THE OCCURRENCE OF DESMOSTEROL IN NEURONAL AND GLIAL FRACTIONS FROM DEVELOPING RAT BRAIN

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Received 30 May 1974

1. Introduction

Desmosterol (24-dehydrocholesterol) is found in the developing brains of many species but is absent, or present at low levels, in the mature nervous system [1]. In particular, the highest levels are detected just prior to and during the early stages of myelination and loss of desmosterol, with concomitant increase in cholesterol, occurs during the period of rapid myelination.

At present the reason for the accumulation of this sterol is unclear. It has been suggested [2] that the increased concentration of desmosterol induces its own reduction and thus controls the rate of cholesterol biosynthesis and myelin formation.

With the advent of techniques for the separation of neuronal and glial fractions it has been possible to measure the concentrations of cholesterol and desmosterol in these two cell populations. Using one of these methods we have made the observation that desmosterol is found not only in glial fractions but also in neurones and that the proportion of desmosterol to cholesterol is the same in the two cell types regardless of the absolute amounts. This observation necessitates a revision of ideas concerning the accumulation of desmosterol in developing brain.

2. Methods

2.1. *Separation of neuronal and glial fractions*

Neuronal and glial fractions were isolated from whole pooled brains of Carworth–Europe strain CFHB rats of various postnatal ages as described [3]. The purified neuronal and glial fractions were washed free of the protein from the separating media and resuspended in a known volume of 0.9% NaCl solution. Aliquots were taken for the determination of cholesterol and desmosterol, carbonic anhydrase and for phase-contrast microscopy.

2.2. *Purity of fractions*

The two fractions were judged pure as seen by phase-contrast microscopy, the neuronal fraction consisted of neuronal perikarya shorn of their axons with clearly visible nuclei whilst the smaller glial cells had extremely granular cytoplasm and were uncontaminated by neuronal perikarya. Carbonic anhydrase has been suggested as a possible glial cell marker [4] since micro-dissection of Dieter's nucleus has revealed 100–120 fold more activity in the glia than the neurones. In this study carbonic anhydrase activity was assayed as described [5] and the average ratio of glial to neuronal activity was 90:1. Rose [4] gives a value of 100:1 for neurones and glia isolated from adult rat cortex. Thus it is assumed that the fractions were pure using the above criteria, however, since it is likely that the glial fraction includes oligodendroglia, astrocytes, ependymal cells, dendrites, and synaptic material, we will refer to this fraction as neuropil.

2.3. *Isolation and estimation of cholesterol and desmosterol*

Samples of neurones and neuropil were freeze dried and then saponified at 70°C for 1 hr in 5–7 ml of 10% (w/v) KOH in ethanol. After the addition of 5 ml of water the non-saponifiable material was extracted 3 times with 10 ml volumes of *n*-hexane.

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The hexane extracts were combined, washed with water and then evaporated to dryness under nitrogen. The residue was dissolved in a small quantity of cyclohexane and desmosterol was separated from cholesterol by GLC using a column of 1% SE-30 on Gas Chrom Q 100/120 mesh at 220°C, carrier gas nitrogen, 50 ml/min. The relative retention time of desmosterol to cholesterol was 1.1. Estimation of sterols was by triangulation using an internal standard of lanosterol.

3. Results and discussion

Fig. 1 shows the ratio of desmosterol to cholesterol in neurones and neuropil isolated from rat brain during early post-natal development and also the ratio in whole brain for comparison. Statistical analysis reveals no significant differences between the distributions; for neurones and neuropil $\chi^2_{22} = 0.32 < 8.643$ ($p = 0.995$), for whole brain and neurones $\chi^2_9 = 0.08 < 1.65$ ($p = 0.995$). Hence it is concluded that the ratio of desmosterol to cholesterol is identical for these two cell populations over the range studied. The absolute amounts of desmosterol and cholesterol in neurones and neuropil are shown in fig. 2. As expected the amounts of cholesterol and desmosterol recovered from the neuropil are greater than that from neurones since not only do glial cells outnumber neurones but they have a much greater capacity for cholesterol biosynthesis, especially during myelination [6]. However, regardless of the absolute amounts the ratios of these compounds are the same throughout early post-natal development.

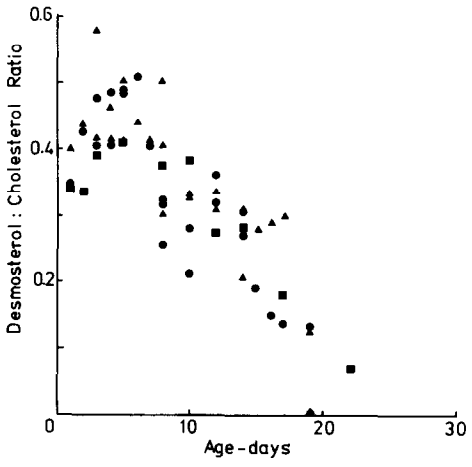

Fig. 1. The ratio of desmosterol to cholesterol in neurones (■), neuropil (▲) and whole brain (●) in the rat during early postnatal development. Sterols were isolated and determined by GLC as described in Methods.

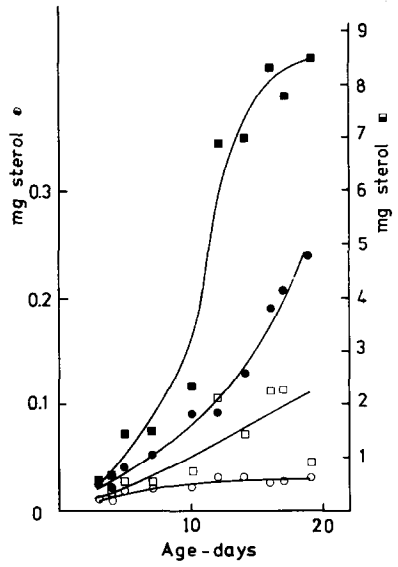

Fig. 2. Amounts of cholesterol and desmosterol in isolated neurones and neuropil from developing rat brain. (■) cholesterol in neuropil (□) desmosterol in neuropil; (●) cholesterol in neurones; (○) desmosterol in neurones. Sterols were isolated and determined by GLC as described in Methods.

This identity can in principle arise in three different ways. Firstly it could be that the separation procedure does not give reasonably pure neuronal and glial fractions. However, if it is assumed that neurones do not contain any desmosterol and that the desmosterol observed is derived from contaminating neuropil it must be conceded that under these circumstances it would be highly improbable that the ratios would be the same. The evidence from microscopic analysis and the distribution of the putative glial marker enzyme carbonic anhydrase would seem to rule out this unlikely explanation.

Secondly it could be that desmosterol is entirely synthesised in either neurones or glia and is then transported into the other type of cell. Intracellular movement of sterol from myelin to glial cytoplasm

and vice versa has been observed [7] as has axoplasmic flow of cholesterol [8] and it is also known that for the synthesis of steryl esters by isolated neuropil the addition of neurones is required [6]. However, there is as yet no evidence for intercellular transfer of sterols from glia to neurones or vice versa.

Thirdly, the explanation which we feel interprets the above results satisfactorily is that both neurones and glia synthesise desmosterol and that the occurrence of desmosterol had biological significance with regard to the nature of developing myelin and neuronal membranes. Considering that desmosterol, with its extra Δ^{24}-double bond, may constitute up to 30% of the total brain sterol content during development it is likely that this can alter the properties of cerebral membranes in some way that is essential for the process of myelination.

It is now known that the composition of myelin alters during development [9], changing from a composition resembling oligodendroglial plasma membranes, to a composition intermediate between plasma membrane and mature myelin ('early-myelin'), finally becoming mature myelin. This process of maturation is accompanied by alterations in lipid composition including increases in the chain lengths of fatty acids and a decrease in their degree of unsaturation [10] and it is now established that such changes, coupled with changes in sterol composition, can alter the properties of membranes [11]. Many of these changes influence the fluidity and hence the mobility of components within membranes and we suggest that the presence of a large amount of desmosterol in developing myelin and neuronal membranes assists the mobility of membrane components and facilitates the alterations in composition that are known to occur during brain development.

Hinse and Shah [2] have postulated that desmosterol induces desmosterol reductase thereby increasing the rate of cholesterol synthesis and hence myelin formation. However, the fact that increased levels of desmosterol are associated with increased activity of demosterol reductase does not necessarily imply a causal relationship. Their explanation does not account for the presence of desmosterol in myelin [12] nor does it suggest any possible role for desmosterol in membranes. Furthermore it has been proposed that the development of the brain is under strict genetic control [13] with 'temporal genes' regulating differentiation, migration, enzyme biosynthesis, etc. Thus the increased levels of desmosterol reductase which are observed during postnatal development in rat brain may be directly controlled by the genome and to suggest enzyme induction by desmosterol may be unnecessary.

Acknowledgements

The authors wish to thank Mr R. V. Hughes for helpful advice. The authors wish to acknowledge financial from the University of Liverpool (R.G.D.) and the Medical Research Council.

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