

Fructose 2,6-bisphosphate, sugar phosphates and adenine nucleotides in the regulation of glucose metabolism in the lactating rat mammary gland

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Fructose 2,6-bisphosphate is present in the rat mammary gland, rising from a value of 1.4 nmol/g in pregnancy to 4.3 nmol/g tissue at 14 days lactation; the equivalent values calculated/ml intracellular water are 5.2 and 11.6 nmol, respectively. The tissue content of fructose 6-phosphate, fructose 1,6-bisphosphate, ATP and phosphoenolpyruvate remain relatively constant in the transition from pregnancy to the height of lactation. The changes in AMP, cyclic AMP, and citrate content of the mammary gland during lactation are such as to promote an increase in fructose 2,6-bisphosphate formation and flux through phosphofructokinase.

<i>Fructose 2,6-bisphosphate</i>	<i>Phosphofructokinase</i> <i>Sugar phosphate</i>	<i>Rat mammary gland</i> <i>Citrate</i>	<i>Lactation</i>
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1. INTRODUCTION

During the lactation cycle the mammary gland changes from a metabolism geared to a growth phase during pregnancy to one of intense biosynthetic activity and secretion during lactation. In the rat the milk yield increases almost linearly during lactation by some 6-fold from the 1st to the 14th day of lactation [1] and the glucose utilization at this peak of lactation may rise to as much as 30 mmol/24 h [2]. The increased demand for energy and for precursors for lipid synthesis, both acetyl-CoA and glycerol 3-phosphate, during lactation requires an increased flux through PFK1 (EC 2.7.1.11).

Recent studies have emphasized the importance of Fru-2,6-P₂ in the regulation of PFK1 (see [3,4]). It was, therefore, of interest to determine the profile of change of Fru-2,6-P₂ in the rat mammary

gland in pregnancy and lactation and to compare these changes with those of other known effectors of PFK1 and PFK2.

2. MATERIALS AND METHODS

2.1. *Animals*

Primiparous rats of the Wistar strain were taken on the 21st day of pregnancy or the 1st or 14th day of lactation; the litter size was restricted to 8 pups in the lactating groups. The rats were anaesthetized with sodium pentobarbital (60 mg/kg body wt, administered i.p.) and the abdominal mammary glands quickly removed and freeze clamped, using liquid nitrogen, for measurement of metabolites, including Fru-2,6-P₂.

2.2. *Materials*

Fru-2,6-P₂ was a gift from Dr E. van Schaftingen (University of Louvain, Belgium). Enzymes, substrates and cofactors, used in the measurement of metabolites and assay of PFK1, were obtained from Boehringer Corp. London Ltd

Abbreviations: Fru-1,6-P₂, fructose 1,6-bisphosphate; Fru-2,6-P₂, fructose 2,6-bisphosphate; PFK1, 6-phosphofructo-1-kinase; PFK2, 6-phosphofructo-2-kinase

(Lewes, England). Potato tuber PP_i:fructose-6-phosphate 1-phosphotransferase, used in the assay of Fru-2,6-P₂, was prepared as in [5].

2.3. Measurement of metabolites

Mammary gland Fru-2,6-P₂ was assayed by its stimulatory action on potato tuber PP_i:fructose-6-phosphate 1-phosphotransferase as in [5], using neutralised alkaline extracts of mammary gland. Sugar phosphates, citrate, adenine nucleotides and cyclic AMP were measured in neutralised perchloric acid extracts of mammary gland as in [6–8].

2.4. Assay of phosphofructokinase (PFK1)

Mammary gland homogenates were prepared in a medium containing 0.25 M sucrose, 20 mM triethanolamine buffer (pH 7.4) and 0.1 mM dithiothreitol and the activity of PFK1 was determined spectrophotometrically as in [9] using the dialysed high-speed supernatant fraction prepared by centrifugation at 105 000 × g for 40 min. The present reported activity of PFK1 is closely similar to that reported in [10] for lactating rat mammary

gland. One unit of activity is defined as the conversion of 1 μmol substrate/min at 25°C.

2.5. Expression of results

The values for metabolites are given as nmol/g tissue and as nmol/ml intracellular water. The latter mode is used in order to facilitate comparison of mammary gland content of Fru-2,6-P₂ and sugar phosphates at different stages of lactation and with reported values for other tissues e.g. liver. The method of converting metabolite content/g mammary gland to metabolite concentration/ml intracellular water has previously been described [1,7,11].

3. RESULTS

3.1. Rat mammary gland content of fructose 2,6-bisphosphate and phosphofructokinase during lactation

Fru-2,6-P₂ appears to be present in significant amounts in the rat mammary gland in pregnancy and lactation (table 1). Based on the level of Fru-2,6-P₂/g tissue, uncorrected for the milk or fat

Table 1
Changes in fructose 2,6-bisphosphate in rat mammary gland during lactation and the relationship to phosphofructokinase (PFK1) activity

	Pregnant (20 days)	Lactating	
		1 day	14 days
Mammary gland weight ^a (g)	3.71 ± 0.23	3.56 ± 0.32	4.32 ± 0.35
Fru-2,6-P ₂ content			
nmol/g gland	1.4 ± 0.1	2.4 ± 0.2	4.1 ± 0.4
nmol/ml intracellular H ₂ O ^b	5.2 ± 0.3	6.2 ± 0.6	11.6 ± 1.1
PFK1 activity			
units/g gland	1.31 ± 0.10	1.79 ± 0.15	4.89 ± 0.21
Relationship of PFK1 to Fru-2,6-P ₂ ^c			
PFK1/Fru-2,6-P ₂	0.96 ± 0.09	0.75 ± 0.09	1.18 ± 0.10

^a Weight of abdominal mammary gland from one side only

^b Calculated as in [1,7,11], correction being made for the milk and fat content of the gland

^c Quotient calculated from PFK1 (activity/g) and Fru-2,6-P₂ content/g mammary gland

Values given are the means ± SE of not less than 5 values

content of the gland, there is a 3-fold increase from late pregnancy to the height of lactation at 14 days. If the results are expressed/ml intracellular water then the tissue concentration is appreciably higher and falls within the range reported for liver [3,12]. Also shown in table 1 is the activity of PFK1 measured at these same stages of the lactation cycle and a striking positive correlation is observed between the activity of PFK1 and the measured amount of Fru-2,6-P₂ in the mammary gland. The quotient PFK/Fru-2,6-P₂ remains constant at the 3 stages of the lactation cycle studied here (table 1).

3.2. Changes in the metabolite profile of mammary gland during lactation

A number of effectors, both positive and negative, have been shown to act on each of PFK1, PFK2, fructose-1,6-bisphosphatase and fructose-2,6-bisphosphatase. The tissue content of some of these, at different stages of the lactation cycle, is shown in table 2.

There is a significant rise, 2-fold, in the Fru-2,6-P₂ content of mammary gland from late

pregnancy to the peak of lactation. Further, the concentration of Fru-2,6-P₂ present in the mammary gland appears to fall within the critical range required for the activation of PFK1 [3,12–14]. The tissue content of AMP also increases 2-fold over the same time span (table 2). The combined effect of these two changes may be highly significant in the control of glycolytic flux in the mammary gland since, as discussed in [4], the effect of AMP, which acts on PFK1, PFK2 and fructose-1,6-bisphosphatase, is remarkably synergistic with that of Fru-2,6-P₂.

Citrate is an inhibitor of both PFK1 and PFK2. The concentration of citrate in the mammary gland falls significantly during lactation. At 14 days lactation it is about half the value found in the mammary gland from pregnant rats (table 2) and approximates to the K_i for inhibition of PFK1. The PFK1 of mammary gland is relatively sensitive to citrate inhibition and a K_i value of 0.2 mM has been reported for citrate inhibition of this enzyme [10]. It has been shown that the mechanism of citrate inhibition of rat mammary gland PFK1 in-

Table 2

Changes in metabolites involved in the regulation of PFK1 and PFK2 in rat mammary gland from pregnancy to lactation

	Pregnant (20 days)			Lactating				14 days lactation/ 20 days pregnancy		Effect of change on	
				1 day		14 days		(%)	(P)	PFK1	PFK2
Glucose 6-phosphate	193	±	27	220	±	39	190	±	24	98	NS
Fructose 6-phosphate	73	±	9	68	±	9	62	±	8	85	NS
Fru-1,6-P ₂	42	±	4	44	±	4	44	±	5	104	NS
Fru-2,6-P ₂	5.2	±	0.3	6.2	±	0.6	11.6	±	1.1	223	<0.001
Glycerol 3-phosphate	263	±	33	578	±	62	787	±	90	300	<0.001
Phosphoenolpyruvate	13	±	1.1	11	±	1.3	12	±	2.0	92	NS
Citrate	800	±	79	538	±	55	400	±	36	50	<0.001
ATP	1450	±	360	1930	±	380	1470	±	291	101	NS
AMP	540	±	41	507	±	48	1180	±	212	219	<0.01
Cyclic AMP	3.05	±	0.05	1.44	±	0.05	0.60	±	0.04	20	<0.001

The values (expressed as nmol/ml intracellular water) given are the means ± SE of 8 values with the exception of those of Fru-2,6-P₂ which each contain 5 values. The calculation of metabolite content/ml intracellular water is given in [7]. The percentage change in metabolites during lactation is given in column 4, expressed as metabolite content at 14 days lactation/content of 20 days pregnant × 100. Fisher's *P* values are given for the change from 20 days pregnancy to 14 days lactation; NS, not significant. The expected effect of the change in metabolite profile with lactation on the activities of PFK1 and PFK2 is shown in the last column. Values for cyclic AMP are calculated from the data in [8], and for some phosphorylated sugars from [7]

volves potentiation of ATP inhibition and a rise in the K_m for fructose 6-phosphate [10]. Since citrate is also an inhibitor of PFK2 [3,4] it is apparent that the sharp fall in citrate concentration in the lactating gland could be a major effector in increasing the glycolytic flux.

Glycerol 3-phosphate has been reported to activate the phosphatase cleaving Fru-2,6-P₂ [3,4]. The 3-fold increase in the content of this intermediate during lactation could be a significant factor in the down-regulation of Fru-2,6-P₂ in the lactating mammary gland.

An important factor in the regulation of liver Fru-2,6-P₂ is the phosphorylation state of PFK2 and fructose-2,6-bisphosphatase [3,4], these enzymes being regulated by cyclic AMP-dependent protein kinases. If a similar mechanism operates in the mammary gland then the activity of adenylate cyclase and the tissue content of cyclic AMP would be important in the regulation of Fru-2,6-P₂ and glycolytic flux. Authors in [8] have shown a 3-fold decrease in cyclic AMP content/g mammary tissue from late pregnancy to the height of lactation. Calculated per ml of intracellular water this is equivalent to a 5-fold decrease in metabolite content (see table 2). There is also a 10-fold decrease in mammary gland adenylate cyclase activity from 20 days pregnancy to 16 days lactation [8].

Other sugar phosphates, fructose 6-phosphate, Fru-1,6-P₂, phosphoenolpyruvate, and ATP, which are involved in the regulation of PFK1 and/or PFK2 remain relatively unchanged in the rat mammary gland in the transition from late pregnancy to the height of lactation and therefore, presumably, play little direct role in regulating the glycolytic flux.

4. DISCUSSION

The present metabolite data suggest a possible mechanism for the coordination of biosynthetic routes in the lactation cycle in the rat mammary gland. Milk secretion involves the synthesis of protein, fat and lactose, all processes requiring increased generation of ATP and, in the case of lipid synthesis, of acetyl-CoA and glycerol 3-phosphate, requirements necessitating an increased flux through the glycolytic route and PFK1.

A number of the changes associated with increased biosynthetic pathways provide signals for

increased flux through PFK1. Thus, one product of the activation of amino acids for protein synthesis is AMP, which increases sharply during lactation and provides a positive effector of both PFK1 and PFK2, the activity of PFK1 being especially enhanced because of the synergistic effect of the AMP and the Fru-2,6-P₂ produced by PFK2 [4]. Similarly, the increased utilization of citrate for fatty acid synthesis and its secretion into milk (which has a high content of this metabolite) lower the intracellular level of citrate and this, again, provides a mechanism for increased activity of PFK1 and PFK2, this time by the removal of a potent inhibitor [3,4,10].

The fall in the tissue content of cyclic AMP [8] will have widespread effects via its action on cyclic AMP-dependent protein kinases (see [15]). In particular, the low-phosphate form of PFK1 in liver has been shown [14] to be much less sensitive to inhibition by ATP and citrate, shows a lower K_m for fructose 6-phosphate and is more sensitive to activation by AMP and Fru-2,6-P₂ than the high-phosphate form, all properties which, if applicable to the mammary gland enzyme, would lead to a greatly increased flux through PFK1 in lactation. In addition, the opposing effects of cyclic AMP on PFK2 and on fructose-2,6-bisphosphatase [3,4] would serve to increase the tissue level of Fru-2,6-P₂ and hence amplify the stimulation of PFK1.

Further, the low-phosphate form of acetyl-CoA carboxylase, i.e., the activated form [16], may be linked to the fall in the tissue content of citrate in lactation as may, also, the low-phosphate form of ATP-citrate lyase [17] although the physiological significance of the dephosphorylation of this enzyme is not yet clear.

All these changes provide a coordinated pattern of modulation of PFK1 which would have the effect of greatly stimulating the glycolytic flux, via activation of the rate-limiting enzyme of the pathway, and of providing energy and substrates for the synthesis of milk components, so allowing the gland to respond to the increasing requirements of the growing pups.

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