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FATTY ACID TRANSPORT INTO THE BRAIN*

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FATTY ACID TRANSPORT INTO THE BRAIN

SUMMARY

Fifteen μc of $1\text{-}^{14}\text{C}$ palmitate was injected into three groups of animals. Group I served as control animals, Group II animals were subjected to functional hepatectomy and Group III animals received the tracer by an intracarotid injection followed by decapitation fifteen seconds 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 FFA fraction had most of the radioactivity. Under these conditions the uptake of radioactivity by the brain was about six times higher in hepatectomized animals and fourteen times higher in the intracarotid injected animals than in the control group. This indicates that FFA 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 glucose¹. However, Abood and Geiger² 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 to 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 Smith⁴ have indicated that myelin lipids, which at one time were considered metabolically inert, have turnover rates varying from rapid to extremely slow. Sun and Horrocks⁵ 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 metabolic change. Allweis et al.⁶ reported that when albumin bound ¹⁴C-palmitate was perfused, the cat brain was able to oxidize it to labeled CO₂, indicating the oxidative capability of the brain. Previous work from this laboratory⁷⁻¹⁰ 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

1-¹⁴C palmitic acid (DHOM Products, Hollywood, Calif., 46.2 mC/mole) 300 µc was complexed with fatty acid-poor bovine serum albumin as described previously⁸. Fifteen µc of this clear solution was injected into each rat.

Animals

Albino Wistar rats weighing approximately 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 I. P. injection of sodium pentobarbital and the femoral vein was exposed. Fifteen 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 hour.

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. Fifteen μ c of 1-¹⁴C palmitic acid albumin complex was injected intravenously as in Group I and the animals were sacrificed after 1 hour.

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 seconds later as described earlier¹¹.

Lipid extraction

The entire brain and a piece from each lobe of the liver were dissected out as quickly as possible and immersed in chloroform: methanol 2:1. Serum was obtained by centrifugation of the heparinized blood and lipids were extracted by the Folch et al.¹² method as described previously⁷.

Fractionation

Blood lipids: Total lipids obtained from plasma were chromatographed on a TLC plate (20 x 20 cms) 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 Malhotra¹³. 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 SiO₂ column chromatography as described earlier⁷.

Brain lipids: Brain total lipids were first freed from traces of neutral lipids and free cholesterol by SiO_2 column chromatography. When the polar lipid fraction was analyzed by TLC 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 FFA 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 minutes. 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 FFA when tested by TLC. 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 previously⁷. 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 (cpm/mg total lipids), 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

← TABLE I

by decapitation at the very short interval of 15 seconds. In other groups, the animals were kept alive for 1 hour 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 group animals the triglyceride fraction had the most radioactivity, in the other two groups it was the FFA component that was most radioactive.

The uptake of radioactivity 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 Groups 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 II

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 FFA 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 FFA

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 FFA by diazomethane treatment and chromatography. Phosphatidyl choline was the most active component in Groups I and III whereas in hepatectomized animals it was PE that had the highest specific activity. The cerebroside fraction from this group was also relatively more radioactive than in other groups. Both PS 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.

← TABLE III

The polar lipids from the brain were first freed from the FFA 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 the carboxyl carbon in all three groups. The carboxyl carbon activity in stearic acid decreased progressively from Group I to Group III.

← TABLE IV

DISCUSSION

The reason for performing functional hepatectomy on some of the rats was to prevent incorporation of the injected 1-¹⁴C palmitic acid into glycerides and phosphatides and recirculation. Laurell¹⁵ has reported that in the normal rat, a considerable fraction of the injected FFA is recirculated in the blood in the form of glycerides and to a lesser extent in phospholipids. Further, this recirculation, according to Borgström

and Olivecrona¹⁶, 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 remove 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 FFA. This is borne out by the results in Table I showing that 77% of the total radioactivity was associated with the FFA 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 III).

It seemed reasonable that a similar pattern of radioactivity of circulating blood lipids, a preponderance of radioactivity in the FFA, 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 seconds¹¹. 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 FFA. 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 FFA in brain lipids has been reported earlier^{17,18} but the concentration was low (only about 0.8% of the total fatty acids).

Borgstrom¹⁶ 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 FFA is unknown. Direct transport of radioactive palmitic acid from blood into brain tissue, clearly indicated in our earlier work⁷, can now explain the highly radioactive FFA fraction in brain total lipids of animals subjected to hepatectomy (Group II) and those given arterial injection when the blood FFA were still very highly radioactive. Lack of a highly radioactive blood FFA fraction also explains why the control animal brain lipids did not contain any appreciable radioactive FFA component. Calculating from the value of trapped blood in dissected brain tissue reported in the literature^{19,20} and the cpm/ml plasma lipids, only a very minor component of the total radioactivity of brain FFA could have come from the trapped blood.

The specific activities of total polar lipids (freed from FFA fraction), and also the major separated components, is shown in Table III. Relative comparisons of the specific activities of cerebroside, PE, PS, PC, and Sph in the three groups cannot be made because the total polar lipid specific activity was not the same in all the groups. However, the following peculiarities were observed. In the hepatectomized animals the cerebroside fraction had greater radioactivity than that from carotid-injected animals, although the latter had higher activity in the total polar lipid fraction and PE had the maximum radioactivity. In the control group, as well as the carotid-injected group, PC had the maximum radioactivity. PS from the carotid-injected group had a high specific activity similar to that observed in the acetate-injected animals in

earlier studies¹¹. 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 seconds, palmitic acid was incorporated into various brain lipid components supporting the view of rapid metabolism in the brain tissue discussed previously¹¹.

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 data⁷⁻¹⁰ as well as from observations by Kishimoto and Radin²¹, the % RCA 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, 1-¹⁴C palmitic acid, was in the form of FFA and this resulted in greater incorporation into brain lipids as compared to that in control animals, where it was circulating mostly as triglyceride, indicating FFA as a preferred form of fatty acid transport to the brain. Also, in

hepatectomized animals, the injected 1-¹⁴C 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 system²¹⁻²³ and was not transported to the brain after incorporation elsewhere.

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TABLE I

RADIOACTIVITY IN BLOOD LIPIDS AFTER INJECTION OF 1-¹⁴C PALMITIC ACID

Group	Sp. act. (cpm/mg total lipids)	Radio- activity of total lipids (cpm/ml plasma)	% Of given dose recovered in total lipids of 1 ml plasma	Distribution of radioactivity (% of total radioactivity)				
				Choles- terol esters	Tri- glycerides	Free fatty acids (FFA)	Choles- terol	Phospho- lipids
Gr. I; Control	19,521 ± 6,075	54,456 ± 16,951	0.24 ± 0.08	1.5	77.7	10.9	6.0	1.6
Gr. II; Hepatecto- mized	8,649 ± 4,975	22,071 ± 15,302	0.1 ± 0.07	---	10.6	77.4	0.14	11.9
Gr. III; Intra-carotid injection	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 1-¹⁴C PALMITIC ACID

Group	Brain			Liver		
	Sp. act. (cpm/mg total lipids)	Radioactivity of total lipids (cpm/g fresh weight of tissue)	% of given dose recovered in total lipids of 1 g	Sp. act. (cpm/mg total lipids)	Radioactivity of total lipids (cpm/g fresh weight of tissue)	% of given dose recovered in total lipids of 1 g
Gr. I; Control	49	5,242	0.02	6,836	276,188	1.25
	± 9	± 972	± 0.004	± 776	± 31,354	± 0.14
Gr. II; Hepatectomized	314	36,688	0.15	243	9,556	0.04
	± 257	± 27,591	± 0.12	± 190	± 7,829	± 0.03
Gr. III; Intra-carotid injection	878	93,331	0.28	1,831	73,956	0.22
	± 527	± 56,596	± 0.17	± 788	± 31,855	± 0.09

TABLE III

INCORPORATION OF RADIOACTIVITY INTO MAJOR POLAR LIPID FRACTIONS OF BRAIN TOTAL LIPIDS FOLLOWING INJECTION OF 1-¹⁴C PALMITIC ACID

Group	Specific radioactivity (cpm/mg)						
	FFA	Total polar lipids	Cerebro-side	PE	PS	PC	Sph.
Gr. I; Control	—	53	4	27	26	68	13
Gr. II; Hepatectomized	2501	132	41	77	27	57	21
Gr. III; Intra-carotid injection	—	173	26	122	83	181	63

TABLE IV

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

Group	Palmitic acid		Stearic acid	
	Sp. act. cpm/mg	% RCA	Sp. act. cpm/mg	% RCA
Gr. I; Control	404	86	31	43
Gr. II; Hepatectomized	1024	89	70	26
Gr. III; Intra-carotid injection	1304	94	70	14.4

(1) LIST OF ABBREVIATIONS USED

FFA = Free fatty acids
PE = Phosphatidyl ethanolamine
PS = Phosphatidyl serine
PC = Phosphatidyl choline
Sph. = Sphingomyelin