What dictates the accumulation of desmosterol in the developing brain?

Maurice Jansen,[*,‡] Wei Wang,[*,‡] Dario Greco,[†,§] Gian Carlo Bellenchi,[‖] Umberto di Porzio,[‖] Andrew J. Brown,[¶] and Elina Ikonen[*,‡,1]

[*]Institute of Biomedicine, Anatomy, and [†]Research Unit of Molecular Medicine, University of Helsinki, Helsinki, Finland; [‡]Minerva Foundation Institute for Medical Research, Helsinki, Finland; [§]Department of Bioscience and Nutrition, Karolinska Institute, Stockholm, Sweden; [‖]Institute of Genetics and Biophysics, Naples, Italy; and [¶]School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, New South Wales, Australia

ABSTRACT **The brain is the most cholesterol-enriched tissue in the body. During brain development, desmosterol, an immediate precursor of cholesterol, transiently accumulates up to 30% of total brain sterols. This massive desmosterol deposition appears to be present in all mammalian species reported so far, including humans, but how it is achieved is not well understood. Here, we propose that desmosterol accumulation in the developing brain may be primarily caused by post-transcriptional repression of 3β-hydroxysterol 24-reductase (DHCR24) by progesterone. Furthermore, distinct properties of desmosterol may serve to increase the membrane active pool of sterols in the brain: desmosterol cannot be hydroxylated to generate 24*S*-hydroxycholesterol, a brain derived secretory sterol, desmosterol has a reduced propensity to be esterified as compared to cholesterol, and desmosterol may activate LXR to stimulate astrocyte sterol secretion. This regulated accumulation of desmosterol by progesterone-induced suppression of DHCR24 may facilitate the rapid enrichment and distribution of membrane sterols in the developing brain.—Jansen, M., Wang, W., Greco, D., Bellenchi, G. C., di Porzio, U., Brown, A. J., and Ikonen, E. What dictates the accumulation of desmosterol in the developing brain? *FASEB J.* 27, 865–870 (2013). www.fasebj.org**

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Since the early 1960s, it has been known that in the developing mammalian brain, there is a transient accumulation of the cholesterol precursor desmosterol (1, 2). Desmosterol is the penultimate precursor of cholesterol in the Bloch pathway of cholesterol biosynthesis and differs from cholesterol only by an additional double bond in the isooctyl tail, between carbons 24 and 25. This conversion is catalyzed by the enzyme 3β-hydroxysterol 24-reductase (DHCR24; **Fig. 1*A***). Interestingly, brain desmosterol levels change during normal aging (2, 3) and DHCR24 may play a role in neurodegeneration, as it was found to be down-regulated in the brains of patients with Alzheimer disease (DHCR24 is also called seladin-1, for selective Alzheimer's disease indicator 1; refs. 4, 5, but see also ref. 6). The accumulation of desmosterol in the developing brain is remarkable because of its quantity. Desmosterol can accumulate up to 30% of total brain sterols, while cholesterol precursors in other tissues rarely exceed 1%. With this large amount, desmosterol represents an important constituent of membranes and lipoproteins in the developing brain (7).

During the 1960s and 1970s, researchers reported the accumulation of desmosterol in the developing brains of several species, including rats (2, 8), mice (9), rabbits (2), fowl (10), guinea pigs (2) and humans (2, 11). Desmosterol was found to accumulate before the period of myelination (2), but the mechanisms involved and the possible physiological relevance of this phenomenon were not extensively studied. More recently, it has become evident that despite the small structural difference, desmosterol behaves very differently from cholesterol. Disruption of the DHCR24 gene results in the accumulation of desmosterol and is accompanied by multiple congenital anomalies in humans and mice (12, 13). On the molecular level, desmosterol acts as a strong liver X receptor (LXR) agonist (14, 15), has an altered propensity to be hydroxylated (16, 17), and binds less avidly to caveolin (18) as compared to cholesterol. Furthermore, its biophysical membrane behavior differs from that of cho-

Abbreviations: ABCA1, ATP binding cassette transporter A1; ApoE, apolipoprotein E; CHO, Chinese hamster ovary; DAC, 20,25-diazacholesterol; DHCR7, 7-dehydrocholesterol reductase; DHCR24, 3β-hydroxysterol 24-reductase; EV, empty vector; HPLC, high-performance liquid chromatography; HPTLC, high-performance thin-layer chromatography; LXR, liver X receptor; SQLE, squalene epoxidase.

[1] Correspondence: Haartmaninkatu 8, 00290 Helsinki, Finland. E-mail: elina.ikonen@helsinki.fi

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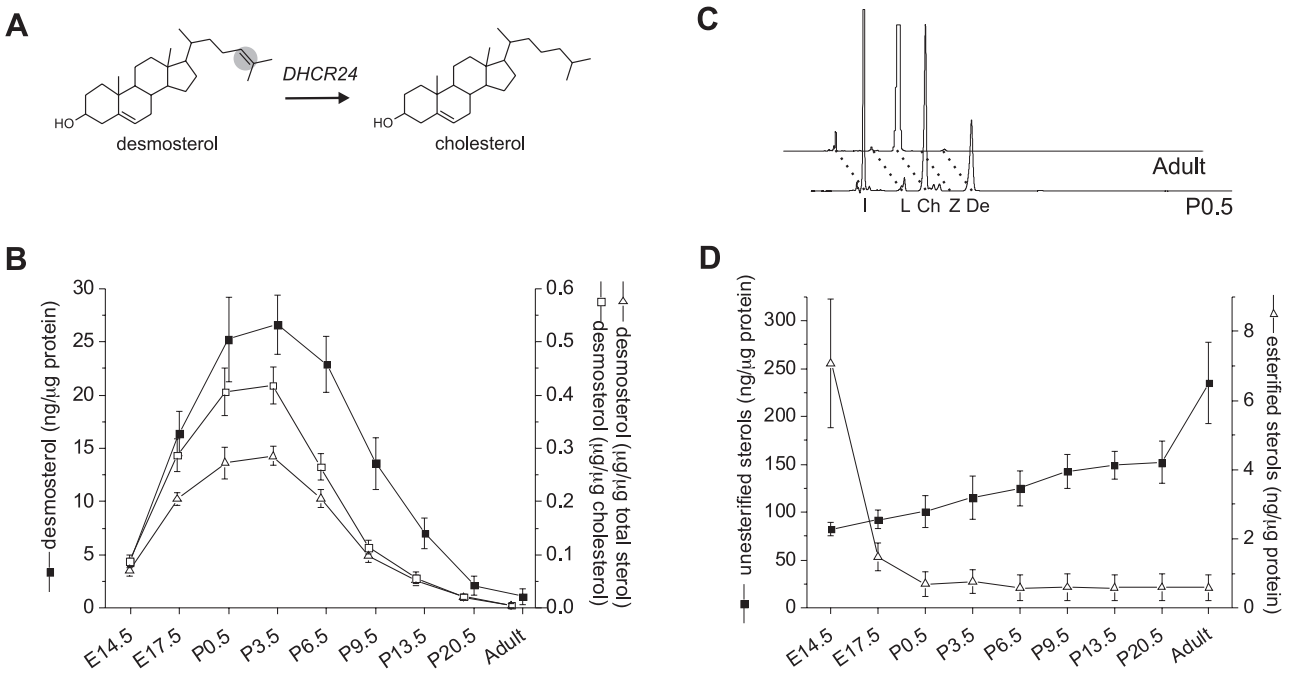

Figure 1. Sterol analysis of the developing mouse brain. *A*) Conversion from desmosterol to cholesterol. Structural difference between cholesterol and desmosterol is indicated with gray shading. *B*) Amount of desmosterol relative to total unesterified sterol, total cholesterol, and total protein (means±SE; n=5-7). *C*) HPLC chromatograms of unesterified sterol fraction in adult and P0.5 mouse brain, I, injection; L, lathosterol; Ch, cholesterol; Z, zymosterol; De, desmosterol. *D*) Amount of total esterified and unesterified sterols relative to total protein (means ± SE; n=5–6).

lesterol, as desmosterol is less efficient in promoting membrane order (19, 20).

Together, these observations point to the possibility that the accumulation of desmosterol in the developing brain is physiologically regulated and functionally relevant. Here, we propose that desmosterol accumulation may be orchestrated by post-transcriptional repression of DHCR24 *via* the major gestational steroid hormone, progesterone. Furthermore, we discuss distinct properties of desmosterol that may assist in increasing the content and facilitating delivery of membrane sterols in the brain.

MATERIALS AND METHODS

Ethics statement

The animal studies were approved by the Experimental Animal Committee of the University of Helsinki.

Brain samples

Brain samples from wild-type C57BL6 mice were snap-frozen in liquid nitrogen and stored at −70°C until use. Whole brains were analyzed from embryonic day 14.5 (E14.5) embryos and whole brains, excluding cerebellum and brain stem, from E17.5 and older animals.

Sterol analysis

Lipids were extracted from cells or tissue homogenate in PBS as reported previously (21). For neutral lipid analysis, extracted lipids were separated by high-performance thin-layer chromatography (HPTLC) in hexane:diethylether:acetic acid (80:20:1, v/v). To separate cholesterol from desmosterol and other precursors, extracted lipids were subjected to Ag-HPTLC in chloroform:acetone (19:1, v/v) as described previously (22), or to Ag–high-performance liquid chromatography (HPLC) in hexane:acetone (8:2, v/v) as described previously (7, 23).

DHCR24 inhibition by progesterone and PCR

Inhibition of DHCR24 by progesterone was analyzed as previously described (22) and quantitative real-time PCR was performed as reported previously (24). A detailed description of these procedures is included in Supplemental Material.

RESULTS AND DISCUSSION

In the developing mouse brain, desmosterol accumulates between E14 and postnatal day 10 (P10), peaking during the first postnatal week (Fig. 1*B*). During this period, no other sterol precursors accumulate significantly (Fig. 1*C*), and the total sterol content increases substantially (Fig. 1*D*). The growth of neuronal cell processes is very active during this time and is followed by the initiation of synaptogenesis.

DHCR24 transcript levels during the period of desmosterol accumulation

To gain insight into the mechanisms leading to the desmosterol accumulation, we analyzed whether the

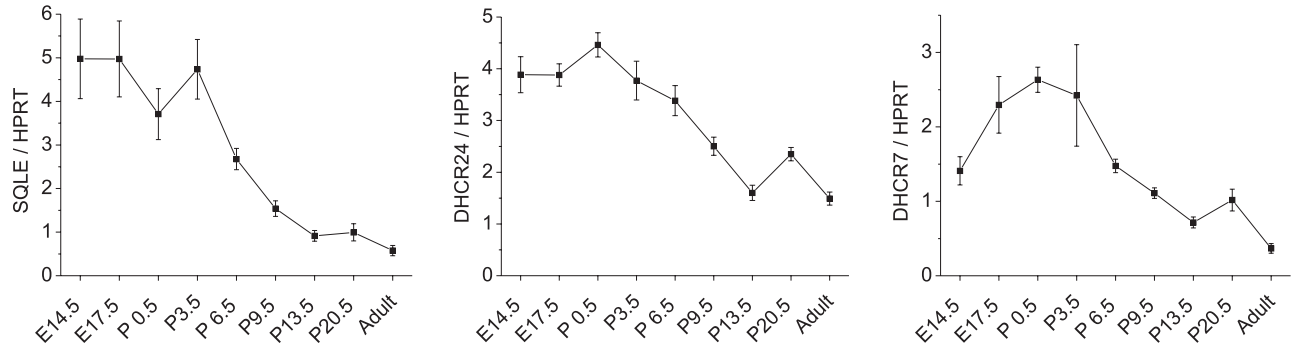

Figure 2. Real-time PCR analysis of the expression of genes involved in late cholesterol biosynthesis in the developing mouse brain (means±SD; $n=6$).

varying desmosterol content around the perinatal period could result from transcriptional regulation of DHCR24. We compared the expression profile of DHCR24 mRNA with that of two other genes involved in cholesterol biosynthesis: squalene epoxidase (SQLE), a rate-controlling enzyme in prelanosterol cholesterol biosynthesis (25), and 7-dehydrocholesterol reductase (DHCR7), the enzyme that reduces the double bond in 7-dehydrocholesterol, which is the immediate precursor of cholesterol in the Kandutsch-Russell pathway of cholesterol synthesis. We found that the expression of DHCR24 remains relatively constant until P3 and then decreases significantly during the next 10 d (**Fig. 2**). The expression profiles of SQLE and DHCR7 were very similar (Fig. 2). These results argue against the idea that transcriptional regulation of DHCR24 is responsible for the accumulation of desmosterol. First, the similar developmental profile of DHCR24 mRNA to those of SQLE and DHCR7 suggests that DHCR24 is not differentially regulated from these prelanosterol and postlanosterol biosynthetic enzymes during desmosterol accumulation. Second, the DHCR24 mRNA decreases from P3 to P12 when the amount of desmosterol decreases. Obviously, it should increase if DHCR24 mRNA variation accounted for the desmosterol reduction. Together, these results suggest that the accumulation of desmosterol is not due to reduced DHCR24 mRNA expression. Of note, since the real-time PCR does not reveal cell-specific changes, we cannot exclude the possibility that DHCR24 is down-regulated in a quantitatively minor cell type that nevertheless substantially contributes to the sterol content. However, the idea that desmosterol accumulation is not due to reduced DHCR24 gene expression is in agreement with the simultaneous up-regulation of desmosterol and DHCR24 activity during rat brain development (8).

Desmosterol accumulation may be caused by progesterone

We next considered whether the accumulation of desmosterol could be caused by post-transcriptional regulation of DHCR24. It has been shown that progesterone and pregnenolone cause the accumulation of desmosterol in cultured cells (26, 27) and that they can affect cellular processes by both transcriptional and post-transcriptional regulation. Because these steroid hormones are present in the developing brain (28), we investigated whether progesterone can inhibit DHCR24 activity *via* a post-transcriptional mechanism. To this end, we used Chinese hamster ovary (CHO) cells stably expressing DHCR24 under a viral promoter (CHO-DHCR24; ref. 22), which induces substantially higher DHCR24 protein levels than those in the corresponding control (empty vector) stable cells (CHO-EV; **Fig. 3**). Notably, progesterone did not decrease DHCR24 protein levels, either ectopic (Fig. 3) or endogenous (not shown,

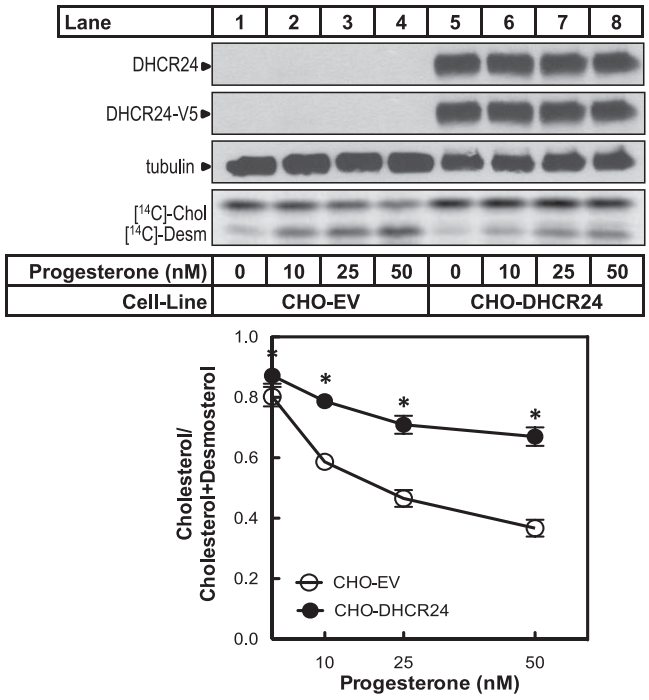

Figure 3. DHCR24 overexpression blunts the inhibitory effect of progesterone on DHCR24 activity. CHO-EV (control) and CHO-DHCR24 cells were treated with progesterone (0-50 nM) and radiolabeled with [^{14}C]-acetate for 4 h. Cell lysates were subjected to immunoblotting with antibodies against DHCR24, V5, and tubulin. Lipid extracts were separated by Ag-TLC, and bands corresponding to cholesterol and desmosterol were visualized by phosphorimager and densitometry (means±SE; $n=8$). *$P < 0.001$; paired Student's t test.

evident on longer exposures of the blot). We pulse-labeled the cells with [^{14}C]-acetate and analyzed sterol biosynthesis in the presence or absence of progesterone. We found that in the presence of progesterone, there was an accumulation of [^{14}C]-desmosterol at the expense of [^{14}C]-cholesterol in CHO-EV cells. This effect was blunted in CHO-DHCR24 cells, *i.e.*, a higher concentration of progesterone was required to achieve a similar degree of enzyme inhibition (Fig. 3 and Supplemental Table S1). These results indicate that DHCR24 activity is reduced in the presence of progesterone and that more enzyme necessitates more progesterone for inhibition. Because DHCR24 protein levels were not reduced by progesterone, and progesterone inhibited enzyme activity produced under a viral promoter, the inhibition of DHCR24 by progesterone is most probably post-transcriptional, possibly *via* direct enzyme inhibition.

In this *in vitro* system, progesterone inhibited DHCR24 at low nanomolar concentrations (Fig. 3). Similar concentrations may be present in the developing brain *in vivo*. In rats, progesterone and pregnenolone are present in the brain at 2 ng/g (~6 nM) and 5 ng/g (~15 nM), respectively, just after birth (28), and their levels may be higher prenatally, as shown for other steroid hormones (29). Progesterone can be synthesized locally in the brain, *e.g.*, by astrocytes (30), and thus, the local effective concentration may be tightly controlled. Notably, astrocytes synthesize cholesterol *via* desmosterol (31), and desmosterol is a far better substrate than cholesterol for pregnenolone formation (32). Furthermore, the time course of progesterone, pregnenolone, and desmosterol reduction postnatally is similar. The levels of progesterone and pregnenolone in rat brain decrease rapidly after birth to 0.5 ng/g by P7, after which their levels remain low (as measured at P14 and 10 wk after birth; ref. 28). This profile is very similar to the decrease in desmosterol after birth in mouse and rat brain. Altogether, these results suggest that the presence of progesterone or related hormones might explain the accumulation of desmosterol during early brain development.

Interestingly, such a mechanism may not be restricted to the developing brain. Desmosterol levels in primate corpus luteum are increased in the early luteal phase when progesterone levels increase (33). Furthermore, one could envisage that some of the neuromodulatory functions of progesterone in the adult brain (see *e.g.*, ref, 34) might result from its effects on DHCR24 activity.

Desmosterol accumulation may serve to prevent 24*S*-hydroxycholesterol generation and sterol deposition as fatty acid esters

Desmosterol accumulates in the brain during its rapid growth phase. During this period, large amounts of sterols are incorporated in the membranes of growing cells. Differently from other organs, sterols in the brain are considered to be synthesized locally (35). Therefore, the brain has to accumulate large amounts of biosynthetic cholesterol in a short time. A major mechanism of cholesterol removal from the brain is its hydroxylation at the carbon 24 position to generate 24*S*-hydroxycholesterol, which then passes the blood-brain barrier to circulation (36). Notably, desmosterol cannot be hydroxylated at C24 (16); therefore, the buildup of desmosterol could serve to prevent brain sterol removal. In addition, the low levels of 24*S*-hydroxylase in the early postnatal brain most likely contribute to this effect (37).

Besides cholesterol secretion, cells can lower their functional sterol pool by esterification and subsequent storage in lipid droplets. We have observed that pharmacological inhibition of DHCR24 and consequent accumulation of desmosterol results in a parallel decrease in the cellular sterol ester content (Supplemental Fig. S1 and unpublished results). This is in line with the finding that liver microsomes esterify desmosterol ~40% less efficiently as compared to cholesterol (38). Therefore, desmosterol accumulation during brain development could also serve to decrease sterol esterification. This idea is supported by the observation that the increase in desmosterol coincides with the decline in sterol esters during mouse brain development (Fig. 1*D*). Together, the observations that desmosterol is a poor substrate for 24-hydroxylation and for sterol esterification suggest that the developmental accumulation of desmosterol may facilitate the rapid accumulation of sterols needed for brain expansion. Of note, a sustained high-desmosterol content does not promote sterol deposition as DHCR24 KO mice actually have a reduced brain sterol content (16).

Desmosterol accumulation may facilitate cellular sterol exchange by stimulating LXR signaling

Desmosterol directly binds and stimulates the LXR in CHO cells (15). In macrophages, it activates LXR while inhibiting SREBP target and inflammatory response genes (14). Also *in vivo*, desmosterol may stimulate LXR, as suggested by the up-regulation of LXR targets in DHCR24-knockout mice (16). Moreover, LXR-mediated sterol secretion has been implicated in brain development. There is strong evidence that astrocytes provide sterols for neurons by secreting cholesterol in apolipoprotein E (ApoE) lipoprotein particles (39–41). It appears likely that during brain development when expanding membranes and synapses need ample cholesterol, such a delivery mechanism would be particularly important. Indeed, LXR-mediated sterol secretion is critical for brain development as evidenced by the severe neuronal defects in newborn mice lacking LXRβ (42).

On the basis of the above observations, it seems plausible that desmosterol accumulation during brain development serves to stimulate LXR signaling. This, in turn, may facilitate sterol secretion from astrocytes. To test this possibility, we analyzed whether desmosterol stimulates LXR *in vitro* in astrocytic cells. We treated

U-251MG astrocytoma cells with the DHCR24 inhibitor 20,25-diazacholesterol (DAC), which by inhibiting DHCR24, results in the accumulation of newly synthesized desmosterol (18). After 2 d of treatment, ~40% of the total sterol was desmosterol, and this was accompanied by increased protein level of the LXR target ATP binding cassette transporter A1 (ABCA1; Supplemental Fig. S1*A*). Moreover, we observed that a DAC-induced desmosterol accumulation was accompanied by an increase in sterols in the medium (Supplemental Fig. S1*B*). Together, these results suggest that desmosterol accumulation stimulates LXR signaling and increases sterol secretion in astrocytic cells. In agreement, exogenously supplemented desmosterol increases ABCA1 protein levels in primary astrocytes (43).

In summary, we propose that progesterone, and possibly related steroids, cause the transient perinatal accumulation of desmosterol during mammalian brain development *via* post-transcriptional repression of DHCR24. Furthermore, we suggest that this phenomenon of desmosterol deposition is evolutionarily conserved because it provides specific advantages: It may help to increase the pool of membrane-active brain sterols during rapid brain growth by preventing the formation of sterol esters and 24*S*-hydroxysterols. It may also facilitate sterol delivery within the central nervous system by stimulating LXR-mediated sterol secretion from astrocytes. These mechanisms may be relevant not only during brain development but also in the adult brain and in neurodegenerative conditions. FJ

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