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Ketone body utilization for energy production and lipid synthesis in isolated rat brain capillaries

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Isolated brain capillaries from 2-month-old rats were incubated for 2 h in the presence of [3-^{14}C]acetoacetate, D-3-hydroxy[3-^{14}C]butyrate, [U-^{14}C]glucose, [1-^{14}C]acetate or [1-^{14}C]butyrate. Labelled CO_2 was collected as an index of oxidative metabolism and incorporation of label precursors into lipids was determined. The rate of CO_2 production from glucose was slightly higher than from the other substrates. Interestingly, acetoacetate was oxidized at nearly the same rate as glucose. This shows that ketone bodies could be used as a source of energy by brain capillaries. Radiolabelled substrates were also used for the synthesis of lipids, which was suppressed by the addition of albumin. The incorporation of [U-^{14}C]glucose in total lipids was 10-times higher than that from other precursors. However, glucose labelled almost exclusively the glycerol backbone of phospholipids, especially of phosphatidylcholine. Ketone bodies as well as glucose were incorporated mainly into phospholipids, whereas acetate and butyrate were mainly incorporated into neutral lipids. The contribuiton to fatty acid synthesis of various substrates was in the following order: butyrate ≥ acetate > ketone bodies ≥ glucose. All precursors except glucose were used for sterol synthesis. Glucose produced almost exclusively the glycerol backbone of phospholipids.

Introduction

Ketone bodies can partly replace glucose as an energy source for the brain [1,2]. Homogenates of adult rat brain utilize ketone bodies for energy production [3]. In addition it has been shown that different cells of developing rat brain (oligodendrocytes [4,13] neurons, astrocytes [5]) use ketone bodies as substrates for energy metabolism as well as precursors for lipid biosynthesis. However, the rate at which these substrates are utilized for different pathways and the activities of the relevant enzymes [17] are not the same for different cell types. Nothing is known about the contribution of endothelial cells to the metabolism of ketone bodies. This is important because these substrates must pass this cellular compartment between the blood and the brain. Thus, to see whether and how endothelial cells of brain capillaries utilize ketone bodies we have performed experiments with isolated rat brain capillaries. We have compared ketone bodies with glucose, acetate and butyrate as precursors for lipid synthesis.

Experimental procedures

Materials

Sprague-Dawley rats were obtained from IFFA-

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CREDO (France). Radiolabelled compounds [3-^{14}C]acetoacetate, 10 mCi/mmol, D-hydroxy[3-^{14}C]butyrate, 50–60 mCi/mmol, [1-^{14}C]acetate, 55 mCi/mmol, [1-^{14}C]butyrate, 56 mCi/mmol, [U-^{14}C]glucose, 335 mCi/mmol and [2-^{14}C]glucose, 55.9 mCi/mmol were bought from Amersham International (Amersham, U.K.) or from new England Nuclear (Boston, MA, U.S.A.). Ethyl [3-^{14}C]acetoacetate was hydrolyzed as described by Koper et al. [6]. Dextran was obtained from Pharmacia (France). Nylon mesh (118 μm) were from Desjobert (Paris, France) and glass beads from B-Baun (Mensugan, F.R.G.). Silica Gel Plates 60 F_{254}, and solvents for thin-layer chromatography were purchased from Merck.

Preparation of purified capillaries

Brain capillaries were isolated from cerebral cortices of 2-months-old rats according to the method of Goldstein et al. [7]. The isolation buffer was oxygen-saturated and contained NaCl (147 mM), KCl (4 mM), $CaCl_2$ (3 mM) Hepes (15 mM), pH 7.4, glucose (5 mM) and 1% (w/v) bovine serum albumin (fraction V). The quality of each preparation was monitored by phase contrast microscopy [9].

Incubations

Isolated brain capillaries were washed twice with incubation medium (modified Krebs buffer) containing MgCl (1.2 mM), $NaHCO_3$ (4.0 mM), KCl (5.4 mM), NaH_2PO_4 (15.7 mM), NaCl (111.2 mM) and glucose (5 mM) [8] which was adjusted to pH 7.4. Incubations were carried out as described previously [9] with various radioactive precursors (5 mM [U-^{14}C]- or [2-^{14}C]glucose, 1 mM [3-^{14}C]acetoacetate, 0.36 mM D-hydroxy[3-^{14}C]butyrate, 0.34 mM [1-^{14}C]butyrate and 0.34 mM [1-^{14}C]acetate) in 0.5 ml of medium. The incubation was then stopped by adding ice-cold buffer. The capillaries were washed twice with Krebs buffer to ensure the removal of free radiolabelled substrates. Lipids were extracted with chloroform/methanol (2 : 1) [10] and sonication [11]. An aliquot of the lipid extract was counted to estimate total incorporation of label into total lipids. Another aliquot was separated into neutral lipids by thin-layer chromatography (TLC) using hexane/diethyl ether/acetic acid (75 : 23 : 2) as developing solvent. Separation of phospholipids into subclasses was achieved using the solvent system described by Vitiello and Zanetta [12]. Lipids were visualized by iodine vapor. Distribution of radioactivity among lipid subclasses was determined by scratching lipid zones from chromatographic plates into counting vials to which 10 ml of Beckman scintillation solution was added. Aliquots of lipids were fractionated after alkaline hydrolysis into a non-saponifiable fraction (sterol) and a saponifiable water-insoluble fraction (fatty acids) [6]. Parallel incubations were carried out to measure $^{14}CO_2$ production [13]. Incubations were carried out under the same conditions in sealed flasks containing a center well for trapping $^{14}CO_2$ on filter paper saturated with 10 M KOH. After 2 h of incubation the reactions were stopped by adding 100 μl of 3 M HCl, and after 30 min of equilibrium the filter papers were removed and trapped radioactivity was measured in a liquid scintillation spectrometer. Samples for background activity were either free of capillaries or contained a capillary aliquot that had been kept on ice. Protein content of the capillaries was determined according to Lowry et al. [14]. The data were analyzed for statistical significance using Student's *t*-test.

Results

Table I summarizes the results on CO_2 production from different radiolabelled substrates by iso-

TABLE I

THE CONVERSION OF VARIOUS ^{14}C-LABELLED SUBSTRATES INTO $^{14}CO_2$ BY ISOLATED RAT BRAIN CAPILLARIES

Incubations were carried out in the presence of bovine serum albumin (1% w/v). Values (means ± S.E.) were compared using Student's *t*-test: * significantly different from butyrate ($P < 0.001$). Values within parentheses represent CO_2 production divided by lipid synthesis (Table II).

Substrate	CO_2 production (nmol/mg protein per h) [a]
Acetoacetate	1.58 ± 0.04 * (88)
3-Hydroxybutyrate	0.99 ± 0.2 (66)
Butyrate	0.84 ± 0.004 (70)
Acetate	1.34 ± 0.25 (34)
Glucose	1.75 ± 0.05 * (11)

[a] nmol substrate converted to $^{14}CO_2$.

lated brain capillaries. The rate of CO_2 production from glucose was higher than from other precursors, but this difference was only significant with butyrate. Similar results were obtained with [2-^{14}C]glucose as tracer (data not shown). It is interesting to note that in the presence of a physiological concentration of glucose, [3-^{14}C]acetoacetate was oxidized at about the same rate as glucose (90% of glucose level). 3-Hydroxybutyrate was oxidized at a much lower rate (56% of glucose level).

The incorporation of radiolabelled precursors into total lipids is shown in Table II. Acetoacetate and 3-hydroxybutyrate were used for lipid biosynthesis in isolated brain capillaries of adult rats. Utilization of acetoacetate was 20% less than that of 3-hydroxybutyrate. Compared to glucose, the incorporation of other precursors was about 10-times lower, but glucose labels almost exclusively the glycerol moiety (see below, Table IV). Biosynthesis of lipids from butyrate was significantly lower than that from other substrates.

When incubations were carried out in the presence of 1% bovine serum albumin, the incorporation of radiolabelled substrates into total lipids was reduced by 44, 63, 52, 20 and 55% for acetoacetate, 3-hydroxybutyrate, butyrate, acetate and glucose, respectively.

The incorporation of various radiolabelled substrates into different lipid classes is shown in Table III. Acetoacetate and 3-hydroxybutyrate showed the same pattern of incorporation. These two substrates were incorporated mainly into phospholipids and glycolipids (approx. 70%). Incorporation of these substrates into glycolipids was verified by multidimensional chromatography [11], and the same results were obtained. Glucose was incorporated into phosholipids (72%), and mainly into phosphatidylcholine (50%). The incorporation of this substrate into neutral lipids occurred mainly in acylglycerols. The incorporation of glucose into free fatty acids was significantly lower than that of ketone bodies. Incorporation of label from butyrate into different lipid classes resembled that of acetate; about 50% of incorporation occurred in neutral lipids, mainly in free fatty acids and acylglycerols. Acetate and butyrate were incorporated into the phospholipid fraction essentially as phosphatidylcholine.

In the presence of 1% bovine serum albumin in the incubation medium, the distribution of radiolabelled substrates into different lipid classes changed mainly in the case of acetate and butyrate as substrates. The percentage incorporation into free fatty acids and triacylglycerols diminished significantly, this decrease being less important for other substrates.

The contribution of different precursors to fatty acids, sterols and the water-soluble fraction is shown in Table IV. The incorporation of ketone bodies into fatty acids and sterol fractions was significantly higher than that of glucose. However, it represents about 35% of total radioactivity and the main incorporation occurred in the water-soluble fraction (66%). Label from glucose was recovered nearly exclusively in the water-soluble fraction (97%). Table IV indicates that butyrate and acetate served mainly as lipogenic precursors (67 and 57%, respectively).

TABLE II

INCORPORATION OF VARIOUS ^{14}C-LABELLED SUBSTRATES INTO TOTAL LIPIDS OF ISOLATED RAT BRAIN CAPILLARIES

Values (means ± S.E.) were compared using Student's *t*-test: ‡ significantly different from glucose; § significantly different from butyrate; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. BSA, bovine serum albumin.

Substrate	Lipid synthesis (nmol/mg protein per h) [a]	
	− BSA	+ BSA
Acetoacetate	0.032 ± 0.002 ***	0.018 ± 0.0003 ‡**
3-Hydroxybutyrate	0.040 ± 0.001 ‡***,§**	0.015 ± 0.0003 ‡**
Butyrate	0.025 ± 0.003 ‡***,§**	0.012 ± 0.002 ‡**
Acetate	0.050 ± 0.01 ‡**	0.040 ± 0.013 ‡**
Glucose	0.353 ± 0.07	0.160 ± 0.045

[a] nmol substrate converted to lipids.

Discussion

Isolated brain capillaries of adult rat brain take up acetoacetate and 3-hydroxybutyrate from incubation medium and oxidize them to CO_2 (Table I). Quantitatively, however, 3-hydroxybutyrate was oxidized at a lower rate (38% lower). The difference between the rate of oxidation of these two precursors suggests that the conversion of 3-hydroxybutyrate into acetoacetate by 3-hydroxy-

TABLE III

THE DISTRIBUTION OF RADIOACTIVITY AMONG LIPID CLASSES OF ISOLATED RAT BRAIN CAPILLARIES INCUBATED IN THE PRESENCE OF [1-^{14}C]ACETATE, [1-^{14}C]BUTYRIC ACID, [3-^{14}C]ACETOACETATE, β-HYDOXY[3-^{14}C]BUTYRATE AND [U-^{14}C]GLUCOSE

Values (calculated as percentage of identified lipids) are the means ± S.E. of at least three observations. Significantly different from [a] acetate; [b] acetoacetate; [c] β-hydroxybutyrate; [d] butyric acid; and [f] between − - and + bovine serum albumin. PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI-PA, phosphatidylinositol-phosphatidic acid. n.d., not detected.

Lipid	− Bovine serum albumin					+ Bovine serum albumin				
	acetate	butyric acid	acetoacetate	β-hydroxy-butyrate	glucose	acetate	butyric acid	acetoacetate	β-hydroxy-butyrate	glucose
PC	16.5 ± 0.4	21.7 ± 1.5 [a,b]	14.0 ± 1.0 [a]	12.3 ± 2.3 [a]	48.0 ± 0.5 [a,b,c,d]	30.0 ± 2.8 [f]	28.7 ± 2.3 [b,c,f]	15.0 ± 3.5 [a]	8.9 ± 3.2 [a]	54.4 ± 1.4 [a,b,c,d]
PE	6.4 ± 0.4	6.4 ± 0.9 [b,c]	12.0 ± 1.1 [a]	12.6 ± 0.1	6.5 ± 0.1 [b,c]	11.7 ± 2.0	8.4 ± 1.9	11.6 ± 1.0	13.7 ± 1.6	7.2 ± 1.1 [c]
PS	2.8 ± 0.2	5.0 ± 0.5 [a,b,c]	8.8 ± 0.4 [a]	9.5 ± 0.6 [a]	7.0 ± 0.3 [a,b,c]	10.6 ± 1.7 [f]	7.6 ± 3.0	9.7 ± 0.8	9.8 ± 1.8	7.5 ± 0.3
PI-PA	7.5 ± 2.1	5.6 ± 0.8 [b]	12.0 ± 1.8 [a]	7.6 ± 2.0 [b]	10.5 ± 1.0 [b,d]	11.7 ± 2.0	8.4 ± 2.3	7.5 ± 1.3	15.2 ± 0.4 [b]	16.5 ± 1.3 [b,d,f]
Sphingomyelin	4.3 ± 0.2	4.7 ± 0.8	4.7 ± 1.1 [a]	7.0 ± 1.1 [a]	6.8 ± 2.0	7.8 ± 2.4	7.8 ± 1.2	4.4 ± 0.6	5.2 ± 2.8	3.9 ± 1.6
Sulphatides	3.6 ± 0.4	5.6 ± 0.8 [b,c]	12.1 ± 1.2 [a]	11.2 ± 1.8 [a]	3.2 ± 0.9 [b,c,d]	2.9 ± 0.8	6.0 ± 1.6 [c]	8.5 ± 2.0 [a]	14.3 ± 0.9 [a]	1.6 ± 0.3 [b,c]
Cerebrosides	4.4 ± 0.5	6.0 ± 0.7 [c]	7.0 ± 1.4	15.1 ± 3.1 [a,b]	3.2 ± 1.4 [b,c]	3.2 ± 0.9	9.5 ± 2.4 [a,c]	13.2 ± 0.5 [a]	18.4 ± 3.2 [a]	4.7 ± 0.6 [b,d]
Diacylglycerols	4.2 ± 0.5	3.7 ± 0.7	4.6 ± 0.6	4.5 ± 1.1	3.8 ± 0.4	4.2 ± 0.5	5.6 ± 1.0	3.9 ± 0.9	6.0 ± 2.7	1.8 ± 0.3 [a,d,f]
Triacylglycerols	12.6 ± 1.6	8.5 ± 2.6	4.2 ± 1.3	6.3 ± 0.9	6.3 ± 0.0	3.7 ± 0.7 [f]	3.6 ± 0.5	8.9 ± 0.2	2.7 ± 0.6	1.8 ± 0.3 [d,f]
Free fatty acids	31.6 ± 1.6	24.8 ± 1.3 [bc]	11.8 ± 1.6 [a]	9.5 ± 2.8 [c]	1.6 ± 0.1 [a,b,c]	7.5 ± 1.4 [f]	7.2 ± 1.7 [f]	9.8 ± 1.8	2.4 ± 0.9 [f]	0.3 ± 0.1 [a,b,c]
Cholesterol	4.0 ± 0.3	3.5 ± 0.7	4.6 ± 0.4	4.9 ± 1.0	1.9 ± 0.3 [a,b,c,d]	5.3 ± 1.7	4.9 ± 1.6	5.9 ± 0.0	2.2 ± 0.8	0.3 ± 0.1 [a,b,c,d]
Cholesteryl esters	2.1 ± 0.3	4.5 ± 1.5	1.9 ± 0.5	1.9 ± 1.5	1.2 ± 0.3 [d]	1.4 ± 0.4	2.3 ± 1.0	1.6 ± 0.0	1.2 ± 0.7	n.d.

TABLE IV

DISTRIBUTION OF LABEL FROM [3-^{14}C]ACETOACETATE, 3-HYDROXY[3-^{14}C]BUTYRATE, [1-^{14}C]BUTYRATE, [1-^{14}C]ACETATE AND [U-^{14}C]GLUCOSE AMONG FATTY ACIDS, STEROL AND GLYCEROL FRACTIONS OF TOTAL LIPIDS FROM ISOLATED RAT BRAIN CAPILLARIES.

Values are the percentage of total radioactivity (means ± S.E.).

Substrate	Fatty acids	Sterol	Water-soluble
Acetoacetate	29.7 ± 0.7	3.7 ± 2.7	66.5 ± 3.5
3-Hydroxybutyrate	31.9 ± 0.4	1.8 ± 0.3	66.4 ± 0.1
Butyrate	67.6 ± 5.3	2.5 ± 1.0	29.9 ± 6.3
Acetate	57.8 ± 9.8	2.7 ± 0.7	39.6 ± 10.4
Glucose	2.9 ± 1.0	0.2 ± 0.1	97.0 ± 1.0

butyrate dehydrogenase is probably the rate-limiting step in the metabolism of 3-hydroxybutyrate in isolated brain capillaries, as is the case in adult rat brain homogenate [3].

It should be mentioned also that the cerebral extraction ratio of acetoacetate is higher (3–5-times) than that of 3-hydroxybutyrate in adult rats [15].

The metabolic rate of isolated capillaries obtained in these experiments is low compared to those reported in whole brain homogenate [3] and brain cells in culture [5]. However, the metabolic rate of endothelial cells of brain capillaries may differ from that of other brain cells in culture. It might also be due to the low metabolic rate of endothelial cells of isolated brain capillaries.

Our data on lipid synthesis from various radiolabelled precursors by isolated brain capillaries show that these substrates are incorporated slowly into lipids compared to their utilization for oxidative metabolism. The ratios of CO_2 production to lipid synthesis were 88, 66, 70, 34 and 11 for acetoacetate, 3-hydroxybutyrate, butyrate, acetate and glucose, respectively (Table I). It is interesting to note that acetoacetate was largely used in oxidative pathways. The similar distribution of label from acetoacetate and 3-hydroxybutyrate among lipid classes suggests that both ketone bodies are equivalent substrates for lipid synthesis. The incorporation of ketone bodies into fatty acids and sterol was significantly higher than the incorporation of glucose (Table IV). Unexpectedly, a high percentage of the label from ketone bodies was recovered in the water-soluble fraction after saponification of the total lipids, indicating an important contribution to the glycerol moieties of complex lipids. Lopes-Cardozo et al. [5] have reported a similar finding for neurons and astrocytes in culture. They suggested that this might result from exchange reaction rather than from synthesis.

The rate of acetate and butyrate utilization for lipid synthesis was about twice the rate observed with ketone bodies. This shows that short-chain fatty acids are better precursors for fatty acid synthesis.

Lipid synthesis in isolated brain capillaries was lowered by the presence of 1% bovine serum albumin in the medium. Especially the fraction of free fatty acids was diminished by the presence of bovine serum albumin (Table III). Data obtained by fractionation of total lipids into sterol and fatty acids also confirmed that bovine serum albumin reduced the rate of lipogenesis (data not shown). This phenomenon can be explained by the inhibitory effect of fatty acids bound to serum albumin on fatty acid synthesis in isolated brain capillaries [16]. Furthermore, Simmons et al. [18] recently showed that defatted albumin reduced metabolic activity in endoneurium. However, the inhibitory effect of bovine serum albumin on glucose utilization might not only be due to the presence of fatty acids bound to albumin but also to other contaminating substances present in commercially prepared bovine serum albumin used in these experiments which may reduce glucose utilization in isolated brain capillaries.

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