EFFECT OF PROLACTIN ON PATHWAYS OF GLUCOSE OXIDATION IN EXPLANTS FROM RABBIT MAMMARY GLAND

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1. Introduction

Studies of pathways of glucose metabolism in mammary gland slices from pregnant and lactating rats and rabbits have shown that with the onset of lactation there is a marked increase in the utilization of glucose via the pentose phosphate pathway [1, 2]. The activities of two of the enzymes of this pathway, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, are higher during lactation than during pregnancy in several species [1, 3–6]. *In vivo* studies have indicated an involvement of prolactin in this response [7, 8].

The *in vitro* effect of prolactin on pathways of carbohydrate metabolism in mammary gland has been investigated by several workers, using tissue slices [9–11], but the results of these studies are conflicting. McLean [9] found that prolactin stimulated the oxidation of glucose carbon atoms 1 and 6 to an equal extent in mammary gland slices from pregnant rats, but had no effect on slices from lactating animals. In contrast, Weinberg et al. [10] reported that prolactin increased the rate of oxidation of 1-^{14}C–glucose only, the effect being greater on mammary gland slices from lactating than from pregnant animals. Jarret and Field [11], however, were unable to detect any *in vitro* effect of prolactin on $^{14}CO_2$ yields from 1-^{14}C-glucose.

In the present study, mammary gland explants from pseudopregnant rabbits were maintained in organ culture, and the presence of prolactin in the culture medium has been shown to result in a significant increase in the ratio:

$$\frac{\text{yield of } ^{14}CO_2 \text{ from 1-}^{14}\text{C-glucose}}{\text{yield of } ^{14}CO_2 \text{ from 6-}^{14}\text{C-glucose}} \quad \left(\frac{C1}{C6} \text{ ratio}\right)$$

This suggests a preferential stimulation by prolactin of glucose oxidation via the pentose phosphate pathway.

2. Methods

Explants of approx. 1 mm^3 and weighing about 0.5 mg each were taken from the mammary glands of virgin Dutch Rabbits, about 6 months old, on the 11th day of a pseudopregnancy induced by intravenous injection of 50 I.U. of Chorulon (Organon Laboratories, Morden, Surrey). The explants in groups of 32 were cultured on stainless steel rafts in plastic, disposable Petri dishes (Sterilin, 5 cm) containing 5 ml medium 199 (Glaxo Laboratories, Greenford, Middlesex) with 0.5 mg glucose per ml. The medium was buffered with bicarbonate (1.3 mg/ml) and pencillin was added to give a concentration of 50 I.U./ml. One group of explants was cultured with insulin (5 μg/ml, donated by Dr. O.K.Behrens, Eli Lilly) and corticosterone (1 μg/ml, provided by Merck, Sharp and Dohme). A second group of explants was cultured with insulin, corticosterone and prolactin (1 μg/ml, NIH-PS-6 ovine). The explants were maintained at 37° in an atmosphere of 95% oxygen, 5% carbon dioxide. After 3 days the culture medium was removed and the explants were washed with Krebs-Henseleit-Ringer solution. Histological examination of serial sections of 2 explants from each group was carried out after culture.

The remaining explants were transferred in batches of 5 to the outer compartments of 25 ml conical flasks fitted with centre wells. Each flask contained 2 μmoles of glucose (0.5 μCi) labelled with ^{14}C in the carbon 1 or carbon 6 position in 2 ml Krebs-Hanseleit-Ringer solution. The explants were incubated with insulin and corticosterone, or with insulin, corticosterone and prolactin, as indicated in table 1. The flasks were gassed with a 95% oxygen, 5% carbon dioxide mixture and stoppered with self-sealing rubber caps. Incubation was carried out at 37° with shaking at 80 strokes/min. After 3 hr, 0.2 ml of KOH was injected into each centre well and 0.35 ml of 6 N H_2SO_4 was injected into the outer compartment of each flask to terminate the reaction and drive off CO_2 from the medium. The flasks were incubated for a further 1 hr to collect the CO_2. The KOH was transferred to scintillation vials and the $^{14}CO_2$ produced was determined in a liquid scintillation counter.

3. Results and discussion

The results are summarised in table 1. The presence of prolactin during the 3-day culture period caused a significant increase in the formation of labelled CO_2 from both 1-^{14}C-glucose and 6-^{14}C-glucose (table 2a). However, 1-^{14}C-glucose oxidation was stimulated to a greater extent than 6-^{14}C-glucose oxidation, resulting in an increase in the C1/C6 ratio from 2 to 5. Prolactin had no effect on the oxidation of labelled glucose when present during the three hour incubation period (table 2b). Results of four further experiments, in which only two or three of the four possible treatments indicated in table 1 were used, confirmed these findings. These results suggest that prolactin when present in the culture medium preferentially stimulates glucose oxidation via the pentose phosphate pathway.

Histological examination of the explants after culture showed good survival with both hormonal treatments. After culturing in the presence of prolactin, large amounts of secretion appeared in the mammary alveoli.

Jones and Forsyth [12], Rivera [13] and Leader and Barry [14] found increases in the levels of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase when mammary gland explants from mid-pregnant mice were cultured in the presence of prolactin. Thus the increase in pentose phosphate pathway activity observed in the present study could be due to a direct effect of prolactin on the activities of the enzymes of this pathway. Preliminary studies suggest that prolactin may also act by stimulating pathways of reoxidation of NADP; McLean [15] has shown that the pentose phosphate pathway in the mammary gland is limited by the availability of the oxidised form of this coenzyme.

McLean [9] and Weinberg et al. [10] have reported that prolactin stimulates the rate of oxidation of glucose by mammary gland slices during incubation in Ringer solution for up to 1½ hr. These workers used concentrations of prolactin between 5 μg/ml and

Table 1
Oxidation of 1-^{14}C-glucose and 6-^{14}C-glucose by rabbit mammary gland explants under different conditions of culture and incubation (means of 4 experiments).

Hormones* present in		% Yield of $^{14}CO_2$ from		$\frac{C1}{C6}$
Culture medium (0–72 hr)	Incubation medium (72–75 hr)	1-^{14}C-glucose	6-^{14}C-glucose	
I + B	I + B	0.253	0.153	1.778
I + B	I + B + PL	0.283	0.150	1.915
I + B + PL	I + B	1.165	0.219	5.052
I + B + PL	I + B + PL	1.115	0.230	4.968

* Concentrations of hormones: Insulin (I) 5 μg/ml; cortisterone (B) 1 μg/ml; prolactin (PL) 1 μg/ml.

Table 2
Comparison of the effect of prolactin in the culture medium and in the incubation medium on subsequent metabolism of explants (statistical analysis of results presented in table 1).

Hormones present*	Mean % yield of $^{14}CO_2$ from		$\frac{C1}{C6}$
	1-^{14}C-glucose	6-^{14}C-glucose	
a) During culture (0–72 hr)			
I + B	0.268	0.152	1.846
I + B + PL	1.140	0.224	5.010
Difference ± S.E.M.	0.872 ± 0.185, $p < 0.01$	0.072 ± 0.028, $p < 0.05$	3.164 ± 0393, $p < 0.001$
b) During incubation (72–75 hr)			
I + B	0.709	0.186	3.415
I + B + PL	0.699	0.190	3.442
Difference ± S.E.M.	0.010 ± 0.185, n.s.	0.004 ± 0.028, n.s.	0.027 ± 0.393, n.s.

* Hormone concentrations as in table 1.

600 μg/ml. The results of the present study show that the presence of prolactin at a concentration of 1 μg/ml during a three hr incubation had no significant effect on glucose oxidation by mammary gland explants, whether or not these had been previously cultured with prolactin. Thus, the expression of the effect which prolactin exerts during culture is not dependent upon the continued presence of this hormone during the three hr incubation period.

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