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Some characteristics of mitochondrial fatty acid oxidation in the liver of the neonatal pig : preliminary results

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Despite an increased concentration of plasma non-esterified fatty acid, sucking piglets do not show an increased level of circulating ketone bodies (Pégorier *et al.*, 1981). This low ketonemia is not due to a high rate of ketone body utilization but to a low rate of hepatic ketone body production (Pégorier *et al.*, 1983). The low rate of ketone body production also results from a limited capacity for fatty acid oxidation associated with a huge capacity for fatty acid esterification in the liver of newborn pigs, whatever their nutritional status (Pégorier *et al.*, 1983).

The purpose of the present study was to determine the capacity of isolated mitochondria to oxidize different fatty acid esters.

Pigs of the Large White strain farrowed in the « Institut National de la Recherche Agronomique » (Jouy-en-Josas, France) were used. Starved newborn pigs were separated from the mother immediately after birth and maintained at 34 °C for 48 h. After anesthesia (15 mg of sodium thiopental/kg body wgt., Specia, Paris, France), the mitochondria were isolated according to Mersmann *et al.* (1972). The mitochondrial pellet was suspended in sucrose-Tris buffer to give a mitochondrial protein concentration in the range of 50-100 mg/ml.

To assess the integrity of the mitochondria, the respiratory control ratio and the ADP : O ratio from succinate were measured systematically. Oxygen utilization was measured by an oxygen electrode in a 2 ml water-jacketed chamber maintained at 30 °C (Oxygraph Gilson, model 5/6 H). After succinate (10 mM) was added to the respiratory medium containing 1 mg of mitochondrial protein, the rates of oxygen utilization were determined in the absence (state 4) or the presence (state 3) of ADP. The respiratory control ratio (state 3/state 4) was high (5.8 ± 0.2), indicating that the mitochondrial membranes were well preserved. This was confirmed by the ADP : O ratio (1.72 ± 0.10) which was close to the theoretical value. Liver mitochondria were incubated at 30 °C in a modified Krebs-Henseleit buffer containing ATP (4 mM), ADP (1 mM), CoASH (50 µM), reduced gluthatione (250 µM) and L-carnitine (200 µM). The rate of ketone body production was determined. Whatever the substrate used to measure ketone body production by liver mitochondria (*i.e.* oleate 0.1 mM, octanoate 0.2 mM, palmitoylcarnitine 0.1 mM, previously bound to fatty acid-free dialysed albumin), the rate of ketone body synthesis (1.2 ± 0.5 to 2.5 ± 0.6 nmol.ketone bodies.min^{-1}.mg protein^{-1}) was 5 to 10-fold lower than the corresponding value measured in the same conditions in isolated mitochondria from 48-hour old starved rabbits (10.6 ± 0.9 to 15.5 ± 0.8 nmol. ketone bodies.min^{-1}.mg protein^{-1}) which exhibited high rates of ketogenesis (Duée *et al.*, 1985).

Palmitoylcarnitine oxidation was also measured polarographically. Taking into account the rate of oxygen utilization due to the oxidation of palmitoylcarnitine (10 μM) and the theoretical value of Δ0/Δ-palmitoylcarnitine according to Shepherd, Yates and Garland (1965), the rate of palmitoylcarnitine utilization could be measured in conditions where the end-products were well-defined.

In the presence of malonate (10 mM), an inhibitor of succinate dehydrogenase, the end-product of β-oxidation was acetoacetate ; in this case, the rate of palmitoylcarnitine utilization was : 2.5 ± 0.7 nmol.min^{-1}.mg protein^{-1} (n = 5), *i.e.* 45 % of the corresponding value in 48-hour old starved rabbits. The addition of an uncoupler (2,4 dinitrophenol, 40 mM) did not modify the rate of palmitoylcarnitine utilization, suggesting that fatty acid oxidation was not limited by the capacity of the respiratory chain. In the presence of malate (2.5 mM), the end-product of β-oxidation was citrate ; in this case, the rate of palmitoylcarnitine utilization strongly increased (7.5 ± 1.0 nmol.min^{-1}.mg protein^{-1}, n = 5) and was similar to that in 48-hour old starved rabbits.

In conclusion, these preliminary observations indicate that the limited rate of fatty acid oxidation in the liver of neonatal pigs was not due to the carnitine-acyltransferase system, but seemed to depend on the metabolic fate of the acetyl-CoA produced.

Duée P. H., Pégorier J. P., Kohl C., Girard J., 1985. *Reprod. Nutr. Dévelop.*, **25**, 331-332.
Mersmann H. J., Goodman J., Houk J. M., Anderson S., 1972. *J. Cell Biol.*, **53**, 335-347.
Pégorier J. P., Duée P. H., Assan R., Peret J., Girard J., 1981. *J. Dev. Physiol.*, **3**, 203-217.
Pégorier J. P., Duée P. H., Girard J., Peret J., 1983. *Biochem. J.*, **212**, 93-97.
Shepherd D., Yates D. W., Garland P. B., 1965. *Biochem. J.*, **97**, 38c-40c.