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Alterations of cholesterol synthesis precursors (7-dehydrocholesterol, 7-dehydrodesmosterol, desmosterol) in dysmyelinating neurological mutant mouse (quaking, shiverer and trembler) in the PNS and the CNS

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In brain, levels of cholesterol, desmosterol and 7-dehydrodesmosterol are reduced in shiverer and quaking, but not in trembler 60-day-old dysmyelinating mutant mice. Very interestingly, 7-dehydrocholesterol is not altered in any mutant. The amount of cholesterol is similar in the different normal control mouse strains and in rat. In contrast, levels of precursors are not the same. In sciatic nerve, cholesterol is slightly reduced in shiverer, reduced 2-fold in quaking, and dramatically reduced in trembler (10-fold). 7-Dehydrocholesterol is affected in all mutants.

The lipid composition of unmyelinated nerve tissue does not deviate very much from that of other tissues. However, as soon as the process of myelinogenesis begins, the concentration of cholesterol increases rapidly and its deposition rate reaches a maximum shortly after the onset of myelination. Myelin is a membrane synthesized by oligodendrocytes and is very rich in cholesterol, which is in keeping with the high level of cholesterol found in membranes (80–90% of total cell cholesterol is in the plasma membrane of the eukaryotic cell [1]). The effect of sterol structure on membrane function has been described [2].

The nervous system has been known to contain cholesterol for many years. Desmosterol (24-dehydrocholesterol) is present in the brain [3–6] and peripheral nervous system (PNS) [7] of young animals. The amount of brain desmosterol increases during the first 2 weeks of life, and thereafter decreases rapidly [3,4]. It has been associated with the myelination process, since no desmosterol has been detected in the brains of adult rats [8] or humans [9] nor is any present in the brain of the newborn guinea pig, which is fully myelinated at birth [8].

However, the exact course of the individual metabolic steps leading from lanosterol to cholesterol in nerve tissue is only vaguely known [10]. The question of the origin and localization of the desmosterol present in the brain of the young rat has not been investigated to date, although desmosterol has been found in myelin from young animals [11]. The desmosterol in brains of neonatal rats is thought to accumulate as a result of a slow step in the reduction of the delta-24 unsaturation, which becomes rate-limiting at the time of rapid cholesterol synthesis during myelination: the relatively high content of lanosterol and desmosterol in the immature optic nerve could be due to a reduction in the intermediate biosynthetic steps for transformation of lanosterol into cholesterol [12].

Nineteen discrete reactions are involved in the conversion of lanosterol to cholesterol, and the side-chain saturation can be either the first or the last reaction in the sequence [13,14]; utilization of various hypocholesterolemic drugs has shown accumulation of precursors [7,15,16]. Although accumulating in adult PNS during treatment with hypocholesterolemic drugs, 7-dehydrodesmosterol and 7-dehydrocholesterol have not been detected in normal PNS [7]. The ratio of cholesterol to desmosterol in developing brains of quaking and jimpy dysmyelinating mutants has been shown to be normal [17]. Thus, this work was undertaken to measure the

Abbreviations: CNS, central nervous system; PNS, peripheral nervous system.

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immediate precursors of cholesterol. Comparisons were performed with brain tissue from normal controls and dysmyelinating neurological mutants.

Examination of dysmyelinating mutants as a tool for studying myelination has recently been reviewed [18,19]. The alterations found in the brain of the quaking mutant seem most consistent with a failure in maturation of the myelin sheath that results from an arrest in myelinogenesis. The prominent change in the brain of the shiverer is the paucity of myelin and an associated virtual absence of the major dense line on electron microscopy. The trembler mouse is characterized by a dominantly inherited hypomyelinating neuropathy with onion bulb formations and it is presumably caused by a primary metabolic disorder of Schwann cells due to unknown reasons [20].

The trembler mutant (B6-CBA strain) was derived from the Scottish mutation (obtained from Dr. Guesnet's Laboratory, Pasteur Institute); the shiverer (C3H-SWV strain) originated from Washington University (Dr. Bird); quaking (C57-B6 black strain) originated from Jackson Laboratories. These mutants were bred in our laboratory. 60-day-old animals were compared with their normal-appearing littermates, except for shiverer mutants, which were compared with a control strain (heterozygotes are affected).

Adult 60-day-old Sprague Dawley rats were obtained from IFFA-Credo (L'Arbresle, France) and bred further in our laboratory. Animals were fed standard chow (UAR diet No. 04, Villemoison-sur-Orge, France).

Tissues were extracted using hexane/isopropanol 3/2, v/v, 20 ml/g brain, fresh weight [21]. An internal standard was added: 7-dehydro cholesterol for cholesterol and desmosterol in PNS and CNS, tocopherol acetate for 7-dehydro compounds in PNS and CNS. Saponification (only for cholesterol and desmosterol measurements) was eventually performed (methanolic 20% KOH/70°C, 3 h) and lipids were extracted with hexane, dried, solubilized in methanol/water 95 : 5, v/v and analyzed, on a C-18 column in an LKB HPLC apparatus. Solvent was methanol/water 95 : 5, flow rate was 1 ml/min modified from Rodriguez and Parks [22]. Detection was performed at 206 nm for cholesterol and desmosterol, and 280 nm for dehydro compounds. Quantitation was carried out with an ENICA 21 integrator (Delsi, Argenteuil, France). Two determinations were performed on the two nerves from the same animal. At least four animals were used for each strain. Statistical analysis was performed using Student's *t*-test.

In whole brain, the level of cholesterol was very similar in rat and in all normal control mice if related to fresh weight; however, if related to dry weight, controls from shiverer presented slightly lower values (Table I). In agreement with previously published data, the concentration of cholesterol was reduced in shiverer and quaking, but not in trembler [20,23–25].

The concentration of desmosterol was similar in all control mice, the value was approx 3-times lower than in rat. It was reduced by approx 30% in shiverer and quaking and was normal in trembler. 7-Dehydrodesmosterol was also reduced by approx. 30% in shiverer and quaking if based upon fresh weight (approx. 20% if based upon dry weight), and it was normal in trembler. The concentration in the normal controls was within a range of 3-fold according to the strain.

Very interestingly, the concentration of 7-dehydrocholesterol was normal in all three mouse mutants. This

TABLE I

Cholesterol, desmosterol, 7-dehydrodesmosterol, 7-dehydrocholesterol in the brain of normal control rats and mice and in neurological dysmyelinating mutants

Brain	Cholesterol (mg/g)		Desmosterol (mg/g)		7-dehydrodesmosterol (μg/g)		7-dehydrocholesterol (μg/g)	
	FW	DW	FW	DW	FW	DW	FW	DW
Rat ($n = 4$)	17.3±0.2	81.9±0.7	0.32 ±0.02	1.5 ±0.1	2.5 ±0.3	12.3±17	1.8±0.3	8.9±1.4
Control ($n = 5$)	17.3±1.1	78 ±4	0.10 ±0.02	0.44±0.07	1.05±0.16	4.8±0.7	0.5±0.1	2.5±0.5
Trembler ($n = 5$)	17.0±1.2	77 ±5	0.10 ±0.03	0.48±0.13	1.03±0.17	4.7±0.8	0.6±0.1	2.6±0.5
Tr/N	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	98.3%	98.7%	100.0%	109.0%	98.0%	97.9%	120%	104%
Control ($n = 4$)	15.4±1.5	68 ±5	0.14 ±0.01	0.62±0.02	1.80±0.08	8.3±0.4	0.8±0.1	3.6±0.6
Shiverer ($n = 4$)	11.1±0.6	54 ±2	0.093±0.02	0.45±0.02	1.3 ±0.1	6.8±0.7	0.9±0.4	4.3±1.5
Shi/N	$P < 0.01$	$P < 0.01$	$P < 0.001$	$P < 0.001$	$P < 0.01$	$0.01 < P < 0.02$	n.s.	n.s.
	72.1%	79.4%	66.4%	72.6%	72.2%	81.9%	112.0%	119.0%
Control ($n = 5$)	15.9±1.1	74 ±5	0.10 ±0.01	0.46±0.04	0.9 ±0.1	4.1±0.6	0.7±0.1	3.3±0.4
Quaking ($n = 5$)	10.7±0.5	56 ±3	0.06 ±0.01	0.32±0.06	0.60±0.02	3.3±0.1	0.7±0.1	3.7±0.5
Qk/N	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.01$	$P < 0.01$	$0.02 < P < 0.05$	n.s.	n.s.
	67.3%	75.6%	60.0%	69.5%	68.6%	80.5%	100.0%	112.1%

TABLE II

Cholesterol, desmosterol, 7-dehydrodesmosterol, 7-dehydrocholesterol in the sciatic nerve from normal control rat and mice and from dysmyelinating neurological mutants

Sciatic nerve	Cholesterol (mg/g)		Desmosterol (mg/g)		7-Dehydrodesmosterol (μg/g)		7-Dehydrocholesterol (μg/g)	
	FW	DW	FW	DW	FW	DW	FW	DW
Rat ($n = 4$)	35.7 ± 0.6	112 ± 1	0.20 ± 0.01	0.60 ± 0.04	2.8 ± 0.5	8.3 ± 1.6	26 ± 5	76 ± 16
Control ($n = 4$)	50 ± 2	140 ± 4	0.40 ± 0.05	1.1 ± 0.1	2.9 ± 0.3	8.0 ± 0.8	45 ± 2	127 ± 12
Trembler ($n = 4$)	5.4 ± 0.6	25 ± 2	0.05 ± 0.01	0.20 ± 0.01	< 0.2	< 0.6	6 ± 2	27 ± 11
Tr/N	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$			$P < 0.01$	$P < 0.01$
	10.8%	17.8%	12.5%	18.2%	–	–	13.3%	21.2%
Control ($n = 4$)	44.9 ± 3.4	116 ± 8	0.05 ± 0.01	0.14 ± 0.03	3.0 ± 0.8	7.4 ± 1.5	53 ± 9	130 ± 15
Shiverer ($n = 4$)	33.2 ± 0.7	106 ± 5	0.05 ± 0.02	0.15 ± 0.06	3.0 ± 0.2	7.9 ± 0.3	19 ± 5	41 ± 10
Shi/N	$P < 0.01$	n.s.	n.s.	n.s.	n.s.	n.s.	$P < 0.01$	$P < 0.01$
	73.9%	91.0%	100%	107%	100%	106%	35.8%	31.5%
Control ($n = 5$)	44.2 ± 3.5	120 ± 11	0.11 ± 0.02	0.30 ± 0.06	5.7 ± 1.9	15.9 ± 5.5	58 ± 8	161 ± 25
Quaking ($n = 5$)	28.0 ± 1.7	96 ± 7	0.07 ± 0.01	0.24 ± 0.06	1.4 ± 0.2	4.7 ± 0.6	30 ± 4	100 ± 13
Qk/N	$P < 0.001$	$0.01 < P < 0.02$	n.s.	n.s.	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.01$
	63.3%	80%	63.6%	80.0%	24.6%	29.6%	51.7%	62.1%

probably means that in normal mice the 7-dehydrocholesterol pathway is not operative in the brain, at least in the oligodendrocytes, unless the activity in astrocytes and neurons is much higher than in oligodendrocytes (and normal in the mutants), thus masking a low and possibly affected metabolism in the mutant oligodendrocytes.

It must be noted that desmosterol, 7-dehydrodesmosterol, and 7-dehydrocholesterol levels were higher in brain from rat than in brain from mouse, although the concentration of cholesterol was similar.

In sciatic nerve, the level of cholesterol varied according to the control strain. It was drastically reduced in trembler, which is in agreement with our previous results [26]. Cholesterol was also reduced in the quaking mutant, in parallel with the reduced amount of myelin. It was unexpected to find that cholesterol was reduced in the sciatic nerve from shiverer only if based upon the fresh weight, but not the dry weight (in agreement with Inouye et al. [27]). Demosterol varied according to the control strain, and it was drastically reduced in trembler, slightly reduced in quaking, and normal in shiverer. 7-Dehydrodesmosterol was not detectable in trembler, drastically reduced in quaking, and normal in shiverer. 7-Dehydrocholesterol was affected in all three mutants: it probably plays an important role in Schwann cell metabolism.

Moreover, the concentration of cholesterol related to the fresh weight in normal 60-day-old control animals was approx. 2-times higher in the PNS than in the CNS; in contrast, levels of 7-dehydrocholesterol were 14-, 90-, 66- and 82-times higher in the PNS from rat, control trembler, control shiverer and control quaking, respectively (data from Tables I and II).

Synthesis of cholesterol via the 7-dehydrocholesterol pathway needs fully differentiated myelinating Schwann cells: in the trembler, cholesterol and 7-dehydrocholesterol are not increased, as could be expected considering the increased number of Schwann cells in the mutant. Indeed the synthesis of cholesterol is active in the peripheral nerve, and is altered during development, degeneration and regeneration [28,29].

This work raises the problem of the origin of the cholesterol in the nervous system. It appears in the brain as a result of two processes: in situ synthesis and transfer of cholesterol from plasma. Cholesterol which appears in the brain by synthesis disappears from this organ by transfer into the plasma [30]. Indeed, the hypothesis of a 'blood-brain barrier' represented by cerebral capillaries does not seem to be valid for cholesterol [31], though cholesterol seems poorly transported from the blood to the brain [32]. Why oligodendrocytes probably use 7-dehydrodesmosterol and the desmosterol pathway and Schwann cells the 7-dehydrocholesterol pathway remains to be elucidated. As the decrease in levels of cholesterol and all three precursors are of the same order of magnitude in the mutant mouse, the regulatory step is probably not at the level of their synthesis and shows that indeed the primary defects in these animals very probable do not lie in some aspect of sterol metabolism.

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