*Neurochem. Int.* Vol. 13, No. 4, pp. 429–433, 1988
Printed in Great Britain. 

0197-0186/88 $3.00 + 0.00


# ACETOACETATE, D-3-HYDROXYBUTYRATE AND GLUCOSE UTILIZATION BY CAPILLARIES ISOLATED FROM DEVELOPING RAT BRAIN

P. HOMAYOUN* and J. M. BOURRE
INSERM U.26, Hôpital F. Widal, 200 rue du Fg. St-Denis, 75475 Paris Cedex 10, France

(*Received* 1 *February* 1988; *accepted* 14 *April* 1988)

**Abstract**—Isolated cerebral capillaries from developing rats utilize glucose as well as ketone bodies essentially for oxidative metabolism. However, $CO_2$ production from [U-$^{14}$C]glucose was significantly greater than from ketone bodies (except at 5 mM). Ketone body utilization (in the presence of 5 mM glucose in the incubation medium) was concentration-dependent (up to 5 mM). Lipid synthesis from ketone bodies was comparable to that from glucose up to 1 mM. At concentrations $\geq$ 1 mM, acetoacetate incorporation into total lipids and fatty acids was higher than other substrates, however, this difference was statistically significant only at 5 mM. Incorporation of substrates into sterols was very low (< 1 pmol/h/mg protein).

Ketone bodies, acetoacetate and D-3-hydroxybutyrate are important sources of energy for metabolism in developing rat brain and also as precursors for lipids synthesis (De Vivo, 1980; Robinson and Williamson, 1980). *In vitro* studies of different brain cells in culture (Koper *et al.*, 1984; Lopes-Cardozo *et al.*, 1986; Sykes *et al.*, 1986) have shown that the rate of ketone body utilization for energy production and lipid synthesis as well as the activities of relevant enzymes (Chechik *et al.*, 1987) vary with cell type. Nevertheless, these studies have been performed on astrocytes, neurons and oligodendrocytes, and very little information is available on the contribution of endothelial cells to the metabolism of ketone bodies in the brain. In our previous work (Homayoun and Bourre, 1987) we have reported that endothelial cells of capillaries isolated from adult rat brain metabolize both acetoacetate and D-3-hydroxybutyrate and utilize both substrates for energy production. During development of the rat, both transport of ketone bodies into the brain and their metabolism increase (Cremer *et al.*, 1976; Moore *et al.*, 1976; Daniel *et al.*, 1971; Patel and Owen, 1977). Therefore, it seemed of interest to study ketone body utilization in capillaries isolated from developing rat brain to answer the following questions: (1) does ketone body utilization increase during development in brain capillaries? (2) what is the quantitative relation between ketone bodies and glucose as precursors for lipid synthesis and energy production? (3) are ketone bodies used preferentially for fatty acid and cholesterol synthesis compared to glucose?

*Present address: Department of Neurology, School of Medicine, Case Western Reserve University, 2074 Abington Road, Cleveland, OH 44106, U.S.A.

## EXPERIMENTAL PROCEDURES

### *Materials*

Sprague–Dawley rats were obtained from IFFA CREDO (France). Chemicals and materials were obtained as follows: [3-$^{14}$C]acetoacetate, 10 mCi/mmol; D-[3-$^{14}$C]hydroxybutyrate, 50–60 mCi/mmol; [U-$^{14}$C]glucose, 335 mCi/mmol (Amersham International, U.K. or CEA, France); dextran (Pharmacia, France); nylon mesh (118 $\mu$m) (Desjobert, Paris, France); glass beads (B-Baun, Mensugen, F.R.G.); Silica Gel Plates 60 $F_{254}$, and solvents for thin-layer chromatography (Merck); ethyl [3-$^{14}$C]acetoacetate was hydrolysed as described by Koper *et al.* (1981).

### *Preparation of purified capillaries*

Brain capillaries were isolated from cerebral cortices of 20 day-old rats according to the method of Goldstein *et al.*, (1975). The isolation buffer was oxygen-saturated and contained NaCl (147 mM), KCl (4 mM), $CaCl_2$ (3 mM), N-2 hydroxyethyl-piperazine-N′-2-ethansulfonic acid (HEPES) (15 mM), pH 7.4, glucose (5 mM) and 1% (w/v) bovine serum albumin (BSA, Fraction V). The quality of each preparation was monitored by phase contrast microscopy (Homayoun *et al.*, 1985).

### *Incubations*

Isolated brain capillaries were washed twice with incubation medium (modified Krebs buffer) containing $MgCl_2$ (1.2 mM), $NaHCO_3$ (4.0 mM), KCl (5.4 mM), $NaH_2PO_4$ (15.7 mM), NaCl (111.2 mM) and variable concentrations of glucose (Pleasure and Towfighi, 1972) which was adjusted

to pH 7.4. Incubations were carried out at 37°C as described previously (Homayoun *et al.*, 1987) and contained 10 $\mu$Ci of radiolabelled [U-$^{14}$C]glucose, or [3-$^{14}$C]acetoacetate or D-3-[3-$^{14}$C]hydroxybutyrate and variable concentrations of non-labelled precursors in 0.5 ml of medium. The inhibitory effect of ketone bodies on glucose utilization was studied in the presence of 5 mM glucose with varying concentrations of acetoacetate or D-3-hydroxybutyrate. The incubation was then stopped by adding ice-cold buffer. The capillaries were washed twice with Krebs buffer to ensure the removal of radiolabelled substrates. Lipids were extracted with chloroform–methanol (2:1) (Folch *et al.*, 1957) followed by sonication (Pollet *et al.*, 1978). An aliquot of the lipid extract was counted to estimate total incorporation of label into total lipids. From another aliquot neutral lipids were separated by thin-layer chromatography using hexane–ether–acetic acid (75:23:2) as developing solvent. Separation of phospholipids into subclasses was achieved using a solvent system described by Vitiello and Zanetta (1978). Lipids were visualized by iodine vapor. Distribution of radioactivity among lipid subclasses was determined by scraping lipid zones from chromatographic plates into counting vials to which 10 ml of Beckman scintillation solution (HP) was added. Aliquots of the lipid extracts were fractionated after alkaline hydrolysis into a non-saponifiable fraction (sterol) and a saponifiable water-insoluble fraction (fatty acids) (Koper *et al.*, 1981). Parallel incubations were performed to measure $^{14}CO_2$ production (Sykes *et al.*, 1986). Incubations were carried out under the same conditions in sealed flasks containing a centre 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 $\mu$l of 3 M HCl, after 30 min of equilibrium the filter paper was removed and trapped radioactivity was measured in a liquid scintillation spectrometer. Quenching corrections were made using external standard method, in a Packard Tri-Carb 4530 spectrophotometer. Samples for background activity were incubated either without capillaries or with capillaries but at 0°C. Protein content of the capillaries was determined according to Lowry *et al.* (1951). Data were analysed for statistical significance using Student's *t*-test.

## RESULTS

The metabolism of acetoacetate and D-3-hydroxybutyrate in isolated capillaries from developing rat brain was investigated in the presence of 5 mM glucose (physiological level). Figure 1(A) and (B) shows the utilization of both ketone bodies for energy production and their incorporation into total lipids. The utilization of both substrates increased by increasing their concentration in the medium up to 5 mM. The oxidation of acetoacetate was comparable with that of hydroxybutyrate, whereas its incorporation into total lipids and fatty acids were greater. However, the difference was statistically significant only at 5 mM. Increasing the concentration of ketone bodies in the incubation medium increased their incorporation into total lipids more than their oxidation to $CO_2$. Thus the ratio of $CO_2$/lipid synthesis diminished with increasing substrate concentration (from 20 at 0.2 mM to 2 at 5 mM). Therefore, the oxidation as well as the incorporation of ketone bodies into total lipids were dependent on their concentration in the medium.

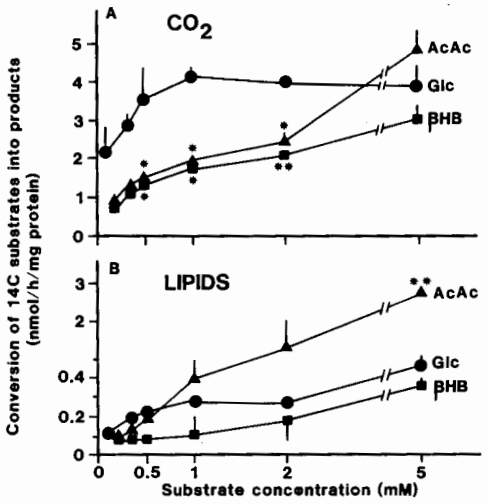


Fig. 1. Comparison of acetoacetate (▲), D-3-hydroxybutyrate (■) and glucose (●) as substrate for brain capillaries of developing rats. The incubation medium contained 10 $\mu$Ci of [3-$^{14}$C]acetoacetate (10 mCi/mmol), D-3-[3-$^{14}$C]hydroxybutyrate (56 mCi/mmol); or [U-$^{14}$C]glucose (355 mCi/mmol) and varying concentrations of unlabelled substrates. Acetoacetate and D-3-hydroxybutyrate utilization were studied in the presence of 5 mM glucose. Results are mean ± SEM ($n = 3$). (A) $CO_2$ production; (B) incorporation into total lipids. Significantly different from glucose; $^*P < 0.05$; $^{**}P < 0.001$.

The utilization of [U-$^{14}$C]glucose was studied in the absence of ketone bodies to determine whether there is a preferential utilization of ketone bodies compared to glucose in isolated brain capillaries of developing rat brain. The oxidation of glucose to $CO_2$ increased sharply [Fig. 1(A)] and reached a plateau at a glucose concentration of 1 mM, whereas its incorporation into total lipids [Fig. 1(B)] continued to rise up to glucose concentration of 5 mM. Compared to ketone bodies, glucose oxidation in isolated capillaries from developing rat brain was significantly higher. However, at 5 mM, $CO_2$ production from acetoacetate was comparable with that of glucose. The incorporation of glucose into total lipids was not significantly different from acetoacetate and 3-hydroxybutyrate at concentrations lower than 1 mM. At higher concentrations (5 mM), acetoacetate incorporation into total lipids was significantly higher than that of glucose and hydroxybutyrate.

Table 1. Distribution of radioactivity among lipid classes of isolated brain capillaries of developing rats

| Substrates | Lipid classes (%) | | |
|---|---|---|---|
| | Phospholipids | Glycolipids | Neutral lipids |
| [3-$^{14}$C]Acetoacetate | 21.1 ± 3.9 | 17.2 ± 1.9 | 54.6 ± 3.7 |
| D-3-[3-$^{14}$C]Hydroxybutyrate | 28.7 ± 4.1 | 13.6 ± 3.7 | 52.2 ± 5.2 |
| [U-$^{14}$C]Glucose | 83.2 ± 0.8** | 4.8 ± 1.5** | 5.9 ± 1.2** |

Values are the percentage of total incorporation, mean ± SEM. Significantly different from ketone bodies; $^{**}P < 0.001$.

Thin-layer chromatography of lipid extracts revealed that ketone bodies were more incorporated into neutral lipids (compared to other lipid classes), while [U-$^{11}$4C]glucose was incorporated mainly into phospholipids (Table 1).

The contribution of ketone bodies and glucose to *de novo* synthesis of total fatty acids is shown in Fig. 2. Fatty acid synthesis increased with increasing concentrations of precursors in the medium. At concentrations $\geq 1$ mM there was a preferential incorporation of acetoacetate into total fatty acids compared to D-3-hydroxybutyrate and glucose, however, this difference was not statistically significant unless at 5 mM. The incorporation of both ketone bodies as well as glucose into cholesterol was very low ($\approx 1$ pmol/h/mg protein).

*Effect of ketone bodies on glucose utilization*

Since *in vivo* both glucose and ketone bodies are available to the developing rat as carbon sources for oxidation and lipogenesis, it was interesting to investigate the effect of ketone bodies on glucose utilization. [U-$^{14}$C]glucose was incubated in the presence of increasing concentrations of acetoacetate or D-3-hydroxybutyrate (0.5–5.0 mM) [Fig. 3(A) and (B)]. The inhibitory effect of acetoacetate and D-3-hydroxybutyrate on glucose utilization was different. Addition of 0.5 mM acetoacetate to the incubation medium [Fig. 3(B)] reduced the incorporation of glucose into total lipids by 60% and there was little changes at higher concentrations of acetoacetate in the medium. $CO_2$ production was less affected and their was only 30% of reduction at 0.5 mM acetoacetate in the medium, and minor changes was observed thereafter.

The inhibitory effect of D-3-hydroxybutyrate was dependent on the concentration of this substrate in the medium. The reduction was about equal for both $CO_2$ production and the lipid synthesis. It was about 40% at 0.5 mM and reached the maximum level ($\approx 60\%$) at 2.5 mM D-3-hydroxybutyrate.

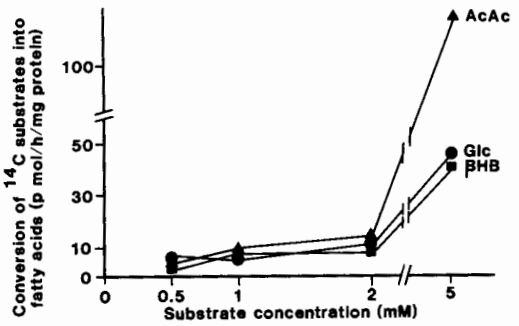


Fig. 2. Conversion of [3-$^{14}$C]acetoacetate (▲), D-3-[3-$^{14}$C]hydroxybutyrate (■) and [U-$^{14}$C]glucose (●) into fatty acids in isolated brain capillaries of developing rats. Incubations were carried out as described (cf. Fig. 1). Results are the mean of three separate experiments, SEMs were 5–10% of the mean values.

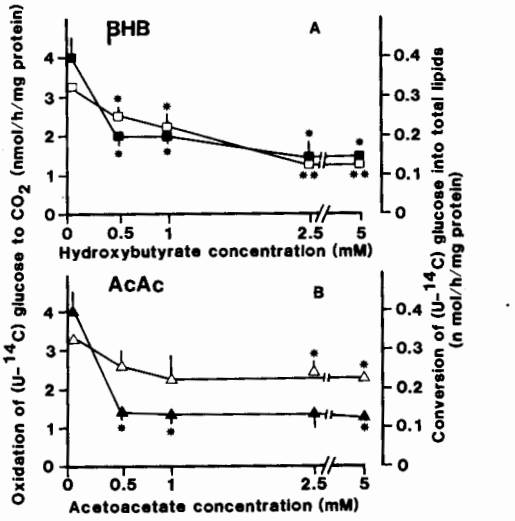


Fig. 3. Effect of D-3-hydroxybutyrate (A) and acetoacetate (B) on the metabolism of [U-$^{14}$C]glucose in isolated brain capillaries of developing rats. Incubations were carried out in the presence of 5 mM glucose and varying concentrations of acetoacetate or hydroxybutyrate. Results are the mean ± SEM ($n = 3$). (□, △), $CO_2$ production; (■, ▲), incorporation into total lipids. Significantly different from control values; $^{*}P < 0.05$; $^{**}P < 0.001$.

## DISCUSSION

Isolated brain capillaries of developing rat brain utilize both ketone bodies for production of energy and lipid synthesis. Oxidation as well as their incorporation into total lipids (Fig. 1) and total fatty acids (Fig. 2) were dependent on the concentration of ketone bodies in the medium even when it is more than their physiological levels (>2 mM). Similar results have been reported for utilization of ketone bodies in brain slices (Patel and Owen, 1977; Ide *et al.*, 1969) and oligodendrocytes (Sykes *et al.*, 1986). The incorporation of acetoacetate into total lipids and total fatty acids was higher than that of D-3-hydroxybutyrate. This difference is probably due to the mitochondrial localization of hydroxybutyrate dehydrogenase (EC 1.1.1.30). Compared to adults (Homayoun and Bourre, 1987) ketone body utilization, especially for lipid synthesis, was significantly greater in cerebral capillaries from developing rats. The higher rate of capillary permeability to ketone bodies during the suckling period (Cremer *et al.*, 1976) may favour their utilization in developing animals. Moreover, the activities of enzymes involving in ketone body metabolism in cerebral capillaries might be greater during the suckling period as is the case in whole brain (Robinson and Williamson, 1980). The incorporation of these substrates into cholesterol was very low. Therefore, as in neurons and astrocytes (Lopes-Cardozo *et al.*, 1986) and oligodendrocytes (Sykes *et al.*, 1986), acetoacetate is not a cholesterogenic precursor in endothelial cells of brain capillaries in developing rats.

Glucose oxidation in capillaries of developing rats was concentration dependent up to 1 mM glucose in the incubation medium, whereas its incorporation into total lipids continued to rise with substrate concentration up to 5 mM. This suggests that the conversion rate of glucose into lipids is under different metabolic control than is the case with ketone bodies (Ide *et al.*, 1969). Compared to ketone bodies, glucose was preferentially used for energy production. However, at higher concentrations (5 mM), $CO_2$ production from ketone bodies was comparable to that from glucose. The incorporation of glucose into total lipids was not significantly different from ketone bodies. Only at high substrate concentrations (5 mM) was it less than acetoacetate. Glucose incorporation into fatty acids was lower than that of acetoacetate but this difference was statistically significant at 5 mM concentration, and glucose labelled mainly the glycerol moiety of glycerolipids. The presence of either acetoacetate or D-3-hydroxybutyrate lowered the utilization of [U-$^{14}$C]glucose. Presence of either 0.5 mM acetoacetate or 2.5 mM D-3-hydroxybutyrate reduced both oxidation of [U-$^{14}$C]glucose and its incorporation into total lipids by about 30 and 60% respectively. Similar results have been obtained with brain slices from 1 week old rats (Patel and Owen, 1977) and other brain cells (Sykes *et al.*, 1986). The lower reduction observed for $CO_2$ production might be due to the high activity of pentose phosphate pathway in brain capillaries (Spatz and Mrsulja, 1982).

*Acknowledgements*—The authors are grateful to Drs P. Ferré and M. Lopes-Cardozo for reviewing this manuscript and to Miss I. Gabelotaud for her technical assistance. This work was supported by CNIEL.

## REFERENCES

Chechik T., Roeder L. M., Tildon J. T. and Poduslo S. E. (1987) Ketone body enzyme activities in purified neurons, astrocytes and oligodendrocytes. *Neurochem. Int.* **10,** 95–99.

Cremer J. E., Braun L. D. and Oldendorf W. H. (1976) Changes during development in transport processes of the blood–brain barrier. *Biochim. biophys. Acta* **448,** 633–637.

De Vivo D. C. (1980) The effect of ketone bodies on glucose utilization. In: *Cerebral Metabolism and Neural Function* (Passoneau J. V., Kawkins R. A., Lust W. D. and Welsh F. A., eds), pp. 243–254. Williams & Wilkins, Baltimore, Md.

Folch J., Lees M. and Sloane-Stanley G. H. (1957) Simple method for the isolation and purification of lipids from animal tissues. *J. biol. Chem.* **226,** 479–509.

Goldstein G. W., Wolinsky J. S., Csejtey J. and Diamond I. (1975) Isolation of metabolically active capillaries from rat brain. *J. Neurochem.* **25,** 715–717.

Homayoun P. and Bourre J. M. (1987) Ketone body utilization for energy production and lipid synthesis in isolated rat brain capillaries. *Biochim. biophys. Acta* **992,** 345–350.

Homayoun P., Roux F., Niel E. and Bourre J. M. (1985) The synthesis of lipids from [1-$^{14}$C]acetate by isolated brain capillaries. *Neurosci. Lett.* **62,** 143–147.

Ide T., Stinke J. and Cahill G. F. (1969) Metabolic interactions of glucose, lactate and $\beta$-hydroxybutyrate in rat brain slices. *Am. J. Physiol.* **217,** 784–792.

Koper J. W., Lopes-Cardozo M. and Van Gold L. M. G. (1981) Preferential utilization of ketone bodies for the synthesis of myelin cholesterol *in vivo*. *Biochim. biophys. Acta* **661,** 411–417.

Koper J. W., Zeinstra E. C., Lopes-Cardozo M. and Van Gold L. M. G. (1984) Acetoacetate and glucose as substrates for lipid synthesis by rat brain oligodendrocyte and astrocytes in serum-free culture. *Biochim. biophys. Acta* **796,** 20–26.

Lopes-Cardozo M., Larsson O. M. and Schousboe A. (1986) Acetoacetate and glucose as lipid precursors and energy substrates in primary cultures of astrocytes and neurons from mouse cerebral cortex. *J. Neurochem.* **46,** 773–778.

Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193,** 265–275.

Moore T. J., Lione A. P., Sugden M. and Regen D. M. (1976) β-Hydroxybutyrate transport in rat brain, developmental and dietary modulations. *Am. J. Physiol.* **230,** 619–630.

Patel M. S. and Owen O. E. (1977) Development and regulation of lipid synthesis from ketone bodies by rat brain. *J. Neurochem.* **28,** 109–114.

Pleasure D. E. and Towfighi J. (1972) Onion bulb neuropathies. *Arch. Neurol.* **26,** 289–301.

Pollet S., Ermidou S., Le Saux F., Monge M. and Baumann N. (1978) Microanalysis of brain lipids employing multiple two dimentional thin-layer chromatography. *Lipids* **19,** 916–921.

Robinson A. M. and Williamson D. H. (1980) Physiological roles of ketone bodies as substrates and signals in mammalian tissues. *Physiol. Rev.* **60,** 143–187.

Spatz M. and Mrsulja B. B. (1982) Progress in cerebral microvascular studies related to the function of blood–brain barrier. In: *Cellular Neurobiology*, Vol. 3, p. 311. Academic Press, New York.

Sykes J. E. C., Lopes-Cardozo M. and Van Den Bergh S. G. (1986) Substrate utilization for energy production and lipid synthesis in oligodendrocyte-enriched cultures prepared from rat brain. *Neurochem. Int.* **8,** 67–75.

Vitiello F. and Zanetta J. P. (1978) Thin-layer chromatography of phospholipids. *J. Chromat.* **166,** 637–640.