Regulation of Lactate Metabolism by Albumin in Rat Neurons and Astrocytes from Primary Culture

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ABSTRACT. The possible role played by albumin in regulating brain metabolism during development has been studied. The effects of fatty acid-free BSA on lactate, glucose, 3-hydroxybutyrate, and glutamine oxidation and lipogenesis by rat neurons and astrocytes from primary culture were studied. The rate of lactate oxidation and lipogenesis by neurons and astrocytes in the presence of BSA greatly exceeded that observed for glucose, 3-hydroxybutyrate, or glutamine, suggesting that lactate may be a key substrate for brain development. BSA strongly stimulated the rate of lactate, 3-hydroxybutyrate, and glutamine incorporation into lipids in both neurons (677%, 726%, and 250%, respectively) and astrocytes (415%, 393%, and 215%, respectively), possibly by binding long-chain acyl-CoA excesses, potent inhibitors of acetyl-CoA carboxylase. However, BSA decreased the rate of lipogenesis from glucose in both neurons (34%) and astrocytes (55%), probably by inhibiting glycerol-borne phospholipid synthesis. BSA significantly increased the rates of lactate (61%) and glucose (32%) oxidation by astrocytes but not those of 3-hydroxybutyrate and glutamine, suggesting that BSA may stimulate pyruvate oxidation. However, in neurons BSA did not affect the rate of oxidation of any of the substrates tested, which suggests that pyruvate oxidation is regulated differently in neurons and astrocytes. The results suggest that lactate is the most important substrate for both neurons and astrocytes, stressing the role played by lactate in brain development. Our results also suggest that serum albumin may control brain development by fostering metabolism for growth and differentiation purposes. (*Pediatr Res* 34: 709–715, 1993)

Abbreviations

DMEM, Dulbecco's modified Eagle's medium
EBS, Earle's balanced solution
CSF, cerebrospinal fluid
GFAP, glial fibrillary acidic protein

Immediately after delivery, the rate of liver glycogenolysis is very low (1–3) and the gluconeogenic capacity of the liver is negligible (3, 4), resulting in very low plasma concentrations of glucose (1, 2). Nevertheless, the rat brain uses ketone bodies

throughout the suckling period, which under these circumstances might supply brain with energy and carbon skeletons (5). Despite this, at birth the newborn rat lacks white adipose tissue (6), thus preventing active ketogenesis until nonesterified fatty acids from the mother's milk become available. Consequently, during the early neonatal period other metabolic substrates are needed to fulfill brain energy requirements. In this sense, lactate accumulates in the blood during late gestation, reaching concentrations higher than 9 mmol/L during the first minutes of extrauterine life (2, 7). However, most of the lactate accumulated is used within the first 2 h of extrauterine life, *i.e.* before the onset of suckling takes place (2, 7). In addition, a number of observations are consistent with the hypothesis that lactate is an important metabolic substrate for the brain during the early neonatal period in several species, including humans (for a review, see ref. 8). Thus, the rate of lactate use by neonatal rat brain slices (9) and isolated cells (10, 11) from early neonatal rat brain is much higher than that of glucose or 3-hydroxybutyrate, suggesting that immediately after delivery lactate is preferred to glucose or ketone bodies as a brain fuel. In addition, lactate transport into the brain is higher during the perinatal period than in adulthood (12, 13), suggesting that lactate may be used by the brain throughout the perinatal period. In this sense, it has been shown (14, 15) that lactate may also be an important substrate for the brain during the early suckling period. Finally, we have recently shown that fetal rat brain may use lactate as the main metabolic substrate during late gestation (16).

Unlike in adults, albumin and other plasma proteins are present in brain cells during development (17–19). In fact, a specific mechanism for the transfer of albumin from blood to CSF that is only active during the early stages of development has been recently reported (20). In addition, albumin uptake by the brain depends on the concentration of albumin in the blood during the early neonatal period but not in adulthood (21). These results are consistent with the notion that albumin uptake by the brain is related to the development of the CNS. However, the function of this protein in the development of the brain is not completely understood. Although albumin can deliver polyunsaturated fatty acids to the brain cells (22, 23), it is reasonable to conjecture that albumin also plays a role in regulating brain cell metabolism during development. Actually, we have recently reported (10) that the utilization of important substrates for the developing rat brain such as lactate, glucose, and 3-hydroxybutyrate was markedly affected by the presence of fatty acid-free BSA.

Because during brain development different types of cell populations are formed, possibly requiring different metabolic substrates for achieving their own final cellular structures, we decided to investigate whether albumin might regulate the utilization of the main metabolic substrates for the developing brain. Consequently, we studied the effect of albumin on lactate, glucose, 3-

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hydroxybutyrate, and glutamine utilization by neurons and astrocytes in primary culture.

MATERIAL AND METHODS

Reagents. DMEM, gentamicin, poly-L-lysine, and cytosine arabinoside were purchased from Sigma Chemical Co. (St. Louis, MO). FCS was obtained from Serva Boehringer Ingelheim (Heidelberg, Germany). Before use, FCS was incubated for 45 min at 60°C to allow complement inactivation. Substrates, coenzymes, and enzymes were purchased from Boehringer (Mannheim, Germany), Sigma Chemical Co., Merck (Darmstadt, Germany), or Serva Feinbiochemica GMBH and Co. (Heidelberg, Germany). Standard analytical grade laboratory reagents were obtained from Merck or Sigma. Fatty acid-free BSA, silicone, and methylbenzethonium hydroxide were purchased from Sigma. Fatty acid-free BSA was dialyzed twice against PBS (11 mmol/L sodium phosphate, 122 mmol/L NaCl, 4.8 mmol/L KCl, 0.4 mmol/L KH_2PO_4 , 1.2 mmol/L MgSO_4 , and 1.3 mmol/L CaCl_2 ; pH, 7.4) for 12 h and filtered through a 0.22- μm filter (Millipore Iberica, Madrid, Spain) before use. Radioactive substrates were purchased from New England Nuclear (Boston, MA). GFAP and neurofilament were detected by a specific antibody coupled to peroxidase (Sigma).

Animals. Albino Wistar rats, fed *ad libitum* on a stock laboratory diet (49.8% carbohydrates, 23.5% protein, 3.7% fat, 5.5% minerals, and added vitamins and amino acids) were used for the experiments. Rats were maintained on a 12-h light-dark cycle. Females with a mean weight of 250 g were caged with males overnight, and conception was considered to occur at 0100 h; this was verified the following morning by the presence of spermatozoa in the vaginal smears. For preparing neurons in primary culture, fetuses at 17.5 d of gestation were delivered by rapid hysterectomy after cervical dislocation of the mother and wiped, and the umbilical cord was cut. Postnatal d 1 newborn rats were used to prepare astrocytes in culture.

Cell culture. Cell isolation was carried out as described previously (10, 11) with some modifications. Briefly, animals were decapitated and their brains immediately excised. After the meninges and blood vessels were removed, the forebrains were placed in Earle's balanced solution containing 20 $\mu\text{g}/\text{mL}$ DNase and 0.3% (wt/vol) BSA. The tissue was minced, washed, centrifuged at $500 \times g$ for 2 min, and incubated in 0.025% trypsin (type III) and 60 $\mu\text{g}/\text{mL}$ DNase I for 15 min at 37°C. Trypsinization was terminated by the addition of DMEM containing 10% FCS. The tissue was then dissociated by gentle trituration, passing it four to eight times through a siliconized Pasteur pipette, and the supernatant cell suspension was recovered. This operation was repeated and the resulting cell suspension was centrifuged at $500 \times g$ for 5 min. The cells were resuspended in a known volume of DMEM supplemented with 10% FCS and 40 $\mu\text{g}/\text{mL}$ gentamicin. For neuron culture, the media were also supplemented with 25 mmol/L KCl. Cells were counted and the test for the exclusion of trypan blue dye showed that cell viability was higher than 90%. The cell suspension was then diluted in DMEM supplemented with 10% FCS and 40 $\mu\text{g}/\text{mL}$ gentamicin and plated onto 10-cm Petri dishes coated with 10 $\mu\text{g}/\text{mL}$ of poly-L-lysine at a density of 1.5×10^5 cells/ cm^2 for neurons and 1.0×10^5 cells/ cm^2 for astrocytes. Finally, the cells were incubated at 37°C in an atmosphere of 95% air/5% CO_2 with 90–95% humidity.

For neuron culture, the medium was replaced by fresh medium containing 10 μM cytosine arabinoside 3 d after plating. After 7 d, neuronal cultures reached confluence, as determined by the number of cells and the amount of protein and DNA per dish (Fig. 1). Antibodies against neurofilament (24) showed that 85% of the cells in the culture were neurons (Fig. 2A).

For astrocyte culture, fresh medium was added after 3 d. This medium was renewed by a fresh one containing 10% FCS twice a week. The number of cells and protein and DNA concentra-

Fig. 1. Time-courses of cell numbers and DNA and protein content in neurons in primary culture. Neurons from brain fetuses at 17.5 d of gestation were grown in serum-supplemented medium. Ten $\mu\text{mol}/\text{L}$ cytosine arabinoside were added 3 d after plating. Results are expressed as number of cells $\times 10^6$ per dish (6 cm), μg of DNA per dish, or mg of protein per dish. Results are means $\pm$ SEM ($n = 5$).

tions increased exponentially until the 10th d of culture (Fig. 3). When confluence was reached, 90–95% of the cells were astrocytes (Fig. 2B), as determined by immunostaining against GFAP (25).

Immunocytochemistry. Neurons and astrocytes (7 and 13 d in culture, respectively) grown in 3.5-cm diameter Petri dishes were stained with the antibodies most commonly used to identify them, *i.e.* neurofilament for neurons (24) and GFAP for astrocytes (25). Cells were fixed in 10% neutral buffered formaline for 30 min, and after washing with PBS endogenous peroxidase was quenched with 3% H_2O_2 for 5 min. A nonspecific blocking solution consisting of 1% normal goat serum was added for 10 min. This was replaced with the monoclonal primary mouse antibody to neurofilament or the monoclonal primary rabbit antibody to GFAP. After 60 min, cells were washed with PBS and biotinylated goat anti-mouse IgG for neurofilament or anti-rabbit IgG for GFAP was added for 20 min. After washing three times with PBS, avidin-conjugated peroxidase was added for 20 min, then the cells were washed and incubated with H_2O_2 and 3-amino-9-ethylcarbazole as chromogen for 10 min. Cells were counterstained with Mayer's hematoxylin and mounted in glycerol. Blanks with normal mouse or rabbit serum instead of primary antibody were carried out in parallel. These preparations were examined with a Nikon inverted microscope and photographed with Kodak Tmax 400 ASA film.

Cell incubation. Neurons were harvested after 7 d in culture and astrocytes on the 13th d. The cells were washed twice with PBS and then with PBS containing 0.05% (wt/vol) trypsin for 1 min at 37°C. After removing this solution, the cells were maintained for 15 min at 37°C and the trypsin activity stopped by the addition of PBS containing 10% FCS. The resulting cell suspension was centrifuged and resuspended in a known volume of oxygen-saturated PBS. The cells were counted and tested for exclusion of trypan blue dye, showing that cell viability was higher than 90%.

The cells ($6\text{--}7 \times 10^6$) were incubated in flasks tightly stoppered with rubber cups as described previously (10, 11). The incubation medium was 0.5 mL of PBS containing 10.5 mmol/L L-lactate + 300–800 dpm/nmol L-[U- ^{14}C]lactate, 5.2 mmol/L D-glucose + 400–800 dpm/nmol D-[U- ^{14}C]glucose, 2.5 mmol/L D-3-hydroxybutyrate + 900–3000 dpm/nmol D-[3- ^{14}C]3-hydroxybutyrate, or 2.0 mmol/L L-glutamine + 2000–3000 dpm/nmol of L-[U- ^{14}C]glutamine in the presence or absence of 2% (wt/vol) BSA (10). The gas phase was pure O_2 . Incubations were stopped after 1 h by injection of 0.1 mL of 12% (vol/vol) HClO_4 , although shaking was continued for an additional 20 min. CO_2 from the acidified medium was recovered in a central well containing 0.5 mL of 1 M methylbenzethonium hydroxide. The whole methylbenzethonium hydroxide well was measured by liquid-scintillation counting (efficiency of counting: 95%). Blanks containing

Fig. 2. Photomicrographs of neurons and astrocytes stained for neurofilament and GFAP, respectively. Neurons were cultured for 7 d and stained with control serum (A) or MAb to neurofilament (B). Astrocytes were cultured for 13 d and stained with control serum (C) or MAb to GFAP (D).

the incubation medium but no cells were carried out in parallel to measure volatile radioactivity, which was subtracted from the sample values. After precipitation, cells were collected by centrifugation and lipids were extracted with chloroform:methanol (2:1). The extracts were washed with 0.3% NaCl saturated with chloroform, evaporated under a stream of N_2 , and dissolved in scintillation fluid for radioactivity counting (efficiency of counting: 98%). Results are expressed as nmol of substrate transformed into CO_2 or lipids/h per 10^7 cells and were calculated by using the specific radioactivity (dpm/nmol) of the substrates assayed.

Under our experimental conditions, neurons and astrocytes oxidized lactate and glucose linearly with the incubation time for at least 90 min ($r = 1.00$; $p < 0.001$). The incorporation of lactate and glucose into lipids was also linear with the incubation time ($r = 0.99$; $p < 0.001$).

Analytical procedures. Substrate concentration in the incubation medium was assayed as follows: L-lactate was measured by the method of Gutmann and Wahlefeld (26), D-glucose as described Bergmeyer *et al.* (27), D-3-hydroxybutyrate by the method of Williamson and Mellanby (28), and L-glutamine by the method of Lund (29). The concentrations of proteins and DNA were determined by the method of Lowry *et al.* (30) and Labarca and Paigen (31), respectively. The number of cells were determined after trypsinization by counting in a hemocytometer. Statistical analyses were carried out using the *t* test.

RESULTS

Cell growth in culture. Neurons grew progressively until the 3rd d in culture, when cytosine arabinose was added to avoid astrocyte proliferation. From this point on, neuron proliferation

ceased, as shown by the maintenance of cell numbers and DNA and protein concentrations (Fig. 1). After 7 d, the culture contained mainly neurons, as shown by positive immunostaining with specific neurofilament antibody (Fig. 2A).

The time course of astrocyte growth was sigmoidal, reaching confluence after about 10 d of culture (Fig. 3). Confluence was clearly onset on the 13th d when astrocytes were used for the experiments. After 13 d, the culture essentially contained astrocytes, as shown by positive immunostaining with specific GFAP antibody (Fig. 2B).

Effect of BSA on time course of lactate utilization by neurons and astrocytes. Figure 4 shows that lactate utilization by neurons and astrocytes was linear for at least 90 min. This phenomenon was observed in the rate of lactate oxidation to CO_2 and in the rate of lactate incorporation into lipids in both neurons and astrocytes. The presence of fatty acid-free BSA greatly enhanced the rate of lipogenesis from lactate in neurons and astrocytes and slightly increased the rate of lactate oxidation in astrocytes but did not modify that in neurons (Fig. 4). The increases observed were linear over time, showing that within the observational period the effect of BSA was nonsaturable. The persistence of the effect of albumin for 90 min confirmed our previous observations showing that the effect of BSA on newborn brain metabolism is not due to osmotic properties of the protein (10); were this not so, an immediate but not constant effect of BSA would be expected.

Lactate utilization by neurons and astrocytes: comparison with glucose, 3-hydroxybutyrate, and glutamine. Table 1 shows that both in the absence and in the presence of BSA in the incubation medium the rate of lactate oxidation by neurons was 2.5-, 5-, and 2-fold ($p < 0.001$) greater than the rates observed for glucose,

Fig. 3. Time courses of cell numbers and DNA and protein content in astrocytes in primary culture. Astrocytes from brains of 1-d-old newborn rats were grown in serum-supplemented medium. Results are expressed as number of cells $\times 10^6$ per dish (6 cm) (a), μg of DNA per dish (b), and mg of protein per dish (c). Results are means $\pm$ SEM ($n = 5$).

Fig. 4. Effect of BSA on the time course of lactate incorporation into CO_2 (oxidation) or lipids (lipogenesis) by neurons and astrocytes from primary culture. Neurons and astrocytes from 7 or 13 d in culture, respectively, were harvested and incubated in 0.5 mL of PBS containing 10.5 mmol/L L-lactate + 300–800 dpm/nmol of L-[U- ^{14}C]lactate in the absence (O) or presence (Δ) of 2% (wt/vol) BSA. Results are expressed in nmol of substrate incorporated in CO_2 or in lipids per 10^7 cells and are means $\pm$ SEM ($n = 3$). a, Lactate oxidation by neurons. O: $r = 0.99$, $p < 0.001$; Δ: $r = 1.00$, $p < 0.001$. b, Lipogenesis from lactate by neurons. O: $r = 1.00$, $p < 0.001$; Δ: $r = 0.96$, $p < 0.001$. c, Lactate oxidation by astrocytes. O: $r = 0.98$, $p < 0.005$; Δ: $r = 0.96$, $p < 0.001$. d, Lipogenesis from lactate by astrocytes. O: $r = 0.97$, $p < 0.005$; Δ: $r = 0.93$, $p < 0.001$.

3-hydroxybutyrate, or glutamine, respectively. Moreover, in the presence of BSA the rate of lipid synthesis from lactate was about 50% ($p < 0.05$) higher than that from glucose and 3- and 45-fold ($p < 0.001$) higher than those from 3-hydroxybutyrate and glutamine, respectively. However, in the absence of BSA in the incubation medium, the rate of lipogenesis from glucose was 8-, 25-, and 160-fold ($p < 0.001$) greater than those from lactate, 3-hydroxybutyrate, and glutamine.

In the presence of BSA, the rate of lactate oxidation by astrocytes was 4.5-, 3-, and 2-fold ($p < 0.001$) higher than those of glucose, 3-hydroxybutyrate, or glutamine, respectively. However, in the absence of BSA, the rate of lactate oxidation was 3.5-fold ($p < 0.001$) higher than that of glucose, about 35% ($p < 0.05$) higher than that of 3-hydroxybutyrate, and similar to that of glutamine. In the presence of BSA, the rate of lipogenesis from lactate in astrocytes was 60% ($p < 0.01$) greater than that of glucose and 2.5- and 12-fold ($p < 0.001$) greater than those of 3-hydroxybutyrate and glutamine, respectively. However, in the absence of BSA, the rate of lipid synthesis from glucose was 7-, 17-, and 56-fold ($p < 0.001$, all) greater than those from lactate, 3-hydroxybutyrate, and glutamine, respectively.

Both in the presence and in the absence of BSA, the rate of total lactate utilization by neurons was 2-, 5-, and 2-fold ($p < 0.001$) higher than those of glucose, 3-hydroxybutyrate, and glutamine, respectively. In the absence of BSA, the rate of total lactate utilization by astrocytes was 2.5-fold ($p < 0.001$) higher than that of glucose, about 35% ($p < 0.05$) higher than that of 3-hydroxybutyrate, and similar to that of glutamine. In the presence of albumin, the rate of total lactate utilization by astrocytes was 4-, 2.5-, and 2-fold ($p < 0.001$) higher than those of glucose, 3-hydroxybutyrate, and glutamine, respectively.

Effect of BSA on lactate, glucose, 3-hydroxybutyrate, and glutamine utilization by neurons and astrocytes in primary culture. The rate of substrate incorporation into lipids was increased by the presence of BSA in the incubation medium both in the case of neurons and in astrocytes (Table 1). In neurons, BSA strongly increased the rate of lipogenesis from lactate (8-fold; $p < 0.001$), 3-hydroxybutyrate (8-fold; $p < 0.001$), and glutamine (4-fold; $p < 0.001$), decreasing the rate of lipid synthesis from glucose by a 35% ($p < 0.001$). Similarly, BSA increased the rate of lipogenesis from lactate (5-fold; $p < 0.001$), 3-hydroxybutyrate (5-fold; $p < 0.001$), and glutamine (3-fold; $p < 0.001$) in astrocytes; however, BSA decreased the rate of lipogenesis from glucose by 55% ($p < 0.001$). Table 1 shows that the presence of BSA in the incubation medium did not affect the rate of lactate, glucose, 3-hydroxybutyrate, or glutamine oxidation by neurons. However, BSA increased the rate of lactate (60%; $p < 0.01$) and glucose (30%; $p < 0.05$) oxidation by astrocytes but slightly decreased the rate of 3-hydroxybutyrate (18%; $p < 0.01$) and glutamine (15%; $p < 0.001$) oxidation.

The rate of total utilization (oxidation plus lipogenesis) of lactate, glucose, and glutamine by neurons was not affected by the presence of BSA, although the protein slightly increased (20%; $p < 0.01$) that of 3-hydroxybutyrate. Total utilization of lactate by astrocytes was significantly increased (65%; $p < 0.001$) by BSA. However, BSA slightly decreased the rates of total utilization of 3-hydroxybutyrate (15%; $p < 0.05$) and glutamine (15%; $p < 0.001$) by astrocytes without affecting the rate of total glucose utilization.

Comparison of neurons and astrocytes for substrate utilization. The rates of lactate utilization were similar in neurons and in astrocytes except for the oxidation of lactate in the presence of BSA, which was about 50% ($p < 0.001$) higher in astrocytes than that observed in neurons. The rates of glucose oxidation were greater in neurons than in astrocytes both in the absence (60%; $p < 0.001$) and in the presence (20%; $p < 0.01$) of BSA. However, the rates of glucose incorporation into lipid were similar in both cell populations. The rates of 3-hydroxybutyrate oxidation were 4- and 3-fold ($p < 0.001$) greater in astrocytes than that observed in neurons in the absence or presence of BSA, respectively.

Table 1. Effect of fatty acid-free BSA on substrate utilization by rat neurons and astrocytes from primary culture*

Additions	Oxidation		Lipogenesis		Total	
	Neurons	Astrocytes	Neurons	Astrocytes	Neurons	Astrocytes
Lactate	72.3 ± 4.41	67.5 ± 6.00	0.81 ± 0.04	0.99 ± 0.15	73.1 ± 4.41	68.5 ± 6.14
Lactate + BSA	72.2 ± 4.27	109 ± 8.65 ^{ce}	6.30 ± 0.68 ^f	5.10 ± 0.82 ^f	78.5 ± 4.39	114.0 ± 9.21 ^{fa}
Glucose	30.9 ± 1.24 ⁱ	18.8 ± 0.94 ^{ci}	6.38 ± 0.21	7.33 ± 0.43	36.8 ± 1.25 ⁱ	26.2 ± 1.34 ^{ci}
Glucose + BSA	29.3 ± 1.84 ⁱ	24.9 ± 1.17 ^{bdi}	4.20 ± 0.25 ^b	3.26 ± 0.22 ^{fk}	33.5 ± 1.83 ⁱ	28.0 ± 2.38 ⁱ
3-Hydroxybutyrate	12.0 ± 1.05 ⁱ	49.4 ± 2.80 ^{ce}	0.27 ± 0.03	0.42 ± 0.03	13.0 ± 1.05 ⁱ	49.8 ± 2.83 ^{ce}
3-Hydroxybutyrate + BSA	13.8 ± 0.91 ⁱ	40.7 ± 1.03 ^{ce}	2.23 ± 0.23 ⁿ	2.07 ± 0.14 ⁿ	16.0 ± 1.10 ^{ei}	42.7 ± 1.14 ^{cdi}
Glutamine	32.3 ± 1.99 ⁱ	62.8 ± 0.81 ^c	0.04 ± 0.01	0.13 ± 0.02 ^b	32.3 ± 1.99 ⁱ	63.0 ± 0.66 ^c
Glutamine + BSA	32.4 ± 1.30 ⁱ	54.7 ± 0.45 ^{bfi}	0.14 ± 0.02 ⁿ	0.41 ± 0.01 ^{cn}	32.6 ± 1.41 ^{ci}	55.1 ± 0.57 ^{cn}

Neurons and astrocytes (7 and 13 d, respectively) in primary culture were harvested and incubated in 0.5 mL of PBS containing 10.5 mmol/L L-lactate + 300-800 dpm/nmol of L-[U-¹⁴C]lactate, 5.2 mmol/L D-glucose + 400-800 dpm/nmol of D-[U-¹⁴C]glucose, 2.5 mmol/L D-3-hydroxybutyrate + 900-3000 dpm/nmol of D-[3-¹⁴C]3-hydroxybutyrate, or 2.0 mmol/L L-glutamine + 2000-3000 dpm/nmol of L-[U-¹⁴C]glutamine in the presence or absence of 2% (wt/vol) BSA. Results are expressed in nmol of substrate incorporated in CO₂ (oxidation), into lipids (lipogenesis), or in both (total) per hour and 10⁷ cells and are means ± SEM (n = 5). Statistical differences between the values obtained in neurons and astrocytes are indicated by superscripts as follows: a, *p* < 0.05; b, *p* < 0.01; c, *p* < 0.001. Statistical differences between the values obtained in the presence or absence of BSA are indicated by superscripts as follows: d, *p* < 0.05; e, *p* < 0.01; f, *p* < 0.001. Statistical differences between the values obtained with lactate compared with those obtained with the other substrates in the absence of BSA are indicated by superscripts as follows: g, *p* < 0.05; h, *p* < 0.01; i, *p* < 0.001. In the presence of BSA: j, *p* < 0.05; k, *p* < 0.01; l, *p* < 0.001.

However, similar rates of lipid synthesis from 3-hydroxybutyrate were obtained in both cell populations. Astrocytes oxidized glutamine at a higher rate than did neurons both in the absence (95%; *p* < 0.001) and in the presence (70%; *p* < 0.01) of BSA. In addition, the rates of lipogenesis from glutamine in astrocytes were 3-fold (*p* < 0.001) greater than those of neurons both in the absence and presence of BSA.

DISCUSSION

Our results show that both cell populations studied—neurons and astrocytes—utilize lactate as the most important energetic and biosynthetic substrate. In this sense, in the presence of BSA the rates of lactate oxidation and lipogenesis by neurons and astrocytes are significantly higher than those of glucose, 3-hydroxybutyrate, or glutamine (Table 1). These results are in agreement with those previously observed in rat brain slices (9, 16, 32) or in isolated rat brain cells (10, 11) and are consistent with the idea that during brain development lactate metabolism plays an important role in providing energy and carbon skeletons for cell proliferation and differentiation. In agreement with this suggestion, Larrabee (33) has shown that lactate can be utilized by neuronal and nonneuronal cells from embryonic sympathetic ganglia. Similarly, the capacity of lactate transport across the blood-brain barrier remains high during the suckling period (34), suggesting that lactate could be utilized by brain cells throughout the development of the brain. In agreement with this, Dombrowski *et al.* (15) have reported that lactate can supply a significant fraction of the energy requirements of the rat brain during the suckling period.

Consequently, it is reasonable to suggest that lactate is actively utilized by the brain during the early neonatal period when other substrates such as glucose and ketone bodies are not available. However, during the suckling period ketone bodies may support the bulk of brain requirements, although lactate is also used to a moderate extent depending on its blood concentrations. In these circumstances, however, the role played by lactate may be important because it would support astrocyte proliferation.

The presence of BSA in the incubation medium strongly increased the rate of lipogenesis from lactate, 3-hydroxybutyrate, or glutamine in both neurons and astrocytes (Table 1). This effect suggests that in the absence of BSA lipogenesis may be limited at the level of the acetyl-CoA carboxylase (EC 6.4.1.2)-catalyzed reaction because albumin presumably removes long-chain acyl-CoA, which is a potent inhibitor of this enzyme in the brain (35). It should be mentioned that brain acetyl-CoA carboxylase may be the same isozyme as that found in the liver

(36), which is very sensitive to the deinhibition by albumin of the acyl-CoA effect (37). Additionally, the mitochondrial citrate carrier might also be deinhibited by albumin because its activity is affected by the presence of long-chain acyl-CoA in the liver (38).

It is intriguing, however, that the effect of BSA of increasing the rate of lipogenesis was observed with lactate, 3-hydroxybutyrate, and glutamine but not with glucose (Table 1). This is unexpected, because lipogenesis from glucose follows the same metabolic pathway as the other substrates assayed. Unlike the other substrates, however, glucose may also be incorporated into glycerol for glycerolipid synthesis. In fact, an important part of the incorporation of glucose into lipids by neurons or astrocytes can be accounted for by the synthesis of glycerol-borne lipids (Tabernero A, Vicario C, Medina JM, unpublished results). It therefore was not surprising that BSA did not stimulate lipogenesis from glucose. Instead, BSA decreased glucose incorporation into lipids (Table 1), suggesting that BSA may inhibit glycerogenesis. Thus, BSA did not inhibit glucose oxidation, in agreement with the idea that the effect of albumin on lipogenesis from glucose is exerted on the derivation of triose phosphate for glycerol synthesis.

The effect of BSA on astrocytes of increasing the oxidation rates of lactate and glucose but not those of 3-hydroxybutyrate or glutamine (Table 1) suggests that the effect of BSA is exerted on the pyruvate dehydrogenase (pyruvate:lipoamide oxidoreductase; EC 1.2.4.1)-catalyzed reaction. Thus, the first common intermediate in glucose and lactate metabolism is pyruvate, and 3-hydroxybutyrate and glutamine enter the tricarboxylic acid cycle, bypassing the pyruvate dehydrogenase-catalyzed reaction. In agreement with this, 3,4-¹⁴C-glucose oxidation, which directly measures the flux through pyruvate the dehydrogenase-catalyzed reaction, was significantly increased by the presence of BSA (Tabernero A, Medina JM, unpublished observations), pinpointing the pyruvate dehydrogenase-catalyzed reaction as the target for the effects of BSA on substrate oxidation by astrocytes. This is not unexpected, because the decrease in acetyl-CoA availability caused by the enhancement of acetyl-CoA carboxylase activity brought about by BSA may increase pyruvate dehydrogenase activity. In this sense, brain pyruvate dehydrogenase activity is controlled by a phosphorylation/dephosphorylation mechanism that is finely regulated by the mitochondrial acetyl-CoA/CoA ratio (39). It should be mentioned that the decrease in acetyl-CoA levels presumably caused by BSA may prevent the diversion of pyruvate through the pyruvate carboxylase (EC 6.4.1.1)-catalyzed reaction, increasing pyruvate oxidation by pyruvate dehydrogenase. Accordingly, the presence of acetyl-CoA is man-

datory for pyruvate carboxylase activity, which is quite high in astrocytes (40–42). In agreement with this suggestion, BSA did not modify substrate oxidation by neurons (Table 1), whose pyruvate carboxylase activity is negligible (40–42). Alternatively, it may be speculated that pyruvate dehydrogenase is not controlled by acetyl-CoA concentrations in the neurons. In fact, lactate oxidation by early neonatal brain slices [composed of about 85% neurons (43)] has proved to be insensitive to dichloroacetate (Fernández E, Medina JM, unpublished observations), which is a strong activator of pyruvate dehydrogenase. In this sense, it has been reported that dichloroacetate increases the dephosphorylated (active) form of the enzyme by inhibiting pyruvate dehydrogenase kinase (44). In agreement with this, it has also been reported that the phosphorylation/dephosphorylation mechanism is not operative during the very early stages of brain development but does increase later, coinciding with astrocyte proliferation (45).

It is interesting to note that BSA increased the rate of total lactate utilization by astrocytes (Table 1) but not by neurons, suggesting a specific role for BSA in lactate metabolism by astrocytes. Thus, the accumulation of lactate in blood at the end of gestation and the early neonatal period (7) coincides with an active proliferation of type I astrocytes (46). Similarly, brain albumin concentrations are very high during the early neonatal period (47) mainly because albumin is actively taken up from blood (19, 20). In fact, albumin brain levels depend on their levels in plasma in the early postnatal period (21). In addition, albumin concentrations in CSF are very high during the perinatal period (18), in agreement with the idea that albumin is translocated by astrocytes (48) and finally secreted (49, 50) into CSF. Consequently, it is tempting to speculate that albumin may specifically stimulate lactate utilization by astrocytes, fueling their proliferation and differentiation. If so, albumin may control brain development not only by a direct proliferating effect (51) but also by fostering metabolism for growth purposes. The role played by lipogenesis in brain cell differentiation is fundamental because both the formation of cell processes and myelinogenesis rely on active lipid synthesis. Whether these phenomena are controlled by the albumin present in the brain remains to be elucidated. However, our results do show that albumin controls neuron and astrocyte metabolism in such situations as that in which metabolism is not only used for the maintenance of ordinary duties but is also focused on obtaining the energy and carbon required for proliferation and differentiation.

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Announcement

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