BBA 75831

FATTY ACID TRANSPORT INTO THE BRAIN

G. A. DHOPESHWARKAR, CAROLE SUBRAMANIAN, DOUGLAS H. McCONNELL* AND JAMES F. MEAD

Laboratory of Nuclear Medicine and Radiation Biology, 900 Veteran Avenue, University of California, Los Angeles, Calif. 90024, and Department of Biological Chemistry, UCLA School of Medicine, Los Angeles, Calif. 90024 (U.S.A.)

(Received August 20th, 1971)

SUMMARY

15 μC of [1-^{14}C]palmitate was injected into three groups of animals. Group I served as control animals, Group II animals were subjected to functional hepatectomy, and animals of both groups received the tracer intravenously and were killed 1 h after injection. Group III animals received the tracer by an intracarotid injection followed by decapitation 15 sec later.

The circulating blood lipids in the control group had most of the radioactivity in the triglyceride fraction whereas in both hepatectomized and carotid injected animals the blood free fatty acid fraction had most of the radioactivity. Under these conditions the uptake of radioactivity by the brain was about 6 times higher in hepatectomized animals and 14 times higher in the intracarotid injected animals than in the control group. This indicates that free fatty acid is a preferred form of fatty acid transport to the brain.

INTRODUCTION

It is well known that most of the energy required by the brain both at rest and during activity is derived from the metabolism of carbohydrate, chiefly glucose[1]. However, ABOOD AND GEIGER[2] showed that during perfusion by glucose free solutions, structural components were used for energy by the brain while the preparation still maintained some of its physiological function, provided the cerebral blood flow was increased 2–3-fold over the normal flow. OWEN *et al.*[3] have reported that during starvation β-hydroxybutyrate and acetoacetate replaced glucose as predominant fuel for brain metabolism. Since this would be considered as an extreme condition, one can assume that compounds other than glucose are normally not used by the brain for energy needs. However, there is no doubt that most of structural lipid components of the brain do undergo turnover at varying rates. Recent studies by SMITH[4] have indicated that myelin lipids, which at one time were considered metabolically inert, have turnover rates varying from rapid to extremely slow. SUN AND HORROCKS[5] have shown that the half life of unesterified palmitic acid injected intracerebrally in mice is very short, which suggests that the brain fatty acids are in a state of continuous

* Department of Surgery, UCLA School of Medicine.

metabolic change. ALLWEIS *et al.*[6] reported that when albumin-bound [^{14}C]palmitate was perfused, the cat brain was able to oxidize it to labeled CO_2, indicating the oxidative capability of the brain. Previous work from this laboratory[7–10] has shown that most fatty acids are taken up by the adult brain directly without prior oxidation to acetate to any appreciable extent. The present study shows that palmitic acid is taken up from the circulating blood by the adult rat brain as the free acid rather than as a component of triglyceride or phospholipid.

MATERIALS AND METHODS

Tracer

300 μC of [1-^{14}C]palmitic acid (DHOM Products, Hollywood, Calif., 46.2 mC per mmole) were complexed with fatty acid-poor bovine serum albumin as described previously[8]. 15 μC in 0.2 ml of this clear solution was injected into each rat.

Animals

Albino Wistar male rats weighing approx. 475–500 g were used in the study; they had free access to food and water. Light anesthesia was maintained between injection and sacrifice.

Group I (control animals, six rats)

Rats were anesthetized by an intraperitoneal injection of sodium pentobarbital 2.85 mg/100 g body weight and the femoral vein was exposed. 15 μC of the tracer was injected intravenously into each animal. A midline incision was made on the abdomen to ensure similarity with the experimental group of animals. The animals were killed at the end of 1 h.

Group II (functionally hepatectomized, seven rats)

Anesthetized animals were operated on to perform functional hepatectomy. The abdomen was opened with a midline incision. The sigmoid colon and its blood supply was doubly ligated and divided. The superior mesenteric artery was first doubly ligated and divided. The porta hepatis containing the hepatic artery and the portal vein was then doubly ligated and divided. The esophagus was also doubly ligated and divided. This permitted removal of the entire intestinal tract from the stomach to the sigmoidal colon in addition to isolating the liver from circulating blood. 15 μC of [1-^{14}C]palmitic acid albumin complex was injected intravenously as in Group I and the animals were sacrificed after 1 h.

Group III (carotid arterial injection, five rats)

Anesthetized animals were given the tracer dose by injection into the surgically exposed carotid artery followed by decapitation 15 sec later as described earlier[11].

Lipid extraction

The entire brain and a piece from each lobe of the liver were dissected within 2 min and immersed in chloroform–methanol (2:1, v/v) immediately. Plasma was obtained by centrifugation of the heparinized blood and lipids were extracted by the FOLCH *et al.*[12] method as described previously[7].

Fractionation

Blood lipids. Plasma total lipids from all animals were extracted separately and aliquots were counted for radioactivity. The remaining portions from each group were pooled and chromatographed on a thin-layer chromatographic plate (20 cm × 20 cm) coated with Silica gel G using 16 % ether and 1 % acetic acid in pentane as developing solvents. After brief exposure to iodine fumes, the areas were marked and then scraped into tubes. Extraction and radioactive counting were done according to the method described by KRITCHEVSKY AND MALHOTRA[13]. This gave the percent distribution of radioactivity in various plasma lipid components.

Liver lipids. Liver total lipids were fractionated into triglycerides, cholesterol and phospholipids using SiO_2 column chromatography as described earlier[7].

Brain lipids. Brain tissue from all animals was extracted separately to obtain total lipids and the radioactivity was determined. Then the total lipids in each group were pooled for chromatography on a SiO_2 column using chloroform to elute neutral lipids (triglycerides, free fatty acid and cholesterol) and methanol to elute polar lipids (phospho- and sphingolipids). When the polar lipid fraction was analyzed by thin layer chromatography in a system that was used for blood total lipids, it was found that brain lipids obtained from animals in Groups II and III (but not in the control animals) contained a highly radioactive free fatty acid fraction. After preliminary experiments with known amounts of [^{14}C]palmitate added to inactive phospholipid fraction, it was found that such contamination could be eliminated by treating the polar lipid fraction with diazomethane in ether, at room temperature for a period of 5 min. This converted the free acids to methyl esters without any effect on the polar lipid components and the methyl esters thus formed were eluted easily with 5 % ether in pentane from a SiO_2 column. The polar lipid fraction obtained after this procedure gave no radioactivity in the area of free fatty acids when tested by thin layer chromatography. One portion of the polar lipids was fractionated by methods described by ROUSER *et al.*[14] and the other portion was subjected to methanolysis and fractionated to give pure palmitic and stearic acids as described previously[7]. The fatty acids were decarboxylated by the Schmidt procedure as described earlier[7]. Radioactivity was determined on all samples using a Packard Tricarb Scintillation Spectrometer Model 574 operating at 80 % efficiency.

RESULTS

Table I shows the specific activity (counts/min per mg total lipids along with standard deviation), radioactivity of total lipids per ml of plasma, percent of dose circulating per ml of plasma and the distribution of radioactivity as percent of the total radioactivity. The specific activity of plasma lipids was maximal in Group III animals which received an intraarterial injection followed by decapitation at the very short interval of 15 sec. In other groups, the animals were kept alive for 1 h during which time much of the tracer would have undergone oxidation, giving lower specific activities. The hepatectomized animals, in contrast, had the lowest specific activity and minimum radioactivity per ml of plasma even though oxidative degradation by the liver was eliminated.

The percent distribution of the radioactivity shows that whereas in control

TABLE I

RADIOACTIVITY IN BLOOD LIPIDS AFTER INJECTION OF [1-^{14}C]PALMITIC ACID

Group	*Spec. act. (counts/min per mg total lipids)*	*Radioactivity of total lipids (counts/min per ml plasma)*	*% of given dose recovered in total lipids of 1 ml plasma*	*Distribution of radioactivity (% of total radioactivity)*				
				Cholesterol esters	*Triglycerides*	*Free fatty acids*	*Cholesterol*	*Phospholipids*
Group I; control (6 rats)	19 521 ± 6075	54 456 ± 16 951	0.24 ± 0.08	1.5	77.7	10.9	6.0	1.6
Group II; hepatectomized (7 rats)	8 649 ± 4975	22 071 ± 15 302	0.1 ± 0.07	—	10.6	77.4	0.14	11.9
Group III; intracarotid injection (5 rats)	109 794 ± 47 636	306 337 ± 132 907	0.92 ± 0.4	0.25	0.65	90.1	4.8	2.3

TABLE II

UPTAKE OF RADIOACTIVITY BY THE BRAIN AND LIVER LIPIDS AFTER INJECTION OF [1-^{14}C]PALMITIC ACID

Group	*Brain*			*Liver*		
	Spec. act. (counts/min per mg total lipids)	*Radioactivity of total lipids (counts/min per g fresh weight of tissue)*	*% of given dose recovered in total lipids of 1 g*	*Spec. act. (counts/min per mg total lipids)*	*Radioactivity of total lipids (counts/min per g fresh weight of tissue)*	*% of given dose recovered in total lipids of 1 g*
Group I; control (6 rats)	49 ± 9	5 242 ± 972	0.02 ± 0.004	6836 ± 776	276 188 ± 31 354	1.25 ± 0.14
Group II; Hepatectomized (7 rats)	314 ± 257	36 688 ± 27 591	0.15 ± 0.12	243 ± 190	9 556 ± 7 829	0.04 ± 0.03
Group III; intra-carotid injection (5 rats)	878 ± 527	93 331 ± 56 596	0.28 ± 0.17	1831 ± 788	73 956 ± 31 855	0.22 ± 0.09

group animals the triglyceride fraction had the most radioactivity, in the other two groups it was the free fatty acid component that was most radioactive.

The uptake of radioactivity (along with the standard deviation) by the brain and liver total lipids is shown in Table II. The uptake of radioactivity in the brain follows the trend of circulating plasma activity in Group I and III, but whereas the circulating plasma radioactivity in the hepatectomized group was low, the uptake by the brain was relatively high. Both in the hepatectomized and carotid injected animals, the percent uptake of the given dose was very much higher than in the control group of animals.

TABLE III

INCORPORATION OF RADIOACTIVITY INTO MAJOR POLAR LIPID FRACTIONS OF BRAIN TOTAL LIPIDS FOLLOWING INJECTION OF [1-^{14}C]PALMITIC ACID

Group	*Specific radioactivity (counts/min per mg)*						
	Free fatty acids	*Total polar lipids*	*Cerebroside*	*Phosphatidyl ethanolamine*	*Phosphatidyl serine*	*Phosphatidyl choline*	*Sphingomyelin*
Group I; control (6 rats, pooled)	—	53	4	27	26	68	13
Group II; hepatectomized (7 rats, pooled)	2501	132	41	77	27	57	21
Group III; intracarotid injection (5 rats, pooled)	—	173	26	122	83	181	63

Liver lipids from the control group of animals had the highest radioactivity, which was about 4-fold higher than in the carotid injected animals. The small amount of radioactivity in the liver of functionally hepatectomized animals must have come from a minor blood supply, possibly *via* the phrenic route.

Highly radioactive free fatty acids were present in brain total lipids of hepatectomized and carotid injected animals but not in control animals. In Group III animals, only trace amounts (but with high radioactivity) of free fatty acids were present and so no specific activity values could be calculated. The specific radioactivity values of the total polar lipid fraction given in Table III are those obtained after removing the free fatty acids by diazomethane treatment and chromatography. Phosphatidyl choline was the most active component in Groups I and III whereas in hepatectomized animals it was phosphatidyl ethanolamine that had the highest specific activity. The cerebroside fraction from this group was also relatively more radioactive than in other groups. Both phosphatidyl serine and sphingomyelin fractions of brain total polar lipids from carotid injected animals were found to be relatively more radioactive than in other groups. However, the total polar lipids themselves in the three groups had varying specific activities.

The polar lipids from the brain were first freed from the free fatty acid fraction and then subjected to methanolysis. The percent distribution of radioactivity in palmitic and stearic acid, shown in Table IV, is thus from fatty acids derived from various polar lipids. Most of the activity of the palmitic acid was associated with

TABLE IV

DISTRIBUTION OF RADIOACTIVITY IN PALMITIC AND STEARIC ACID ISOLATED FROM BRAIN TOTAL POLAR LIPIDS FOLLOWING INJECTION OF [1-^{14}C]PALMITIC ACID

Polar lipids in each group were pooled to obtain fatty acids.

Group	*Palmitic acid*		*Stearic acid*	
	Spec. act. (counts/min per mg)	*% Rel. carboxyl act.*	*Spec. act. (counts/min per mg)*	*% Rel. carboxyl act.*
Group I; control	404	86	31	43
Group II; hepatectomized	1024	89	70	26
Group III; intracarotid injection	1304	94	70	14.4

the carboxyl carbon in all three groups. The carboxyl carbon activity in stearic acid decreased progressively from Group I to Group III.

DISCUSSION

The reason for performing functional hepatectomy on some of the rats was to prevent incorporation of the injected [1-^{14}C]palmitic acid into glycerides and phosphatides and recirculation. LAURELL[15] has reported that in the normal rat, a considerable fraction of the injected free fatty acids is recirculated in the blood in the form of glycerides and to a lesser extent in phospholipids. Further, this recirculation, according to BORGSTRÖM AND OLIVECRONA[16], is almost completely abolished after hepatectomy. These authors have cited the possible role of the small intestinal mucosa in the formation of glycerides and so in our hepatectomy procedure we also removed the entire length of the intestine from the rat after ligating off the liver blood supply. This procedure should then ensure circulation of the injected tracer as free fatty acids. This is borne out by the results in Table I showing that 77 % of the total radioactivity was associated with the free fatty acid fraction. Further, under these conditions, the specific radioactivity of the brain total lipids was about 6 times higher than in the control group of rats, indicating a considerably higher uptake of the given dose (Table II).

It seemed reasonable that a similar pattern of radioactivity of circulating blood lipids, a preponderance of radioactivity in the free fatty acids, could be achieved by reducing the time interval between injection and sacrifice of the animal. Therefore, in Group III animals the tracer was injected into the carotid artery followed by decapitation within 15 sec[11]. The carotid injection, as against femoral intravenous injection, ensures that the tracer goes first to the brain and the short interval minimizes recirculation in the form of glycerides. Table I shows that under these conditions almost 90 % of the radioactivity was circulating as free fatty acids. Examination of uptake of radioactivity by the brain in these animals (Table II) showed that it was 14 times higher than in the control group.

The occurrence of free fatty acids in brain lipids has been reported earlier[17,18] but the concentration was low (only about 0.8 % of the total fatty acids). BORGSTRÖM[16] has reported that hepatectomy resulted in an increased amount of free fatty acids

in the depot fat and blood plasma; however, its effect on brain free fatty acid is unknown. Direct transport of radioactive palmitic acid from blood into brain tissue, clearly indicated in our earlier work[7], can now explain the highly radioactive free fatty acid fraction in brain total lipids of animals subjected to hepatectomy (Group II) and those given arterial injection when the blood free fatty acids were still very highly radioactive. Lack of a highly radioactive blood free fatty acid fraction also explains why the control animal brain lipids did not contain any appreciable radioactive free fatty acid component. Calculating from the value of trapped blood (1.2 ml/100 g) in dissected brain tissue reported in the literature[19,20] and the observed counts/min per ml plasma lipids, only about 1.9, 0.36 and 6.2 % of the total radioactivity of brain total lipids per g of tissue could have come from the trapped blood in Groups III, II and I respectively.

In the hepatectomized animals the cerebroside fraction had greater radioactivity than that from carotid-injected animals and phosphatidyl ethanolamine had the maximum radioactivity. In the control group, as well as the carotid-injected group, phosphatidyl choline had the maximum radioactivity. Phosphatidyl serine from the carotid-injected group had a high specific activity similar to that observed in the acetate-injected animals in earlier studies[11]. The sphingomyelin component from the intracarotid-injected animals also had relatively high radioactivity. It is interesting to note that within a short period of 15 sec, radioactivity from palmitic acid was incorporated into various brain lipid components supporting the view of rapid metabolism in the brain tissue discussed previously[11].

The radioactive component of these various brain polar lipids must be, to a large extent, the injected palmitic acid. Table IV shows that palmitic acid isolated from the polar lipids had about 16 times higher activity than stearic acid; the unsaturated fatty acids had even lower specific activities. Further, the palmitic acid still had most of the activity in the carboxyl carbon proving a direct uptake and metabolic incorporation into complex lipids. The carboxyl carbon activity of stearic acid in the intracarotid injected group was low and closer to the calculated value (1/9 of the total activity) of *de novo* synthesis from radioactive acetate, itself a breakdown product of the injected tracer. However, from our earlier data[7–10] as well as from observations by KISHIMOTO AND RADIN[21], the percent rel. carboxyl act. (radioactivity in COOH × 100/radioactivity in total fatty acid) in stearic acid has been shown to depend on the time interval between administration of the dose and sacrifice of the animals.

In conclusion, it can be seen that in the hepatectomized animals, as well as in the carotid-injected animals, the circulating radioactive tracer, [1-^{14}C]palmitic acid, was in the form of free fatty acids and this resulted in greater incorporation into brain lipids as compared to that in control animals, where it was circulating mostly as triglyceride, indicating free fatty acid as a preferred form of fatty acid transport to the brain. Also, in hepatectomized animals, the injected [1-^{14}C]palmitate was directly taken up by the brain tissue and incorporated into complex lipids *in situ* by enzymatic reactions already known to occur in the central nervous system[21–23] and was not transported to the brain after incorporation elsewhere. However, the possibility of fatty acid incorporation into blood vessel endothelial cells in the brain itself followed by formation of complex lipids cannot be ruled out. Such reactions in the capillary cells leading to complex lipids unique to the central nervous system have not yet been reported.

ACKNOWLEDGEMENTS

This investigation was supported in part by Contract AT(04-1)GEN-12 between the Atomic Energy Commission and the University of California and by the U.S. Public Health Service Research Career Award No. GM-K6-19, 177 from the Division of General Medical Sciences, National Institute of Health.

REFERENCES

1 S. S. KETTY, in D. RICHTER, *Metabolism of the Nervous System*, Pergamon Press, New York, 1957, p. 221.
2 L. G. ABOOD AND A. GEIGER, *Am. J. Physiol.*, 182 (1955) 557.
3 O. E. OWEN, A. P. MORGAN, H. G. KEMP, J. M. SULLIVAN, M. G. HERRERA AND G. F. CAHILL Jr., *J. Clin. Invest.*, 46 (1967) 1589.
4 M. E. SMITH in R. PAOLETTI AND D. KRITCHEVSKY, *Advances in Lipid Research*, Vol. 5, Academic Press, New York, 1967, p. 241.
5 G. Y. SUN AND L. A. HORROCKS, *J. Neurochem.*, 16 (1969) 181.
6 C. ALLWEIS, T. LANDAU, M. ABELES AND J. MAGNES, *J. Neurochem.*, 13 (1966) 795.
7 G. A. DHOPESHWARKAR AND J. F. MEAD, *Biochim. Biophys. Acta*, 187 (1969) 461.
8 G. A. DHOPESHWARKAR AND J. F. MEAD, *Biochim. Biophys. Acta*, 210 (1970) 250.
9 G. A. DHOPESHWARKAR, C. SUBRAMANIAN AND J. F. MEAD, *Biochim. Biophys. Acta*, 231 (1971) 8.
10 G. A. DHOPESHWARKAR, C. SUBRAMANIAN AND J. F. MEAD, *Biochim. Biophys. Acta*, 239 (1971) 162.
11 G. A. DHOPESHWARKAR, C. SUBRAMANIAN AND J. F. MEAD, *Biochim. Biophys. Acta*, 248 (1971) 41.
12 J. FOLCH, M. LEES AND G. H. SLOANE STANLEY, *J. Biol. Chem.*, 226 (1957) 497.
13 D. KRITCHEVSKY AND S. MALHOTRA, *J. Chromatogr.*, 52 (1970) 498.
14 G. ROUSER, G. KRITCHEVSKY, D. HELLER AND E. LIEBER, *J. Am. Oil Chemists' Soc.*, 40 (1963) 425.
15 S. LAURELL, *Acta Physiol. Scand.*, 47 (1959) 218.
16 B. BORGSTRÖM AND T. OLIVECRONA, *J. Lipid Res.*, 2 (1961) 263.
17 C. E. ROWE, *Biochim. Biophys. Acta*, 84 (1964) 424.
18 G. G. LUNT AND C. E. ROWE, *Biochim. Biophys. Acta*, 152 (1968) 681.
19 H. DAVSON AND E. SPAZIANI, *J. Physiol.*, 149 (1959) 135.
20 A. LAJTHA, S. FURST, A. GERSTEIN AND H. WAELSCH, *J. Neurochem.*, 1 (1957) 289.
21 Y. KISHIMOTO AND N. S. RADIN, *Lipids*, 1 (1966) 47.
22 R. O. BRADY, *J. Biol. Chem.*, 235 (1960) 3099.
23 E. AEBERHARD AND J. H. MENKES, *J. Biol. Chem.*, 243 (1968) 3834.