Measurements of Substrate Uptake by Mammary Gland of the Rat

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Recent measurements of the enzymes involved in ketone-body metabolism in rat mammary gland (Page & Williamson, 1972) have shown that this tissue is a major potential site of ketone-body utilization during lactation. To assess the actual role of ketone bodies relative to other substrates in supplying the requirements of the lactating gland, measurements have been made of the arteriovenous differences for glucose, lactate, pyruvate, 3-hydroxybutyrate, acetoacetate, free fatty acids and triglycerides across mammary tissue of lactating, starved lactating and starved non-lactating (virgin) rats.

Experimental

Rats of the Wistar strain weighing 305–370 g were used. The period of lactation varied between 10 and 20 days and no attempt was made to keep the litter size (8–12 pups) constant. The starved lactating rats had their food removed for 16 h but were kept with their litters; virgin rats were starved for the same period. The rats were anaesthetized with Nembutal (50 mg/kg body wt.) and kept in their cages with litters (if lactating) for about 30 min before the operative procedure. A femoral arterial catheter was inserted as described by Hawkins *et al.* (1971). The catheter was kept patent with 0.9% NaCl; no heparin was given to these animals. After surgical exposure of one of the pudic–epigastric veins blood (0.5 ml) was removed with a 25-gauge needle. Shortly thereafter (1–2 min) arterial blood (0.5 ml) was collected. The process was repeated with the other pudic–epigastric vein. Thus two arterial and two venous samples were usually collected from each rat. The blood was added to small plastic tubes containing 5 μ l of heparin solution (5 units). A portion (0.2 ml) was removed and added to 3% (w/v) HClO₄, and then the mixture was centrifuged to remove protein and the supernatant fluid was neutralized with KOH. The remainder of the blood was centrifuged to obtain plasma for free fatty acid and triglyceride determinations. The following metabolites were determined by standard enzymic methods: glucose (Slein, 1963); L-lactate and pyruvate (Hohorst *et al.*, 1959); acetoacetate and D-3-hydroxybutyrate (Williamson

et al., 1962); plasma triglycerides (Eggstein & Kreutz, 1966). Plasma free fatty acids were determined colorimetrically (Itaya & Ui, 1965).

Results and discussion

The arterial glucose concentrations were similar in the lactating, starved lactating and starved virgin rats (Table 1), but there were considerable differences in the arteriovenous differences across the mammary gland in the three situations. The arteriovenous difference for glucose was about 50% less in the starved lactating rat than in the fed lactating rat, and there was no significant arteriovenous difference in the starved non-lactating rat (Table 1). The decreased uptake of glucose in the starved lactating rat was accompanied by a significant output of lactate and pyruvate (Table 1); thus the net utilization of carbon derived from glucose by the gland must be considerably less in this situation. Presumably part of this decreased uptake of glucose is due to the lower circulating concentrations of insulin in starvation.

The arterial concentration of ketone bodies (acetoacetate + 3-hydroxybutyrate) approximately doubled on starvation (16 h) of the lactating rat, but this increase was only about 50% of that observed in the starved non-lactating rat (Table 1). In agreement with the general concept that the uptake of ketone bodies is related to their concentrations in the circulation (see Williamson & Hems, 1970), there was an approximate doubling of the ketone-body uptake by mammary gland on starvation of the lactating rat (Table 1). However, despite the higher concentration in the circulation, the uptake of ketone bodies by the starved non-lactating rat was actually less than for the starved lactating rat. The differences in ketone-body uptake between the three situations become still greater if the uptake is calculated for the total mammary gland, by using the blood flow rates and weights of tissue reported by Chatwin *et al.* (1969). Thus the ketone-body uptakes expressed as μ mol/min per total mammary-gland tissue are 2.50, 5.95 and 0.19 for lactating, starved lactating and starved non-lactating rats respectively. The value for starved lactating rats may be too high because it is based on the assumption that the blood flow to the mammary gland does not decrease on starvation,

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Table 1. Concentrations of blood metabolites and arteriovenous differences across inguinal mammary gland of lactating and non-lactating rats

The results are mean values $\pm$ S.E.M. expressed as μ mol or μ equiv./ml of whole blood (or plasma for free fatty acids and triglycerides) with the numbers of paired blood samples shown in parentheses. Arterial blood was taken from the femoral artery and venous blood from the pudic-epigastric vein. Two arterial samples and two venous samples (left and right side) were usually taken from each rat. Arteriovenous differences that are significant are indicated by: * $P < 0.01$. The symbols + and - indicate production or uptake of the metabolite. For other experimental details see the text.

Condition of rats	Blood	Glucose	Lactate	Pyruvate	3-Hydroxybutyrate	Acetoacetate	Free fatty acids	Triglyceride
Fed lactating (18)	Arterial	4.59 $\pm$ 0.12	0.93 $\pm$ 0.13	0.10 $\pm$ 0.01	0.14 $\pm$ 0.02	0.20 $\pm$ 0.02	0.61 $\pm$ 0.11	0.91 $\pm$ 0.06
	Venous	3.42 $\pm$ 0.20	0.80 $\pm$ 0.08	0.08 $\pm$ 0.01	0.08 $\pm$ 0.02	0.10 $\pm$ 0.01	0.46 $\pm$ 0.08	0.50 $\pm$ 0.05
	Difference	-1.16 $\pm$ 0.16*	-0.13 $\pm$ 0.09	-0.02 $\pm$ 0.02	-0.06 $\pm$ 0.02*	-0.10 $\pm$ 0.02*	-0.23 $\pm$ 0.04*	-0.41 $\pm$ 0.04*
16h-starved lactating (14)	Arterial	4.34 $\pm$ 0.10	0.43 $\pm$ 0.05	0.07 $\pm$ 0.01	0.29 $\pm$ 0.03	0.41 $\pm$ 0.05	0.78 $\pm$ 0.06	0.60 $\pm$ 0.05
	Venous	3.74 $\pm$ 0.14	0.64 $\pm$ 0.07	0.12 $\pm$ 0.01	0.13 $\pm$ 0.02	0.17 $\pm$ 0.02	0.59 $\pm$ 0.04	0.40 $\pm$ 0.05
	Difference	-0.60 $\pm$ 0.12*	+0.21 $\pm$ 0.07*	+0.05 $\pm$ 0.01*	-0.17 $\pm$ 0.02*	-0.23 $\pm$ 0.03*	-0.19 $\pm$ 0.06*	-0.20 $\pm$ 0.01*
16h-starved non-lactating (7)	Arterial	4.67 $\pm$ 0.12	0.65 $\pm$ 0.11	0.06 $\pm$ 0.01	0.70 $\pm$ 0.11	0.61 $\pm$ 0.07		
	Venous	4.52 $\pm$ 0.15	0.95 $\pm$ 0.20	0.07 $\pm$ 0.01	0.58 $\pm$ 0.11	0.46 $\pm$ 0.06		
	Difference	-0.15 $\pm$ 0.14	+0.30 $\pm$ 0.12	+0.01 $\pm$ 0.00	-0.13 $\pm$ 0.02*	-0.15 $\pm$ 0.03*		

and this has been shown not to hold for the goat (Annison *et al.*, 1968). The high rate of ketone-body uptake by the mammary gland of the lactating rat fits well with the high activities of the enzymes of ketone-body utilization (Page & Williamson, 1972), and, on the assumption that production of ketone bodies by the liver is similar to that of a normal rat (1.7 μ mol/100g body wt.; Bates *et al.*, 1968), approx. 40% of the hepatic output is taken up by the gland in the fed state. This suggests that the lower concentrations of ketone bodies in the circulation in starved lactating rats compared with starved non-lactating rats may be due to the high capacity of lactating mammary tissue to remove ketone bodies. The high uptake of ketone bodies by mammary tissue from starved lactating rats may also play a role in decreasing glucose utilization, as in the case of heart (Williamson & Krebs, 1961; Randle *et al.*, 1964).

There was an appreciable uptake of triglycerides and free fatty acids by the lactating mammary gland of fed rats (Table 1). On starvation the uptake of triglycerides decreased by about 50%, whereas the free fatty acid uptake remained relatively unchanged (Table 1). This net uptake of free fatty acids by rat mammary gland is in contrast with the fed goat, where it is difficult to detect arteriovenous differences for free fatty acids, although isotope studies have shown that there is uptake and release of free fatty acids from goat mammary gland (Annison *et al.*, 1967). However, on starvation of the goat there is an appreciable arteriovenous difference for free fatty acids across the mammary gland (Annison *et al.*, 1968), and it would seem that free fatty acids may play a role similar to that postulated for ketone bodies in mammary gland of starved rats, namely replacing glucose carbon for lipid synthesis and energy supply.

The value of the technique of measuring arteriovenous differences has its limitations (see Barry, 1961), especially where the animals are stressed by anaesthesia and surgery (Linzell, 1960). Nevertheless the results give the order of magnitude of the substrate uptake by rat mammary gland, and it is of interest to compare the relative importance of the various substrates for the rat mammary tissue with similar studies carried out on pig (Spencer *et al.*, 1969) and goat (Linzell, 1968).

In the goat and rat the uptake of substrates other than glucose plays a major role in supplying the mammary gland, whereas in the sow the situation is reversed and glucose is the main substrate (Table 2). In general, the relative importance of a particular substrate is related to its availability in the arterial blood, and thus there are no significant arteriovenous differences for acetoacetate in the goat or sow, where the arterial concentrations of acetoacetate are very low. On the other hand 3-hydroxybutyrate makes a definite contribution to the substrate supply of goat mammary gland.

Table 2. Comparison of the arterial concentrations and relative uptakes of blood metabolites by the lactating mammary gland of rat, goat and sow

The arterial concentrations are expressed as $\mu\text{mol/ml}$ of whole blood (or plasma for triglycerides). The relative uptakes are expressed as percentages of the total uptake calculated on the basis of μmol of carbon. The values for pig (fed) have been calculated from the results of Spincer *et al.* (1969), for the goat (fed) from the results summarized by Linzell (1968) and for the rat from the results presented in Table 1. An average molecular weight for triglycerides of 800 has been chosen. The value 0 indicates that no significant arteriovenous difference was observed.

Metabolite	Arterial concentration ($\mu\text{mol/ml}$)			Relative uptake by mammary gland (%)		
	Rat	Goat	Sow	Rat	Goat	Sow
Glucose	4.59	2.52	6.80	24	29	70
Lactate	0.92	0.75	1.33	0	5	3.5
Acetate	—	1.48	0.22	—	10	1
3-Hydroxybutyrate	0.14	0.56	0.18	0.9	8	0.5
Acetoacetate	0.20	0.02	0.06	1.4	0	0
Triglycerides	0.91	0.31	0.43	73	48	25

The results of the measurements of arteriovenous differences reported here confirm the previous suggestion that the lactating mammary gland of the rat is a major site of ketone-body utilization (Page & Williamson, 1972), although their actual contribution to the total substrate uptake of the gland is a small one. Further experiments are required to decide whether alterations in the concentrations of ketone bodies, e.g. in starvation, may play a regulatory role in controlling glucose uptake by the mammary gland.

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