

Precursors for Cholesterol Synthesis (7-Dehydrocholesterol, 7-Dehydrodesmosterol, and Desmosterol): Cholesterol/7-Dehydrocholesterol Ratio as an Index of Development and Aging in PNS but Not in CNS

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Abstract: In rat sciatic nerve, the 7-dehydrocholesterol content decreased dramatically during the postnatal period and slowly during adulthood and aging. In contrast, the 7-dehydrodesmosterol content peaked at 14 days and was nearly undetectable after 60 days. The desmosterol content peaked at 21 days and was nearly undetectable after 1 year. The cholesterol content increased up to 21 days and remained nearly constant thereafter. In brain (in contrast to sciatic nerve), 7-dehydrodesmosterol and desmosterol contents decreased dramatically during development and slightly during adulthood and aging; the 7-dehydrocholesterol content peaked at 21 days and remained constant during aging. Only 7-dehydrocholesterol was dramatically more concentrated in PNS than in CNS. In brain, the cholesterol/7-dehydrocho-

lesterol ratio increased during development and remained stable after 6 months. In contrast, in sciatic nerve, this ratio continuously increased during development and aging (950-fold between 5 days and 18 months). Thus, the cholesterol/7-dehydrocholesterol ratio is a useful biochemical index of development and aging in the PNS. **Key Words:** Cholesterol — 7-Dehydrocholesterol — 7-Dehydrodesmosterol — Desmosterol — Brain — Sciatic nerve — PNS — CNS — Development — Aging. Bourre J. M. et al. Precursors for cholesterol synthesis (7-dehydrocholesterol, 7-dehydrodesmosterol, and desmosterol): Cholesterol/7-dehydrocholesterol ratio as an index of development and aging in PNS but not in CNS. *J. Neurochem.* **54**, 1196–1199 (1990).

The PNS, as well as the CNS, has been known for many years to contain cholesterol. It is actively synthesized during peripheral nerve development, and this synthesis is altered during degeneration and regeneration (Yao, 1988). However, the occurrence of desmosterol in the PNS was not demonstrated until 1971 by Ramsey et al. (1971). In contrast, desmosterol (24-dehydrocholesterol) was found in the brain of the young rat and mouse (Kritchevsky and Holmes, 1962; Paoletti et al., 1965) and in fetal human brain (Fumagalli and Paoletti, 1963). It has been associated with the myelination process, because no desmosterol has been detected in the brains of adult rats (Kritchevsky et al., 1965) or humans (Fumagalli and Paoletti, 1963), nor is any present in the brain of the newborn guinea pig, which is fully myelinated at birth (Kritchevsky et al., 1965). Recent reexamination of sterol content in the nervous system has shown that the following free sterols

are found in both the optic nerve and cerebral white matter: cholesterol, desmosterol, lanosterol, two dimethylsterols (4,4-dimethyl-5- α -cholesterol-8,24-dienes-3- β -ol and 4- α ,14 α -dimethyl-5- α -cholesterol-7-ene-3- β -ol), and probably cholestene. In contrast with previous results, adult brain has been reported to contain desmosterol; however, tri- and dimethylsterols were not detected (Adamczewska-Goncerzewicz and Trzebny, 1981).

Yao and Rastetter (1985) have developed a high-performance TLC technique showing that lanosterol is synthesized in the PNS.

Desmosterol is synthesized in brain (Holstein et al., 1966; Marco et al., 1985). In neonatal rats, it is thought to accumulate as a result of a slow step at the reduction of the Δ^{24} unsaturation, which becomes rate limiting at the time of rapid cholesterol synthesis during myelination (Hinse and Shah, 1971).

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Cholesterol appears in the brain according to two processes: by *in situ* synthesis and by the transfer of plasma cholesterol. Cholesterol that appears in the brain by synthesis disappears from this organ by transfer into the plasma (Sérougne et al., 1976). The hypothesis of a "blood-brain barrier" represented by the cerebral capillaries is not valid for cholesterol (Sérougne and Chevallier, 1974).

However, the exact course of the individual metabolic steps leading from lanosterol to cholesterol in nervous tissue is only vaguely understood (Marco et al., 1985). Nineteen discrete reactions are used to convert lanosterol to cholesterol, and the side-chain saturation can be either the first or the last reaction in the sequence (Schroepfer, 1982; Rilling and Chayet, 1985). Use of various hypocholesterolemic drugs has shown accumulation of precursors (Ramsey et al., 1971; Suzuki et al., 1974; Ramsey, 1977; Heacock et al., 1984).

7-Dehydridesmosterol and 7-dehydrocholesterol have not been described in the normal PNS, although they accumulate in adult PNS during treatment with hypocholesterolemic drugs (Ramsey, 1977). Thus, this work was undertaken to measure immediate precursors of cholesterol during development and aging of the PNS.

MATERIALS AND METHODS

Adult Sprague-Dawley rats were obtained from IFFA-Credo (L'Arbresle, France) and further bred in our laboratory. They were fed standard chow (diet no. 04; UAR, Villemoisson-Sur-Orge, France).

Tissues were extracted using hexane/isopropanol (3:2 vol/vol), 20 volumes/g of brain fresh weight (Radin, 1981). An internal standard was added (7-dehydrocholesterol for quantification of cholesterol and desmosterol in PNS and CNS; α -tocopherol acetate for quantification of 7-dehydro compounds in PNS and CNS). Saponification (only for cholesterol and desmosterol content measurements) was performed (methanolic 20% KOH at 70°C for 3 h). Lipids were extracted with hexane, dried, solubilized in methanol/water (95:5 vol/vol), and analyzed on a C18 column in an LKB HPLC apparatus. The solvent was methanol/water (95:5 vol/vol) at a flow rate of 1 ml/min (modified from the system of Rodriguez and Parks, 1985). Detection was performed at 206 nm for cholesterol and desmosterol and 280 nm for dehydrocompounds. Quantification was performed with an ENICA 21 integrator (DELSI, Argenteuil, France). Statistical analysis was performed using Student's *t* test.

RESULTS

Figure 1A shows that in sciatic nerve, the 7-dehydrocholesterol content decreased dramatically during the postnatal period (22-fold between 5 and 60 days) and then decreased slowly during adulthood and aging (fourfold between 6 and 18 months). In contrast, the 7-dehydridesmosterol content increased by approximately fourfold between 5 and 14 days, then decreased

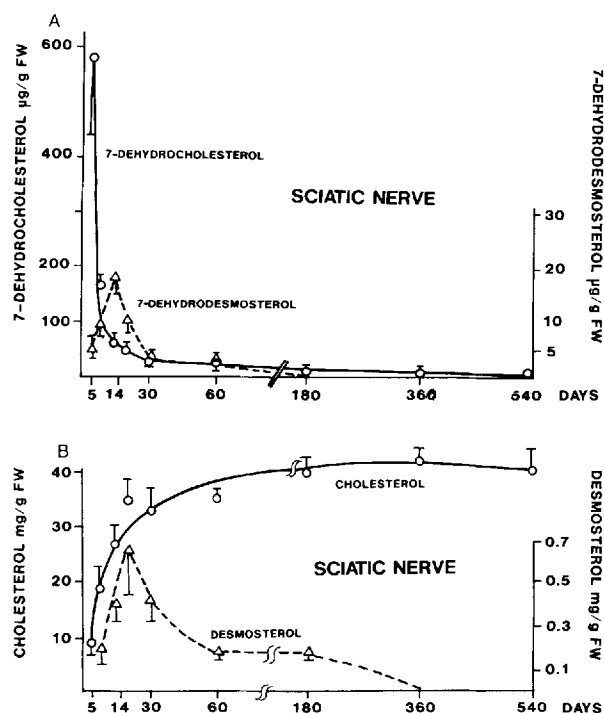


FIG. 1. Levels of (A) 7-dehydrocholesterol and 7-dehydridesmosterol and (B) cholesterol and desmosterol in sciatic nerve during development and aging. FW, fresh weight.

by approximately sevenfold between 14 and 60 days, and was not detectable after 6 months. Figure 1B shows that the desmosterol content increased threefold between 8 and 21 days, then decreased, and was not detectable at 1 year. The cholesterol content increased fourfold between 5 and 21 days and then remained constant.

Figure 2A shows that in brain, in contrast with sciatic nerve, the 7-dehydridesmosterol content decreased 3.2-fold between 4 and 21 days and then decreased slowly during adulthood and aging. The 7-dehydrocholesterol content increased twofold between 4 and 21 days and also decreased slowly during adulthood and aging. Figure 2B shows that the desmosterol content decreased fourfold between 4 and 21 days and was nearly undetectable at 6 months. The cholesterol content increased dramatically during postnatal development between 5 and 21 days but very slightly during adulthood and aging.

DISCUSSION

7-Dehydrocholesterol was dramatically more concentrated in the PNS than in the CNS (40- and threefold at 8 days and 6 months, respectively).

In brain, the desmosterol/7-dehydridesmosterol ratio decreased dramatically during development and remained stable thereafter. In contrast, the cholesterol/

7-dehydrocholesterol ratio increased regularly during development—11-fold between 5 days and 6 months and fourfold between 21 days and 6 months—and was constant during aging (Fig. 3). In sciatic nerve, this ratio continuously increased during development and aging: 950-fold between 5 days and 18 months and 20-fold between 21 days and 18 months ($r = 0.98$).

As the desmosterol concentration was 10 times higher than the 7-dehydrodesmosterol and 7-dehydrocholesterol concentrations (but 10 times lower than the cholesterol concentration), it could be speculated that Δ^{24} -desaturase functions poorly with desmosterol (and better with zymosterol) and thus desmosterol accumulates and is incorporated into membrane, that the two pathways from zymosterol are not found in the same cells and are regulated differently, or that 7-desaturase acts much more rapidly than Δ^{24} -desaturase (with 7-dehydrodesmosterol and 7-dehydrocholesterol being rapidly transformed into desmosterol and cholesterol, respectively). Moreover, the possibility that Δ^{24} -desaturase may synthesize 7-dehydrocholesterol from 7-dehydrodesmosterol is not excluded.

Examination of the effect of hypocholesterolemic agents on PNS sterol composition has shown that sterol deposition in the PNS and CNS was the same under analogous conditions; however, the accumulation of 7-dehydrodesmosterol as a result of diazocholesterol

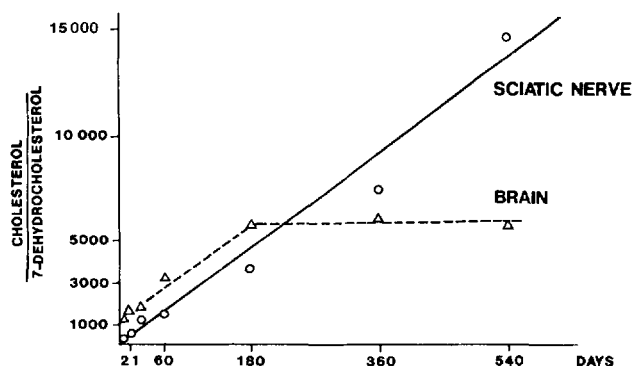


FIG. 3. Cholesterol/7-dehydrocholesterol ratio during development and aging in PNS and CNS.

treatment was specific to the PNS (Ramsey, 1977). The present study shows that the CNS (and probably oligodendrocytes) could use the 7-dehydrodesmosterol pathway to synthesize cholesterol. In contrast, the PNS (and thus the Schwann cell) could use the 7-dehydrocholesterol pathway. This is in agreement with other results showing that the 7-dehydrocholesterol content is normal in the CNS from dysmyelinating mutants (quaking, shiverer, and trembler), although myelination and thus cholesterol content are dramatically reduced (in shiverer and trembler). In contrast, the 7-dehydrocholesterol content is profoundly abnormal in the PNS from all mutants (Bourre et al., 1989).

Finally, we suggest that the cholesterol/7-dehydrocholesterol ratio could be a useful index of development and aging in the PNS.

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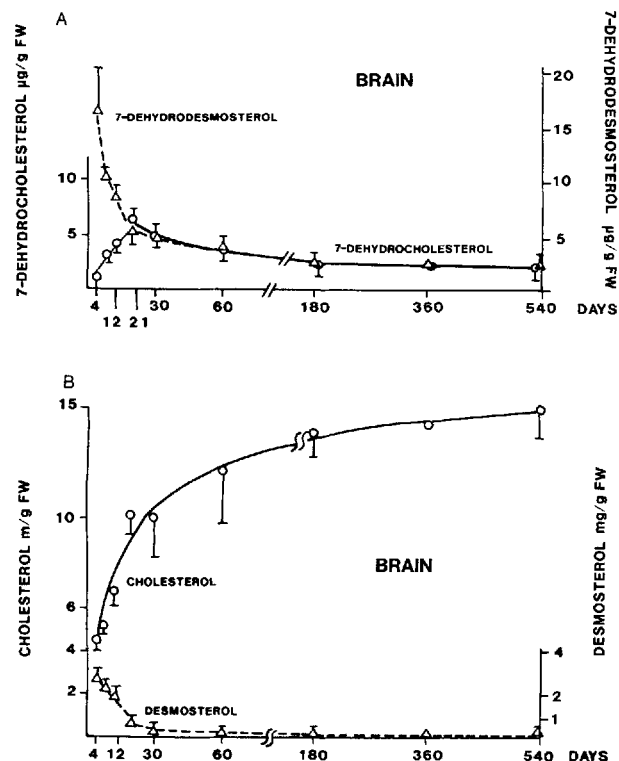


FIG. 2. Levels of (A) 7-dehydrocholesterol and 7-dehydrodesmosterol and (B) cholesterol and desmosterol in forebrain during development and aging. FW, fresh weight.

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