Lactating Mammary Gland of the Rat: a Potential Major Site of Ketone-Body Utilization

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The lactating mammary gland of the ruminant can metabolize large amounts of D(–)-3-hydroxybutyrate for the synthesis of the lipid constituents of milk or as a fuel of respiration (Shaw & Knodt, 1941; Kumar *et al.*, 1959; Barry *et al.*, 1963; Kronfeld *et al.*, 1968). However, until the demonstration by Kinsella (1970) that cultured rat mammary-gland cells can utilize 3-hydroxybutyrate for lipid synthesis, metabolism of ketone bodies by rat mammary gland had not been studied. The present paper gives the results of measurements of the activities of enzymes involved in ketone-body utilization in rat mammary gland at different stages of lactation. The results indicate that during lactation the mammary gland has the capacity to be a major site of ketone-body utilization in the rat.

Methods

Pregnant and nursing Wistar rats weighing 280–320 g were kept in individual cages and fed on a commercial diet (Oxoid breeding diet for rats and mice; Oxoid Ltd., London S.E.1, U.K.). The rats were killed by decapitation (without anaesthesia) and blood was collected into tubes containing heparin. A sample of blood (0.2 ml) was deproteinized as described previously (Williamson *et al.*, 1969) and used for the determination of ketone bodies (Williamson *et al.*, 1962). Homogenates of inguinal mammary gland were prepared in the medium of Lehninger *et al.* (1960) in all-glass homogenizers. The homogenate was then exposed to ultrasonic treatment, and appropriate fractions of the homogenate were used for the determination of the activities of the enzymes of ketone-body utilization (Williamson *et al.*, 1971), namely D(–)-3-hydroxybutyrate dehydrogenase (EC 1.1.1.30), 3-oxo acid CoA-transferase (EC 2.8.3.5) and acetoacetyl-CoA thiolase (EC 2.3.1.9) (reactions 1, 2 and 3):

All measurements of enzyme activity were carried out at 25°C. One unit of enzyme activity is defined as the amount of enzyme that transforms 1 μmol of substrate/min at 25°C and specific activity as units/g

fresh wt. of tissue. For full details of the experimental techniques see Williamson *et al.* (1971).

Results and discussion

The specific activities of the enzymes of ketone-body utilization in mammary gland from rats at different stages of lactation are given in Table 1. The specific activities of all three enzymes were low before parturition, but increased four- to eight-fold after suckling commenced (1st–5th. day) and reached peak activities between the 11th. and the 20th. day of lactation (Table 1). Within 5 days of weaning the specific activities in the mammary gland had returned to the pre-partum values. If the total activities of the individual enzymes of ketone-body utilization in lactating mammary gland are compared with those of other tissues (Table 2) it is clear that the lactating gland is a potential major site of ketone-body utilization. Thus the total activity of 3-oxo acid CoA-transferase in the lactating gland is approximately equal to the combined activities of the other major tissues. This point is further emphasized by the fact that the lactating gland has a high total tissue blood flow (Table 2) and receives about 10% of the cardiac output (Chatwin *et al.*, 1969).

The concentration of ketone bodies (acetoacetate + 3-hydroxybutyrate) in the blood of fed lactating rats was low (0.26 mM; mean of five observations) and was similar to that of virgin rats (0.23 mM). Thus it would appear that ketone bodies do not play an important role in the metabolism of mammary gland in the fed state. This raises the question as to the reason for the high potential capacity of the lactating mammary gland to utilize ketone bodies. A possible explanation for the presence of the enzymes is that they may represent a safety mechanism in case the availability of carbohydrate is restricted. During starvation the concentration of circulating ketone bodies rises (to 2–3 mM), and as their utilization is concentration-dependent (see Williamson & Hems, 1970) ketone bodies could become an important substrate for the mammary gland. If, in addition, ketone bodies restricted the utilization of the available glucose by mammary gland, as they do in rat heart (Williamson & Krebs, 1961; Randle *et al.*, 1964), they might then replace part of the carbohydrate in supplying the carbon required for milk-fat synthesis. Some evidence

Table 1. *Specific activities of enzymes of ketone-body utilization in rat mammary gland*

The values are expressed in units/g fresh wt. of tissue (not corrected for lactose content) and are means with two to six observations in each group. For other experimental details see the text.

Condition of rat	Stage (days)	Specific activity (units/g)		
		3-Hydroxybutyrate dehydrogenase	3-Oxo acid CoA-transferase	Acetoacetyl-CoA thiolase
Pregnant	17-18	0.06	0.60	0.51
Lactating	1-5	0.49	3.05	1.91
Lactating	6-10	0.73	4.13	2.59
Lactating	11-15	0.77	5.71	2.60
Lactating	16-20	0.74	5.73	2.95
Lactating	21-25	0.68	2.44	1.75
Involuting	5	0.02	0.67	0.13

Table 2. *Total activities of enzymes of ketone-body utilization in tissues of lactating rats*

The total activities of the enzymes in each tissue were calculated by multiplication of the mean specific activities (units/g fresh wt. of tissue) by the weight of tissue. The activities of the enzymes in individual tissues were obtained from Williamson *et al.* (1971) and Table 1 of the present paper. The mean weights of tissues of lactating rats (315g body wt.) and the tissue blood flows were obtained from Chatwin *et al.* (1969); total muscle weight was estimated to be 49% of body wt.

Tissue	Total activity (units/tissue)			Tissue blood flow (ml/min)
	3-Hydroxybutyrate dehydrogenase	3-Oxo acid CoA-transferase	Acetoacetyl-CoA thiolase	
Brain	0.97	3.6	3.6	1.1
Heart	0.83	23	8.3	2.5
Kidney	3.22	56	32	4.8
Muscle	—	72	94	38
Mammary gland	18.6	139	63	14

that milk production continues during short-term starvation is provided by the fact that suckling rats do not lose weight if the mother is starved for 24h and her weight loss is approximately twice that of a non-lactating rat.

Thus, like the reciprocal post-partum changes of the specific activities of lipoprotein lipase in adipose tissue and mammary gland of the rat (Hamosh *et al.*, 1970), which have the effect of increasing the availability of triglycerides to mammary tissue, the high specific activities of the enzymes of ketone-body utilization in the lactating gland can be considered a mechanism for directing alternative substrates for lipid synthesis and energy production to this tissue when required.

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