Lactate as an oxidizable substrate for rat brain *in vitro* during the perinatal period

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(Received 11 April 1983/Accepted 15 June 1983)

Foetal brain slices showed a high capacity for lactate oxidation *in vitro* during late gestation. This capacity remained high during the very early postnatal period, suggesting that lactate may play an important role as an energy substrate in the brain during the early neonatal period. The capacity for lactate oxidation decreased markedly during the first 2 days of extra-uterine life and thereafter remained low.

During the first hours after delivery, newborn rats rapidly remove the lactate accumulated in large amounts in foetal plasma during late gestation (Girard *et al.*, 1973; Cuezva *et al.*, 1980). Because it is an excellent gluconeogenic substrate, it has generally been assumed that lactate is metabolized through the gluconeogenic pathway (Snell & Walker, 1973). Thus the main liver gluconeogenic enzymes are induced after delivery (Ballard & Hanson, 1967; Vernon & Walker, 1968) and the gluconeogenic capacity of suckling rat liver is high *in vivo* (Vernon & Walker, 1972; Ferre *et al.*, 1977) and *in vitro* (Ballard & Oliver, 1963). However, plasma lactate is mostly depleted before the enhancement of the activities of the main gluconeogenic enzymes, i.e. phosphoenolpyruvate carboxykinase, fructose biphosphatase and glucose 6-phosphatase, takes place (Cuezva *et al.*, 1980). In addition, it has been reported (Medina *et al.*, 1980) that gluconeogenesis from lactate is insignificant during the first 2 h after delivery, even though plasma lactate is almost completely removed during this period (Cuezva *et al.*, 1980). Moreover, the radioactive lactate injected is principally converted into respired CO₂ in these circumstances, a fact consistent with the idea that plasma lactate is consumed as a consequence of its oxidation through the tricarboxylic acid cycle (Medina *et al.*, 1980). On the other hand, Cremer *et al.* (1976) have reported that lactate uptake by the brain is very high during the suckling period. In addition, Hellman *et al.* (1982) have more recently suggested that

neonatal dog brain may utilize lactate as an energy substrate.

The aim of the present work was to study the possible contribution of the brain to plasma lactate removal observed immediately after delivery. Consequently, lactate oxidation by rat brain slices from foetuses and early newborns has been studied, together with the dependence of lactate oxidation on substrate concentration and the oxygen concentration of the gas phase.

Experimental

Albino Wistar rats, fed on a stock laboratory diet and of known gestational age, were used for the experiments. Females were caged overnight with males and conception was considered to occur at 01:00 h. Pregnant rats of 19.5–21.5 days of gestation were killed by cervical dislocation without anaesthesia and the foetuses were delivered by rapid hysterectomy. This operation was carried out in a Perspex cabin at 37°C. The foetuses were weighed and those that presented low weight for the average of the litter or a discrepancy with the corresponding gestational age were discarded. Newborns delivered by caesarian section were maintained for 2 h in an incubator at 37°C and in a continuous stream of water-saturated air, without feeding. Naturally delivered newborns were maintained with their mothers up to the time of the experiments. Foetuses and newborns were killed by decapitation and the brains used for the experiments.

The forebrain was sliced in a water-saturated cabin, taking one slice from each hemisphere. The weight of the slices was standardized to 70–80 mg

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wet wt. of tissue. When the weight of the whole hemisphere was less than 80 mg, the hemisphere was cut partially and opened to increase the surface of contact with the incubation medium. The rate of lactate oxidation was linear, with the wet weight of the slice in the range 40–100 mg in all experimental conditions tested ($r = +0.93$; $P < 0.001$ for foetal brains of 21.5 days). After rapid weighing, slices were incubated at 37°C in 25 ml Erlenmeyer flasks with a central well containing 0.1 ml of 20% (w/v) KOH. The incubation medium was 2 ml of phosphate physiological saline (Elliot, 1960) containing $0.5 \mu\text{Ci}$ of L-[U- ^{14}C]lactate (The Radiochemical Centre, Amersham, Bucks., U.K.) and L-lactate at the desired concentration. The gas phase, unless stated otherwise, was pure oxygen. The incubations were stopped after 1 h by injection into the main well of 0.2 ml of 20% (v/v) HClO_4 , although shaking was continued for a further 30 min. The rate of lactate oxidation was linear with the time of incubation up to 2 h ($r = +1.00$; $P < 0.001$ for foetal brain of 21.5 days). The radioactive CO_2 trapped by KOH was measured by liquid-scintillation counting. L-Lactate was measured by the method of Hohorst (1965). The initial specific radioactivity of lactate was used for calculations.

Results and discussion

The rate of lactate oxidation depended on the oxygen concentration of the gas phase respired by

Fig. 1. Dependence of lactate oxidation on substrate concentration and on the gas phase respired (insert) by slices from foetal rat brain

Foetal (21.5 days post coitus) brain slices were incubated at 37°C in phosphate-physiological saline containing $0.5 \mu\text{Ci}$ of L-[U- ^{14}C]lactate and radioactive CO_2 was trapped in KOH. Unless stated otherwise, the concentration of L-lactate was 5 mM (insert) and pure oxygen was the gas phase. For experimental details see the text. Results are means $\pm$ S.E.M. (indicated by the bars) ($n = 20$ –30).

the slice (Fig. 1, insert). Lactate oxidation was maximal in the presence of pure oxygen, about 30% lower in the presence of air and very low in the presence of N_2 . It should be mentioned that the incubation medium was not oxygen-free, a fact that may account for the detectable oxidation of lactate observed when N_2 was used as the gas phase.

The rate of lactate oxidation approached saturation at concentrations of lactate comparable with those observed *in vivo* at birth (Fig. 1; Girard *et al.*, 1973; Cuezva *et al.*, 1980), suggesting that the lactate oxidation observed in our experiments may have physiological relevance.

Foetal brain showed a substantial capacity for lactate oxidation during late gestation (Fig. 2). These results might suggest that foetal brain utilizes plasma lactate produced by other foetal tissues. However, foetal blood oxygen concentrations were very low (Cuezva *et al.*, 1981), a fact that may decrease the rate of lactate oxidation in such circumstances (Fig. 1, insert).

However, the rate of lactate oxidation observed in foetal brain at delivery remained high during the first

Fig. 2. Effect of perinatal age on the capacity for lactate oxidation by rat brain *in vitro*

Brain slices were incubated at 37°C in phosphate-physiological saline containing $0.5 \mu\text{Ci}$ of L-[U- ^{14}C]lactate and 12 mM-L-lactate with pure oxygen as the gas phase. Caesarian-delivered newborns (2h) were maintained at 37°C in a continuous stream of water-saturated air without feeding. Naturally delivered newborns were maintained with their mothers up to the time of the experiments. For experimental details see the text. Results are means $\pm$ S.E.M. (indicated by the bars) ($n = 15$ –20).

two postnatal hours (Fig. 2). In these circumstances, the observed increase in blood oxygen concentrations (Cuezva *et al.*, 1981) may result in the enhancement of lactate oxidation by the neonatal brain *in vivo*. This fact is consistent with the idea that lactate oxidation may play an important role in the energy homeostasis of neonatal brain during the presuckling period. Actually, at 2–3 h after delivery the onset of suckling took place and ketone bodies supplied the bulk of energy and carbon requirement of the brain (Krebs *et al.*, 1964; Williamson & Buckley, 1973). It should be mentioned that the maximal rate of lactate oxidation observed in our experiments (Fig. 1) is quantitatively similar to that reported for ketone-body utilization by the brain during the suckling period (Itoh & Quastel, 1970; Patel & Clark, 1980). Consequently, it may be that lactate plays an important role as an alternative substrate to glucose and ketone bodies during the early neonatal period. In fact, immediately after delivery, blood glucose concentration is very low and plasma ketone bodies are virtually undetectable (Cuezva *et al.*, 1980).

The rate of lactate oxidation decreased sharply during the first 2 days of extra-uterine life, remaining low thereafter (Fig. 2). This result is consistent with the idea that lactate is an alternative substrate to glucose and/or ketone bodies, since the capacity of lactate oxidation decreased concurrently with the increase of the availability of glucose and ketone bodies (Krebs *et al.*, 1964). However, our results are insufficient to conclude that the decrease in the rate of lactate oxidation observed after delivery (Fig. 2) results in a parallel decrease in the lactate uptake by the brain. Conversely, the capacity of lactate transport to cross the blood/brain barrier remained high throughout the suckling period (Cremer *et al.*, 1976, 1979), a fact that suggests that lactate is actively used during this period. If so, the decrease in the rate of lactate oxidation observed after delivery may result in the utilization of lactate within the brain as a source of carbon skeletons rather than as an energy-yielding substrate.

In conclusion, our results suggest that lactate may be an alternative substrate for the brain during the early neonatal period. During the short presuckling period, lactate would replace glucose, which is not available owing to the interruption of placental supply, and before ketone bodies take up the role as the major brain fuels during the suckling period. If

so, the inhibition of lactate oxidation occurring when ketone bodies are available may reserve lactate as a source of carbon skeletons in the brain.

We are indebted to Professor F. Mayor for his encouragement. This work was partly supported by a grant from 'Función Ramón Areces', Spain.

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