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Lactate utilization by the neonatal rat brain *in vitro*

Competition with glucose and 3-hydroxybutyrate

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The maximum rates of lactate oxidation and lipogenesis from lactate by early-neonatal brain slices were considerably greater than those for utilization of glucose and 3-hydroxybutyrate at physiological concentrations. Lactate inhibited glucose utilization, but enhanced 3-hydroxybutyrate utilization. 3-Hydroxybutyrate inhibited lactate and glucose utilization. Glucose slightly inhibited oxidation of lactate and 3-hydroxybutyrate, but scarcely enhanced lipogenesis from these substrates.

INTRODUCTION

During the first postnatal hours the newborn rat develops a profound hypoglycaemia (Girard *et al.*, 1973; Di Marco *et al.*, 1978; Cuezva *et al.*, 1980) as a consequence of the low rates of glucose production by glycogenolysis and gluconeogenesis (Cake *et al.*, 1971; Snell & Walker, 1973; Pearce *et al.*, 1974; Medina *et al.*, 1980; Fernández *et al.*, 1983). Accordingly, it may be suggested that during the first 1–2 h of extra-uterine life the supply of glucose is not sufficient to provide the energy requirements of the brain. Since ketone bodies are actively utilized by the brain during the suckling period (Krebs *et al.*, 1964; Lockwood & Bailey, 1971; Williamson & Buckley, 1973), it might be suggested that, during the first hours after delivery, glucose is replaced by ketone bodies as the main metabolic substrate for the brain. However, the rat is almost lacking in white adipose tissue at birth (Girard & Ferré, 1982), and thus ketogenesis can only occur when non-esterified fatty acids from the mother's milk are available. Moreover, the low concentrations of ketone bodies observed during the pre-suckling period (Girard *et al.*, 1973; Cuezva *et al.*, 1980) seem to be a consequence of a transient inhibition of ketogenesis caused by the lack of ketogenic precursors and/or cofactors (Girard & Ferré, 1982; Ferré *et al.*, 1983) rather than an increase in ketone-body utilization. Consequently, during the early neonatal period the brain has to be supplied by substances other than ketone bodies.

A putative substrate for the brain during the pre-suckling period may be lactate, which is actively removed from the blood in these circumstances (Girard *et al.*, 1973; Cuezva *et al.*, 1980; Fernández *et al.*, 1983). Gluconeogenesis from lactate is thus negligible during the first 2 h after delivery (Medina *et al.*, 1980; Fernández *et al.*, 1983), whereas lactate is mostly consumed through its oxidation by the tricarboxylic acid cycle (Medina *et al.*, 1980). Moreover, lactate is actively oxidized by brain *in vitro* (Arizmendi & Medina, 1983), a fact consistent with the idea that lactate may be an alternative substrate to glucose in the early-neonatal brain.

The aims of the present work were to establish the possible role of lactate as an energy substrate and as a source of carbon skeletons for lipogenesis *de novo* in neonatal brain slices at birth. We also studied the competition of lactate with glucose and 3-hydroxybutyrate in order to evaluate the importance of lactate as a substrate for the brain during the early neonatal period.

EXPERIMENTAL

Albino Wistar rats fed on stock laboratory diet (carbohydrate 58.7%, protein 17.0%, fat 3.0% and added salts and vitamins) and of known gestational age were used for the experiments. Females with a mean weight of 250 g were caged with males overnight, and conception was considered to occur at 01:00 h; this was verified the following morning by the presence of spermatozoa in vaginal smears. Foetuses weighing 5.52 ± 0.10 g (mean ± S.E.M.) were delivered at day 21.5 of gestation (21.7 days to full gestation) by rapid hysterectomy after cervical dislocation of the mother. Newborns were kept in an incubator at 37 °C in a continuous stream of water-saturated air without feeding. After 1–2 h, the newborns were decapitated and the right hemispheres of the forebrain were removed and immediately used for the experiments.

Brain slices were prepared and incubated as previously described (Arizmendi & Medina, 1983). The incubation medium was 2 ml of phosphate physiological saline (Elliot, 1969) containing 0.5 μCi of L-[U-^{14}C]lactate, D-[6-^{14}C]glucose or D-3-hydroxy[3-^{14}C]butyrate (New England Nuclear, Boston, MA, U.S.A.) and the desired concentrations of unlabelled substrates. The gas phase was pure O_2. Incubations were stopped after 1 h by injection into the main well of 0.2 ml of 20% (v/v) $HClO_4$, though shaking was continued for a further 20 min. The radioactive CO_2 trapped by KOH was measured by liquid-scintillation counting. Blanks without slices were carried out in parallel to measure volatile radioactivity, which was subtracted from other values. Lactate was measured by the method of Hohorst (1965), D-glucose as described by Bergmeyer *et al.* (1974), and D-3-hydroxybutyrate by the method of Williamson *et al.* (1962).

After incubation the brain slices were frozen and powdered in liquid N_2. Lipids were extracted with

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chloroform/methanol (2:1, v/v), and the extract was washed once with 0.8 ml of 0.3% NaCl saturated with chloroform. Since only a negligible amount of radioactivity was found in a second washing layer, the organic phase was not washed any further; 0.2 ml of this organic phase was used for liquid-scintillation counting. The specific radioactivity of substrates found in the blanks was used for the calculations.

RESULTS AND DISCUSSION

The maximum rate of lactate oxidation (4.1 μmol/h per g wet wt.) was 14-fold and 3-fold greater than the rates of oxidation observed with 5 mM-glucose and 2 mM-3-hydroxybutyrate respectively (Figs. 1–3). Furthermore, lipogenesis from lactate was also substantially greater than that from well-recognized lipogenic substrates for the brain such as glucose and 3-hydroxybutyrate. Thus the rate of lipogenesis from 12 mM-lactate was 2.2 μmol/h per g wet wt., i.e. about 2-fold and 4-fold greater than those with 5 mM-glucose and 2 mM-3-hydroxybutyrate respectively (Figs. 1–3). These findings strongly suggest that lactate may be the main metabolic substrate for the brain during the early neonatal period. Accordingly, lactate may be utilized by the brain not only as an energy-yielding substrate but also as a source of carbon skeletons for lipid synthesis.

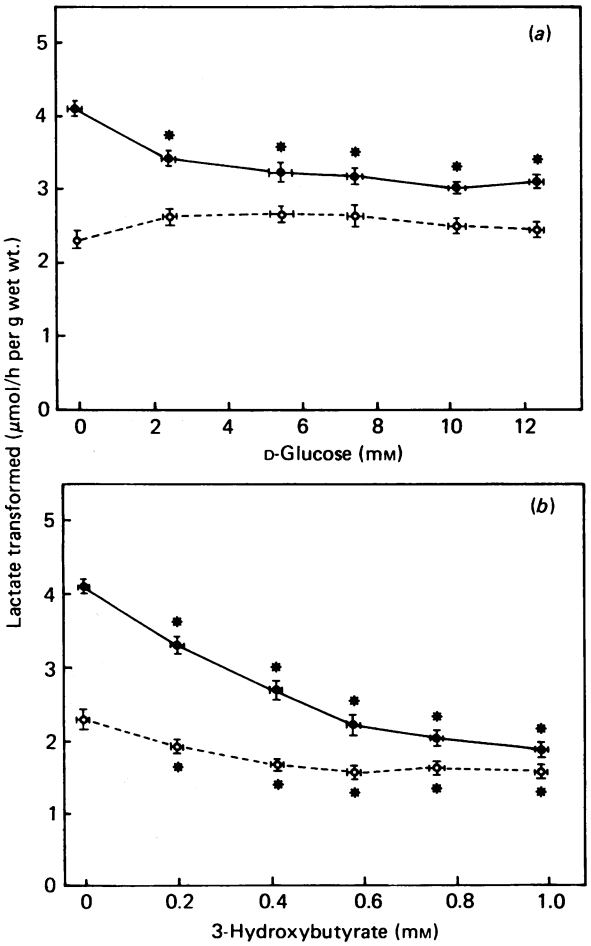

Fig. 1. Lactate utilization by neonatal brain slices

Early neonatal (1–2 h) brain slices were incubated with 12 mM-lactate and 0.5 μCi of L-[U-^{14}C]lactate in the absence or the presence of increasing concentrations of D-glucose (*a*) or D-3-hydroxybutyrate (*b*). Lactate oxidation (●) and lipogenesis from lactate (○) were measured as described in the Experimental section. Results are means ± S.E.M. for brain slices from at least 14 newborns; *significant ($P < 0.01$) versus initial values.

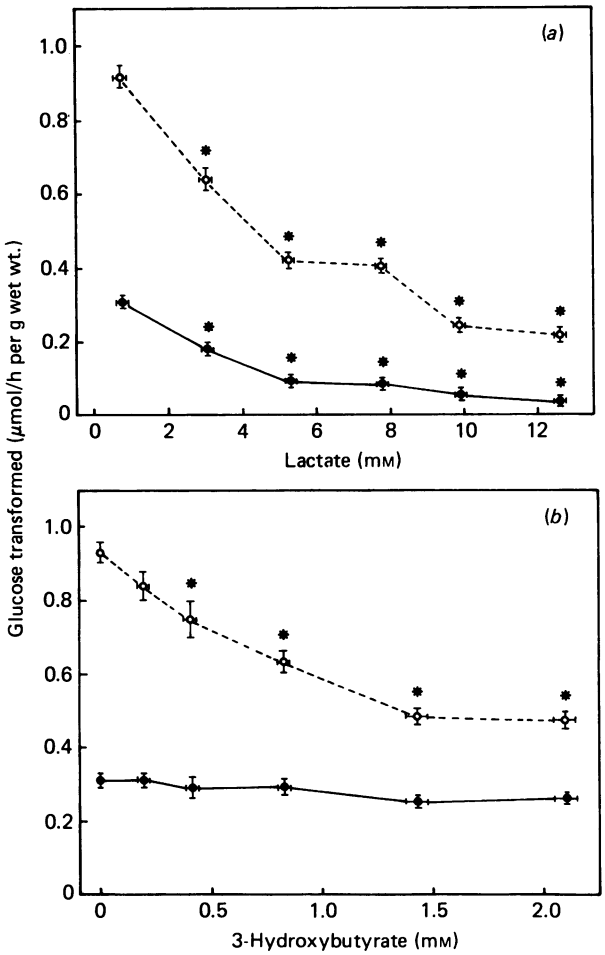

Fig. 2. Glucose utilization by neonatal brain slices

Early neonatal (1–2 h) brain slices were incubated with 5 mM-D-glucose and 0.5 μCi of D-[6-^{14}C]glucose in the absence or the presence of increasing concentrations of L-lactate (*a*) or D-3-hydroxybutyrate (*b*). Glucose oxidation (●) and lipogenesis from glucose (○) were measured as described in the Experimental section. Results are means ± S.E.M. for brain slices from at least 14 newborns; *significant ($P < 0.01$) versus initial values.

The presence of 5 mM-glucose in the incubation medium slightly inhibited lactate oxidation, but no further inhibition was observed up to 12 mM-glucose. The rate of lipogenesis from lactate remained unchanged in the presence of up to 12 mM-glucose (Fig. 1*a*). These results may suggest that lactate can be utilized by neonatal brain *in vivo* regardless of blood glucose concentrations. However, lactate oxidation was strongly inhibited by the presence of 3-hydroxybutyrate in the incubation medium (Fig. 1*b*). Such inhibition was observable from 0.2 mM-3-hydroxybutyrate, reaching maximal inhibition (about 50%) at 0.6 mM-3-hydroxybutyrate. Lipogenesis from lactate was slightly inhibited

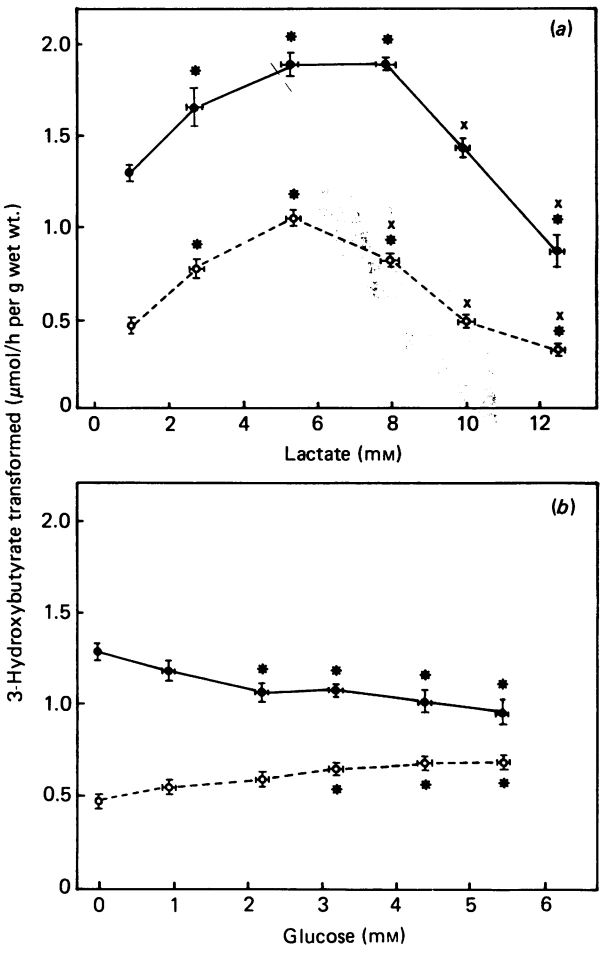

Fig. 3. 3-Hydroxybutyrate utilization by neonatal brain slices

Early neonatal (1–2 h) brain slices were incubated with 2 mM-D-3-hydroxybutyrate and 0.5 μCi of D-3-hydroxy-[3-^{14}C]butyrate in the absence or the presence of increasing concentrations of L-lactate (*a*) or D-glucose (*b*). 3-Hydroxybutyrate oxidation (●) and lipogenesis from 3-hydroxybutyrate (○) were measured as described in the Experimental section. Results are means ± S.E.M. for brain slices from at least 14 newborns: *significant ($P < 0.01$) versus initial values; x significant ($P < 0.01$) versus maximal values.

by the presence of 0.2 mM-3-hydroxybutyrate, with maximum inhibition (about 35%) at 0.4 mM-3-hydroxybutyrate. Whether the inhibition of lactate utilization caused by 3-hydroxybutyrate may be accomplished by the inhibition of pyruvate dehydrogenase (Land *et al.*, 1977; Booth & Clark, 1981) or through competition with carrier-mediated transport of lactate (Olendorf, 1973; Cremer *et al.*, 1976, 1979) remains to be elucidated.

The presence of 5.3 mM-lactate in the incubation medium inhibited glucose oxidation (Fig. 2*a*) by about 70%, although inhibition was already observable (37%) at about 3 mM-lactate. In addition, lipogenesis from glucose was strongly inhibited (54%) by 5.3 mM-lactate and almost completely abolished in the presence of 12 mM-lactate (Fig. 2*a*). These results are in agreement with the suggestion that, immediately after birth, the brain utilizes lactate as its major metabolic substrate, channelling glucose to neonatal tissues that utilize it as the sole metabolic substrate. No changes in the rate of glucose oxidation were observed in the presence of 3-hydroxybutyrate up to 2 mM (Fig. 2*b*). However, the presence of 3-hydroxybutyrate progressively inhibited lipogenesis from glucose, reaching maximum inhibition (48%) at 1.5 mM-3-hydroxybutyrate. Since glucose oxidation is not affected by the presence of 3-hydroxybutyrate, it is unlikely that the inhibition of lipogenesis from glucose under the same circumstances is achieved either by the inhibition of pyruvate dehydrogenase (Booth & Clark, 1981) or by competition for a putative common carrier (Cremer & Heath, 1974; Gjedde & Crone, 1975; Moore *et al.*, 1976; Conn *et al.*, 1983). However, it should be noted that ketone bodies can be utilized in neonatal brain through an exclusive pathway (Williamson & Buckley, 1973). Whether the inhibition of lipogenesis from glucose observed in our experiments may be due to the activity of this pathway remains to be elucidated.

The presence of lactate in the incubation medium up to 6–8 mM enhanced 3-hydroxybutyrate oxidation by 50% (Fig. 3*a*), whereas concentrations of lactate higher than 8 mM inhibited its oxidation, to a maximum inhibition of 55% at 12 mM-lactate. A similar profile was observed for lipogenesis from 3-hydroxybutyrate (Fig. 3*a*). It may be suggested that the enhancement of 3-hydroxybutyrate utilization caused by the presence of low concentrations of lactate in the incubation medium (Fig. 3*a*) may be due to the increase of oxaloacetate synthesis from lactate through the reactions catalysed by lactate dehydrogenase and pyruvate carboxylase. This increase would enhance citrate availability for both the tricarboxylic acid cycle and lipogenesis. In addition, putative inhibition of pyruvate dehydrogenase caused by 3-hydroxybutyrate oxidation (Booth & Clark, 1981) may favour diversion of pyruvate from lactate to oxaloacetate synthesis. The inhibition of lactate utilization observed in the presence of 3-hydroxybutyrate (Fig. 1*b*) could also be accounted for by such a mechanism. The presence of glucose in the incubation medium slightly inhibited 3-hydroxybutyrate oxidation (Fig. 3*b*), but no further inhibition was observed up to 6 mM-glucose. However, the presence of 3.2 mM-glucose in the incubation medium enhanced lipogenesis (about 35%) from 3-hydroxybutyrate, although no further effect was observed up to 5 mM-glucose (Fig. 3*b*). It is noteworthy that in the same circumstances 3-hydroxybutyrate inhibited lipogenesis from glucose (Fig. 2*b*), suggesting that in the presence of both substrates carbon atoms from 3-hydroxybutyrate are mostly diverted to lipid synthesis.

In conclusion, our results suggest that lactate is the major metabolic substrate for the neonatal brain in the absence of 3-hydroxybutyrate. This is the case in the pre-suckling period, during which ketone-body concentrations are negligible as a result of a lack of their main precursors, i.e. non-esterified fatty acids from the mother's milk. However, as from the onset of suckling, when ketone bodies become available, lactate utilization by the brain may cease and be replaced by ketone bodies as the main metabolic substrate. In such circumstances, lactate may be channelled to gluconeogenic tissues. The mechanism to reserve glucose for those tissues that use it as the sole metabolic substrate would be provided by the inhibition of glucose utilization by lactate during the pre-suckling period and by ketone bodies during the longer suckling period.

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