulation of cholestanol. It should be emphasized that the capacity of the sterol 27-hydroxylase mechanism to remove cellular cholesterol (and presumably also cholestanol) appears to be ∼1 order of magnitude lower than that of the HDL-dependent reverse cholesterol transport (25). This sterol 27-hydroxylase mechanism, however, may be more important in some specific tissues, such as tendons and brain, which are the preferential sites for the formation of xanthomas in CTX.

The xanthomas in the brain of the patients with CTX contain not only cholestanol but also cholesterol. The possibility must be considered that the accumulation of cholesterol may be a consequence of the accumulation of cholestanol. We reported previously that feeding of cholestanol to both rats and mice does not have a significant effect on hepatic HMG-CoA reductase activity (26, 27). In fact, there was some tendency toward an upregulation of enzyme activity. In agreement with these findings, Shefer and collaborators (28), in a comparative study of the effects of cholestanol and cholesterol on hepatic sterol metabolism in the rat, reported a 2.6-fold upregulation of HMG-CoA reductase activity. Therefore, it is evident that despite the structural similarity between cholesterol and its 5α-saturated derivative, cholestanol does not exert the same strong feedback inhibition on cholesterol biosynthesis as cholesterol. It may be speculated that the reduced capacity to remove cholesterol by the sterol 27-hydroxylase mechanism and the reduced suppression of cholesterol synthesis may be part of the explanation for the accumulation of cholesterol in xanthomas in patients with CTX. If so, the continuous influx of 7α-hydroxy-4-cholesten-3-one may lead to a slow expansion not only of cholestanol but also of the pool of cholesterol, which is ultimately followed by esterification and the subsequent development of xanthomas.

The current treatment for CTX is chenodeoxycholic acid, which causes a suppression of cholesterol 7α-hydroxylase in the liver and normalizes the levels of both 7α-hydroxy-4-cholesten-3-one and cholestanol (1, 3). This treatment leads to a slow reduction in size of both extracerebral and intracerebral xanthomas (1, 29, 30). It is evident that such a treatment cannot affect the capacity to transport cholesterol and cholestanol from the cells in the form of 27-oxygenated metabolites. It is then tempting to suggest that it is the continuous flux of 7α-hydroxy-4-cholesten-3-one into specific cell populations, and the

subsequent production and esterification of cholestanol, that are the most critical factors. The absence of the sterol 27-hydroxylase may further aggravate the imbalance between cellular influx and efflux. If the flux of the bile acid precursor into the brain is reduced to normal, however, the overall result may be a slow reduction in the size of the xanthomas.▪

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