Rates of cholesterol, ubiquinone, dolichol and dolichyl-P biosynthesis in rat brain slices

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Slices from the brain and liver of rats were prepared and upon incubation exhibited a continuous and high capacity for incorporation of radioactive precursors into proteins and lipids. Using [^{3}H]mevalonate as precursor, the rates of biosynthesis of cholesterol, ubiquinone, dolichol and dolichyl-P in brain slices were determined and found to be 5.5, 0.25, 0.0093 and 0.0091 nmol/h/g, respectively. Dolichol and dolichyl-P accumulate to a limited extent, but almost all of these lipids in the brain originate from de novo synthesis. The calculated half-lives for cholesterol, ubiquinone, dolichol and dolichyl-P were 4076, 90, 1006 and 171 h, respectively. The results indicate that lipids formed via the mevalonate pathway in the brain have an active and independently regulated biosynthesis.

Cholesterol; Dolichol; Ubiquinone; Biosynthetic rate; Brain slice

1. INTRODUCTION

The brain has complete enzymatic systems for biosynthesis of the mevalonate pathway lipids, cholesterol, ubiquinone, dolichol and dolichyl-P [1]. The chemical composition of the brain differs from other organs, since this organ is especially rich in lipids, including the end products of the mevalonate pathway [2–4]. In contrast to several other organs, the uptake of lipids from the circulation by the brain is limited, which emphasizes the importance of endogenous synthesis. Labeling of brain lipids in vivo is difficult, a fact which necessitates the use of other systems for studying the synthesis of various lipids by this organ. Previous investigations have demonstrated that appropriately prepared tissue slices synthesize constitutive components at rates proportional to those observed in vivo [1,5]. Consequently, such a system is suitable for studies on the metabolism of cellular components.

The rates of ubiquinone and polyisoprenoid biosynthesis in brain have not yet been studied, but investigations on cholesterol turnover have been performed, since the large amount of this lipid in brain makes such studies possible. Earlier experiments suggested a very low rate of cholesterol synthesis in brain [6], but more recent studies indicate that a considerable portion of this lipid is continuously renewed [7,8]. It was also proposed that myelin is present in several compartments possessing different turnover times [9]. In the present investigation the rates of synthesis of products of the mevalonate pathway in brain slices were studied. It appears that these lipids are renewed to very different extents in adult rat brain.

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2. MATERIALS AND METHODS

Male Sprague-Dawley rats with ages of 2 and 10 months were used. All experiments were begun at the same timepoint in the day-night cycle. The rats were fed standard pellets ad libitum. They were killed by decapitation and the brains rapidly removed. The brain was cut into cubes 0.5 × 0.5 × 1.0 cm in size and placed in cold modified minimum essential medium (Flow Laboratories, Irvine).

Slices of 500 μm thickness were subsequently prepared with a Polaron H 1200 Vibrating microtome (BioRad, Watford). For histological evaluation a paraffin embedded section of the slices was stained with hematoxylin-eosin. The slices were placed in flasks containing 4 ml medium supplemented with 5 mM glucose and 0.7 mM unlabeled mevalonate. This concentration is required to minimize the effect of dilution by the endogenous pool of mevalonate [5]. Each flask contained approximately 0.5 g brain slices and was supplemented with 0.5 mCi (RS)-5-[^{3}H]mevalonolactone (37.9 Ci/mmol, New England Nuclear). This system was then incubated at 37°C, with exposure to carbogen gas (95% O_2/5% CO_2) and slow shaking. Incubation was terminated by rinsing the slices in cold 0.9% NaCl solution.

For measurement of dolichol and dolichyl-P, the homogenates were supplied with dolichol-23 and dolichyl-P-23 as internal standards and subjected to alkaline hydrolysis, followed by extraction with chloroform/methanol, 2:1. For determination of ubiquinone and cholesterol, the homogenates were supplied with ubiquinone-6 and ergosterol as standards and extracted with chloroform/methanol, 2:1, without alkaline hydrolysis. After silica and reversed phase chromatography, cholesterol, ubiquinone, dolichol and dolichyl-P were separated by HPLC [10,11] and their radioactivity determined by scintillation counting. The rates of synthesis were calculated by dividing the total radioactivity appearing in the different lipids by the specific activity of the [^{3}H]mevalonate added to the incubation flasks. The values obtained were corrected by a factor of 4.4 in order to estimate the rate of in vivo synthesis [1,2]. In order to take into consideration the different numbers of [^{3}H]mevalonate molecules incorporated into dolichol, ubiquinone and cholesterol, the levels of total radioactivity were divided by 19,

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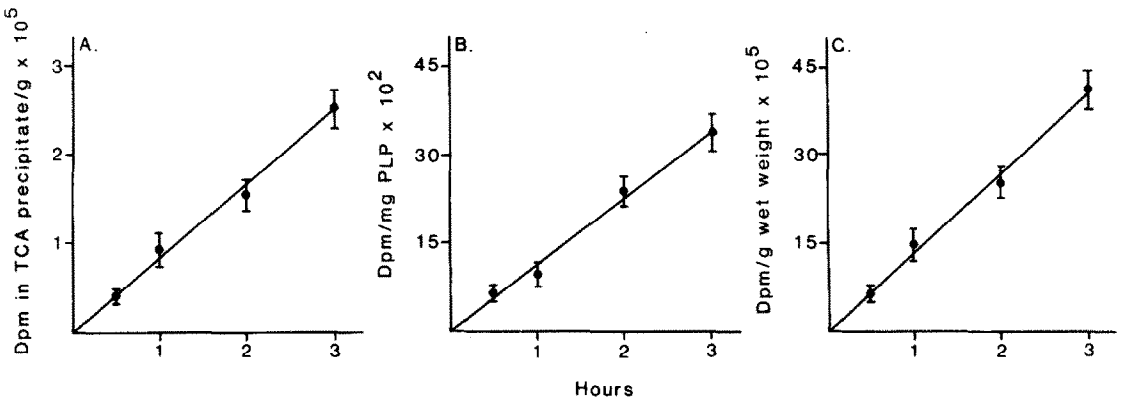

Fig. 1. Incorporation of [^{3}H]leucine into total protein, of [^{3}H]glycerol into total phospholipid and of [^{3}H]mevalonate into cholesterol by brain slices. (A) After incubation with 2.5 μCi [^{3}H]leucine/ml, the slices were rinsed, homogenized, precipitated with 12% cold CCl_3CO_2H and dissolved in 2% sodium dodecyl sulphate in order to measure radioactivity in total protein. (B) After incubation of slices with 2 μCi [^{3}H]glycerol/ml, the homogenates were extracted with $CHCl_3$/MeOH, 2:1, and phospholipids isolated on a silica column. (C) The homogenate lipids were extracted and neutral lipids separated by silica column chromatography followed by isolation of cholesterol on HPLC. The values are the means ± SE of values obtained with slices from 5 rats

9 and 5.5, respectively (factor K). Consequently, the rate of synthesis (4.4 × total lipid dpm)/(spec. act. in the precursor pool × K). Turnover time was calculated by dividing the concentration of lipid in the tissues with the amount of lipid synthesized per hour. Half-life ($t_{1/2}$) was obtained by dividing the turnover time by 1.44 [13].

3. RESULTS AND DISCUSSION

The slices prepared had a constant thickness of 500 μm and upon histological examination were found to possess damaged cells to a limited extent only at the cutting surface. In order to investigate the metabolic intactness of these slices, the time courses of the incorporation of certain precursors into cellular components were monitored. Incorporation of [^{3}H]leucine into trichloroacetic acid-precipitable protein was linear during the 3 h incubation period, demonstrating the presence of active protein synthesis (Fig. 1A). [^{3}H]Glycerol incorporation into total phospholipid is often utilized to follow the biosynthetic rate and a high rate of such incorporation could be obtained in slices (Fig. 1B). The time course of labeling indicates that synthesis of these lipids is also well preserved. Incorporation of [^{3}H]mevalonate into total cholesterol (isolated after alkaline hydrolysis) also occurred in a linear fashion, further demonstrating that the slices prepared are useful for investigation of synthetic processes (Fig. 1C).

In these experiments slices were prepared from the brains of rats 2 and 10 months of age and, therefore, the lipid composition of brain at these ages was determined (Table I). At 2 months of age the two largest components are phospholipid and cholesterol. The ubiquinone content is 23 μg/g wet weight and the polyisoprenoid content is also approximately at this same level, consisting of 5 times more dolichol than dolichyl-P. Eight months later the phospholipid content is unchanged and the levels of ubiquinone and cholesterol decreased to a great and moderate extent, respectively, while dolichol and dolichyl-P levels are increased. For comparison the lipid content of liver was also determined. The changes in liver tissue between 2 and 10 months were similar to those observed in brain with the exception of the cholesterol level, which increased in liver.

The rates of synthesis of various products of the mevalonate pathway using [^{3}H]mevalonate as precursor are shown in Table II. The rate of ubiquinone synthesis in brain slices was 20 times less than that of cholesterol, while the rates of dolichol and dolichyl-P biosynthesis were similar, i.e. around 0.01 nmol/h/g tissue. In comparison with liver on a gram basis, the rate of cholesterol synthesis in brain was considerably less (77 times) and the ratios of the synthetic rates for ubiquinone, dolichol and dolichyl-P in liver versus brain were 7, 27 and 15, respectively.

The values in Table I show that during this 8-month period of the rat's lifespan, 6.4 μg dolichol and 1.4 μg dolichyl-P are accumulated. It can be calculated from the values in Table II that during this same time period

Table I

Lipid composition of rat brain and liver

Lipid	Brain		Liver	
	2 months	10 months	2 months	10 months
Phospholipid[a]	25.2 ± 2.9	24.6 ± 3.1	23.0 ± 2.6	23.1 ± 2.6
Cholesterol[a]	12.5 ± 1.2	9.4 ± 1.0	2.3 ± 0.3	5.6 ± 0.5
Ubiquinone[b]	23.3 ± 2.2	12.8 ± 1.4	42.1 ± 4.7	19.4 ± 2.2
Dolichol[b]	17.7 ± 2.1	24.1 ± 2.9	25.0 ± 2.3	63.5 ± 5.4
Dolichyl-P[b]	3.2 ± 0.4	4.6 ± 0.6	9.0 ± 1.0	8.7 ± 0.9

The values are the means ± SEM of six rats
[a] mg/g wet weight
[b] μg/g wet weight

Table II

Rate of lipid synthesis in brain slices of 2 month-old rats

Lipid	Brain		Liver		Liver/brain
	(dpm/g/h)	(nmol/g/h)	(dpm/g/h)	(nmol/g/h)	
Cholesterol	1326870	5.5 ± 0.6	100360000	416 ± 51	77
Ubiquinone	98900	0.25 ± 0.03	473040	1.8 ± 0.23	7.2
Dolichol	7770	0.0093 ± 0.0009	208000	0.25 ± 0.03	26.9
Dolichyl-P	7580	0.0091 ± 0.0008	116680	0.14 ± 0.02	15.4

The slices were incubated in the presence of [^{3}H]mevalonate. After incubation the lipids were extracted and after silica and reversed phase chromatography, separation was obtained by HPLC and calculations were performed as described in section 2. The values are the means ± SE of 7 experiments

approximately 70 μg dolichol and dolichyl-P are synthesized.

The values obtained in Tables I and II were used to calculate the half-life of lipids in the brain (Table III). Cholesterol has a $t_{1/2}$ of about 6 months, while in the liver this value is 99 h. The values for ubiquinone, dolichol and dolichyl-P are approximately 4, 42 and 7 days, respectively. The $t_{1/2}$ values for these lipids in the liver are between 20 and 50 h.

The results demonstrate that the lipid products of the mevalonate pathway are continuously synthesized in the brain, but that the synthetic rates are very different. In comparison with liver the rate of cholesterol biosynthesis is 77 times slower in brain, but the liver values do not necessarily represent synthesis of membrane constituents only. In the initial phase of synthesis by the liver, cholesterol is produced to a large extent for discharge into the blood and as precursor for the biosynthesis of bile acids. The amount of newly synthesized liver cholesterol which is utilized as a component of cellular membranes most probably represents only a small fraction of the total. Therefore, the amount incorporated into liver membranes may not be very different from that of brain, where cholesterol is not excreted or utilized for the production of active metabolites.

Ubiquinone biosynthesis, on the other hand, is only 7 times faster in liver than in brain, in agreement with the fact that this lipid is discharged into the bile and circulation to only a limited extent [14]. Rates of dolichol and dolichyl-P biosynthesis are 20 times less in brain than in liver, but even in these cases the real differences may be much less. Some export of these lipids to the circulation in association with lipoproteins occurs and considerable amounts of hepatic dolichol and its metabolites are discharged into the bile [14,15].

It appears therefore that the rate of synthesis of the lipid products of the mevalonate pathway in the brain of adult rats occurs at rates which are slower, but nonetheless comparable to those seen in the liver. Some accumulation of both dolichol and dolichyl-P takes place in brain during aging, but this amount is only a few percent of that produced by de novo synthesis. Clearly, a continuous and active pathway for the breakdown of polyisoprenoid compounds must operate in adult rat brain in order to maintain a constant polyisoprenoid level in the presence of a continuous synthesis.

The values obtained for the biosynthetic rates were used to calculate the half-life of the lipids. At present there is no direct evidence that these values calculated for brain slices are identical to those in vivo, but the values for liver are similar to those obtained previously using other approaches [16,17]. While the $t_{1/2}$ values for ubiquinone and dolichyl-P in the brain are relatively short, the $t_{1/2}$ of cholesterol and dolichol are very long. The probable explanation for this finding is that cholesterol and dolichol are present in different pools with very different turnovers. It is also probable that both cholesterol and dolichol are not degraded in brain [18,19] and slow removal may occur through exchange with blood.

Table III

Half-lives of lipids in brain

Lipid	Brain (h)	Liver (h)
Cholesterol	4080	99.2
Ubiquinone	89.6	22.4
Dolichol	1010	52.9
Dolichyl-P	171	31.0

The $t_{1/2}$ was calculated from Tables I and II as described in section 2

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REFERENCES

[1] Jeske, D.J. and Dietschy, J.M. (1980) J. Lipid Res. 21, 364–376.
[2] Sastry, P.S. (1985) Prog. Lipid Res. 24, 69–176.
[3] Andersson, M., Appelqvist, E.L., Kristensson, K. and Dallner, G. (1987) J. Neurochem. 49, 685–691.

[4] Söderberg, M., Edlund, C., Kristensson, K. and Dallner, G. (1990) J. Neurochem. 54, 415–423.
[5] Elmberger, P.G., Kalen, A., Appelkvist, E.L. and Dallner, G. (1987) Eur. J. Biochem. 168, 1–11.
[6] Nicholas, H.J. and Thomas, B.E. (1959) Biochim. Biophys. Acta 36, 583–585.
[7] Turley, S.D., Andersen, J.M. and Dietschy, J.M. (1981) J. Lipid Res. 22, 551–569.
[8] Turley, S.D. and Dietschy, J.M. (1988) in: The Liver Biology and Pathobiology (Arias, I.M., Jakoby, W.B., Popper, H., Schachter, D. and Shafritz, D.A. eds) pp. 617–641, Raven Press, New York.
[9] Morell, P., Quarles, R.H. and Norton, W.T. (1981) in: Basic Neurochemistry (Siegel, G., Agranoff, B., Albers, R.W. and Molinoff, P. eds) pp. 109–136, Raven Press, New York.
[10] Elmberger, P.G., Eggens, I. and Dallner, G. (1989) Biomed. Chromatogr. 3, 20–28.
[11] Tollbom, Ö., Valtersson, C., Chojnacki, T. and Dallner, G. (1988) J. Biol. Chem. 263, 1347–1352.
[12] Adair, W.L. and Keller, R.K. (1982) J. Biol. Chem. 257, 8990–8996.
[13] Comar, C.L. (1955) in: Radioisotopes in Biology and Agriculture, pp. 1–58, McGraw-Hill, New York.
[14] Elmberger, P.G., Kalen, A., Brunk, U. and Dallner, G. (1989) Lipids 24, 919–930.
[15] Kalen, A., Söderberg, M., Elmberger, P.G. and Dallner, G. (1990) Lipids 25, 93–99.
[16] Eriksson, L.C. and Dallner, G. (1973) FEBS Lett. 29, 351–354.
[17] Edlund, C., Brunk, U., Chojnacki, T. and Dallner, G. (1988) Biosci. Rep. 8, 139–146.
[18] Chojnacki, T. and Dallner, G. (1988) Biochem. J. 251, 1–9.
[19] Dhopeshwarkar, G.A. and Subramanian, C. (1981) Lipids 16, 389–392.