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ACETOACETATE IS A CHOLESTEROGENIC PRECURSOR FOR MYELINATING RAT BRAIN AND SPINAL CORD

INCORPORATION OF LABEL FROM [3-^{14}C]ACETOACETATE, [^{14}C]GLUCOSE AND $^{3}H_2O$

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Rat pups, 3 weeks old, were injected i.p. with combinations of $^{3}H_2O$ and either [3-^{14}C]acetoacetate or [^{14}C]glucose. $^{3}H/^{14}C$ incorporation ratios were measured in lipid fractions of homogenates and myelin prepared from whole brain and spinal cord. Spinal cord synthesized at least twice as much fatty acids and 3-fold more sterols than whole brain. Both tissues used acetoacetate preferentially for sterol synthesis, whereas label from [^{14}C]glucose was distributed between fatty acids and sterols in the same way as ^{3}H from $^{3}H_2O$. The relative contributions of acetoacetate to sterol synthesis in whole tissue and in the purified myelin fraction were about the same, both for the cerebrum and for the spinal cord.

Myelin of rat central nervous system (CNS) is formed postnatally at a rate that is maximal in the third week after birth [1]. Cholesterol is a major component of the lipid bilayer of compact myelin contributing to its characteristic stability [2]. The uptake of cholesterol from circulating lipoproteins is a very slow process in adult rat brain [3], and cholesterol synthesis in situ appears to be more important both in young and in adult rat brains [4]. This conclusion is endorsed also by recent experiments in vivo with $^{3}H_2O$ which indicate that brain cholesterol is formed 90% endogenously in adult rats [5] and at least 50% in suckling rats [6]. Degradation of cholesterol does not occur in adult rat brain: endogenous cholesterogensis appears to be balanced by delivery of cholesterol to the blood [7,8].

Young rat pups convert part of ingested milk-fat to ketone bodies which are used to a larger extent than glucose for the synthesis of cholesterol in the brain [9–12]. In the present study we compared $^{3}H_2O$ with either [^{14}C]glucose or [3-^{14}C]acetoacetate as precursors for the synthesis of whole brain and myelin lipids in vivo. As no data are available from double-label studies in vivo on cholesterogenesis and lipogenesis in spinal cord, we included this part of rat CNS in our analysis.

3-week-old Wistar rats were injected i.p. with $^{3}H_2O$ (1 mCi/g body weight) in combination with either [3-^{14}C]acetoacetate (0.5 μCi/g body weight) or [^{14}C]glucose (0.75 μCi/g body weight). Cerebra [11] and spinal cord [13] were removed and homogenized 4 h after injection. Myelin was isolated from the homogenates; total lipids, extracted from the homogenates and the myelin, were fractionated into a saponifiable and a non-saponifiable fraction [11]. 3-β-Hydroxysterols (DPHS, digitonin-precipitable 3-β-hydroxy-sterols) were precipitated from the latter fraction with digitonin [6]. The incorporation rates of ^{3}H and ^{14}C into total fatty acids and into DPHS were measured by liquid scintillation counting.

Abbreviation: DPHS, digitonin-precipitable 3-β-hydroxy sterols.

Table I summarizes the results as ratios of ^3H incorporated (DPHS/fatty acids; column 1) and ratios of ^{14}C incorporated (DPHS/fatty acids; column 2). Ratios turned out to be more reliable and informative than incorporation data in dpm. The rates of incorporation, especially of [3-^{14}C]acetoacetate, varied over a wide range, even between littermates. This is a consequence of variable pool size and turnover rate as discussed before [11].

Incorporation of ^{14}C both from [U-^{14}C]glucose and from [2-^{14}C]glucose resulted in ^{14}C ratios (column 2) which were close to the ^3H ratios (column 1). Apparently, the ^{14}C and the ^3H were distributed in a similar way between fatty acids and DPHS. This is in line with the established pathways of glucose breakdown and synthesis of cholesterol and fatty acids from one pool of acetyl-CoA. In the case of [3-^{14}C]acetoacetate, on the other hand, the ^{14}C ratios were almost twice the ^3H ratios, indicating a preferential incorporation of acetoacetate into cholesterol. The best parameter to substantiate these conclusions turned out to be the cholesterogenic index (column 3). We defined this index as ($^{14}C/^3H$ in DPHS)/($^{14}C/^3H$ in fatty acids) which equals the quotient of the ^{14}C and ^3H (DPHS)/(fatty acids) ratios given in column 2 and 1, respectively. This cholesterogenic index varied less between experiments than the ^3H and ^{14}C ratios. It should be noted that potential sources of variation, such as the recoveries of sterols and fatty acids from total lipids, are eliminated from the cholesterogenic index. The higher ^{14}C ratios for cerebral homogenates and myelin fractions reported by us previously [11] can be explained by a further purification of the nonsaponifiable fraction with digitonin in this study. Table I also shows that the myelin fractions differed from whole brain or spinal cord homogenates with respect to their ratios of DPHS to fatty acids. With ^3H_2O as precursor, this ratio was 5–8-fold higher in cerebral myelin and 3–4-fold higher in spinal cord myelin than in the original homogenates. This could well reflect much higher rates of cholesterogenesis in oligodendrocytes compared to other brain cells of 3-week-old rats. Interestingly, spinal cord appeared to be much more active than cerebrum with respect to incorporation of ^3H from ^3H_2O into DPHS (1.48 ± 0.12 and 0.43 ± 0.04 μmol/g wet weight per h, respectively) and into fatty acids (5.4 ± 0.3 and 2.4 ± 0.2 μmol/g wet weight per h, respectively). It should be noted that very similar results were obtained after 1- and 2-h incubations. It is unlikely therefore that uptake of labeled lipids from the circulation is an important process.

TABLE I

LABEL INCORPORATION FROM [^{14}C]GLUCOSE, [3-^{14}C]ACETOACETATE AND ^3H_2O INTO STEROLS AND FATTY ACIDS OF HOMOGENATES AND MYELIN FRACTIONS PREPARED FROM CEREBRA AND SPINAL CORD

^3H and ^{14}C radioactivities (dpm) were measured in digitonin-precipitable 3-β-hydroxy-sterolds (DPHS) and in total fatty acids. The ratios between the (^{3}H-dpm in DPHS)/(^{3}H-dpm in fatty acids) are presented in column 1, those of the (^{14}C-dpm in DPHS)/(^{14}C-dpm in fatty acids) in column 2. The last column is a cholesterogenic index (($^{14}C/^3H$) in DPHS)/(($^{14}C/^3H$) in fatty acids), which equals the quotient of (column 2)/(column 1). Averages of data from six rats are presented ($\pm$S.D.).

Radioactive substrates	Fraction	Ratio DPHS/fatty acids		Cholesterogenic index
		^3H	^{14}C	
[U-^{14}C]Glucose + ^3H_2O	Cerebral homogenate	0.08 ± 0.01	0.08 ± 0.01	0.99 ± 0.04
	Cerebral myelin	0.66 ± 0.06	0.58 ± 0.05	0.88 ± 0.01
[2-^{14}C]Glucose + ^3H_2O	Cerebral homogenate	0.11 ± 0.01	0.10 ± 0.01	0.93 ± 0.05
	Cerebral myelin	0.51 ± 0.06	0.44 ± 0.06	0.86 ± 0.03
	Spinal cord homogenate	0.21 ± 0.03	0.19 ± 0.03	0.92 ± 0.01
	Spinal cord myelin	0.77 ± 0.09	0.61 ± 0.07	0.79 ± 0.01
[3-^{14}C]Acetoacetate + ^3H_2O	Cerebral homogenate	0.14 ± 0.01	0.28 ± 0.02	1.95 ± 0.07
	Cerebral myelin	0.61 ± 0.04	1.03 ± 0.03	1.70 ± 0.08
	Spinal cord homogenate	0.25 ± 0.01	0.47 ± 0.13	1.90 ± 0.07
	Spinal cord myelin	0.77 ± 0.09	1.28 ± 0.10	1.67 ± 0.07

These data provide strong support for the postulation by Webber and Edmond [9] that developing rat brain can use acetoacetate directly as precursor for cholesterol, bypassing the cytosolic pool of acetyl-CoA.

The conclusions of previous studies on this subject [9–12] were based on a direct comparison of incorporation data from [^{14}C]glucose with data from [^{14}C]ketone bodies. Theoretically, higher ^{14}C ratios of cholesterol to fatty acids with ketone bodies as substrates than with glucose could result either from preferential use of glucose for fatty acid synthesis or from preferential use of ketone bodies for cholesterogenesis. The simultaneous measurement of ^{3}H and ^{14}C ratios in the present study permits a decision between these two possibilities and suggests that the latter possibility is indeed correct. It shows, moreover, that this is the case both for cerebra and for the spinal cord of 3-week-old rats. The spinal cord turned out to be 3.6 ± 0.4 ($n = 16$) -fold more active in sterol synthesis than cerebral tissue and hence also uses more acetoacetate for this biosynthetic path.

A high specific activity of acetoacetyl-CoA synthetase (EC 6.2.1.-), the committed enzyme in the use of intact C_4-units for cholesterogenesis, was reported for isolated calf oligodendrocytes [14]. If the oligodendrocytes of rat pups are also enriched in this activity, a functional relationship between ketone-body utilization and myelination could exist. The present results do not support this notion because the cholesterogenic index was not higher in myelin fractions than in the original homogenates. In fact, slightly lower indices were observed for all myelin preparations, both with [^{14}C]glucose and with [3-^{14}C]acetoacetate as substrates. We observed that primary cultures of astrocytes, neurons or oligodendroglia all have a capacity for the conversion of acetoacetate to cholesterol (Ref. 15 and Koper, J.W., Lopes-Cardozo, M., Schousboe, A. and Sykes, J.E.C., unpublished data). These data support the conclusion drawn from the present results, viz. that the preferential use of acetoacetate for cholesterogenesis is not confined to one cellular compartment but is expressed homogeneously in the brain and spinal cord of 3-week-old rats.

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