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Influence of Prolactin on Metabolism and Energy Production in Perfused Corpus Luteum Bearing Bovine Ovaries*

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Summary. Eight bovine ovaries with a corpus luteum were perfused for 4 h in a haemoglobin-free semi-synthetic perfusion medium in a closed circuit. After an initial prolactin(PRL)-free perfusion phase, 4.7 ng/ml ovine PRL was added in the 1st h, followed by 47 ng/ml in the 2nd h and 470 ng/ml in the 3rd h. Glucose and oxygen consumption and the production of lactate, pyruvate and CO_2 were measured, while perfusion pressure and pH-value were recorded continuously. Under the influence of PRL anaerobic glucose metabolism was stimulated by 40.5% and oxidative phosphorylation was inhibited. Energy production from aerobic glucose metabolism rose by only 0.25%. Unlike PRL, Human Menopausal Gonadotropin (hMG) and Human Chorionic Gonadotropin (hCG) stimulated aerobic metabolism. This may indicate that PRL is the "older" hormone in phylleogenetic terms.

Key words: Bovine ovary – Corpus luteum – In vitro perfusion – Prolactin – Energy metabolism

Introduction

Hyperprolactinaemia is known to inhibit ovulation (L'Hermite et al. 1977; Thorner et al. 1975). The reason for this is unclear. In 1975, Saito and Saxena showed the presence of PRL receptors in the human ovary. In 1974, McNatty et al. demonstrated that luteinized granulosa cells lose their ability to synthesize progesterone in the absence of PRL. The work of Behrmann et al. (1970) showed that PRL maintained the "precursor pool" for the synthesis of progesterone. McNatty et al. (1975) pointed out that women in the early follicular phase have high concentrations of PRL. Jacobs (1975) showed that ovaries in patients with hyperprolactinaemia are refractory to gonadotrophins.

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Armstrong et al. (1969, 1970) demonstrated that PRL stimulated the synthesis of long chain fatty acids from 14-C labelled acetate. Administration of PRL to rats showed a clear decrease in cholesterol ester and free cholesterol levels (Armstrong et al. 1969). Behrmann et al. (1970) showed that PRL may influence the availability of cholesterol for the synthesis of progesterone, possibly by reducing the activity of enzymes like sterol-acyl-transferase and sterol esterase.

In previous experiments, we have demonstrated the influence of luteinizing hormone (LH) and hMG/hCG on the energy metabolism in isolated perfused human and bovine ovaries (Stähler et al. 1971, 1974). In the present experiments on isolated perfused bovine ovaries, we studied whether PRL influences the energy metabolism which is switched on before the steroid metabolism.

Material and Method

The experiments were performed in eight healthy bovine ovaries which contained a corpus luteum and were obtained from cows less than 7 years old. The mean wet weight was 7.2 ± 2.3 g. The ovaries were taken from the slaughter house to the laboratory in ice-cooled perfusion medium. Under aseptic conditions the main artery was cannulated, and ovaries were perfused with a semisynthetic, haemoglobin-free medium at 38° C at a nominal flow rate of 2 ml/min/g tissue in a closed, recirculating system (Stähler and Huch 1971). A continuous record of pH, P_{O_2}, P_{CO_2}, arterial and tissue pressure was made using a multi-channel recorder (Rikadenki, Tokyo, Japan). The experiments lasted about 4.5 h. After an initial period of about 30 min during which an oxygen uptake, pH and perfusion pressure were stabilized, the ovaries were perfused first with a PRL-free medium for 1 h, and then with medium containing increasing amounts of PRL (4.7 ng/ml in the 2nd h, 47 ng/ml in the 3rd h, and 470ng/ml in the 4th h). Glucose, lactate and pyruvate levels were measured serially in the perfusion medium. At the end of perfusion the ovary was frozen in liquid nitrogen and homogenized in perchloric acid. Tissue levels of glucose, lactate, pyruvate, ATP, ADP and AMP were estimated enzymatically by means of ready-made kits (Boehringer, Mannheim, FRG). The results were compared with six unperfused control ovaries. For statistical evaluation the "two tailed Student's *t*-test" for paired and unpaired values was used.

Results

Table 1 shows oxygen consumption and CO_2 production in corpus luteum bearing bovine ovaries perfused with increasing concentrations of PRL. Under these circumstances oxygen consumption decreased, but not significantly. Carbon dioxide production showed a slight, but statistically insignificant increase. Glucose consumption was increased by perfusion with 4.7 ng/ml PRL, and the increase reached statistical significance at 470 ng/ml PRL ($p < 0.02$). PRL also produced a significant increase in lactate production ($p < 0.02$), but pyruvate production was not significantly changed. The lactate/pyruvate ratio showed a slight increase up to 47 ng/ml PRL and remained at this level (Table 2). Table 3 shows the tissue concentrations of glucose, lactate, pyruvate, ATP, ADP and AMP in perfused and unperfused control ovaries. After perfusion with PRL, tissue glucose was found to increase ($p < 0.02$) as was AMP while lactate ($p < 0.05$), pyruvate ($p < 0.001$), ATP and ADP levels ($p < 0.001$) were significantly reduced when compared with unperfused ovaries.

Table 1. Oxygen uptake and carbon dioxide production of bovine ovaries with a corpus luteum before and after perfusion with PRL

PRL ng/ml	Oxygen uptake µmol/g/60 min $n = 8$	CO_2 production µmol/g/60 min $n = 6$
–	25.45 ± 12.89	14.91 ± (15.59)
4.7	24.78 ± 13.80	18.16 ± 15.59
47	24.41 ± 15.63	17.91 ± 9.44
470	24.86 ± 15.05	22.54 ± 13.21

Table 2. Glucose uptake, lactate and pyruvate production of bovine ovaries with a corpus luteum before and after perfusion with PRL ($n = 8$)

PRL ng/ml	Glucose consumption µmol/g/60 min	Lactate production µmol/g/60 min	Pyruvate production µmol/g/60 min	Lactate/ pyruvate ratio
–	28.45 ± 6.67	30.7 ± 17.3	2.56 ± 2.4	11.9
4.7	23.78 ± 10.36	32.8 ± 13.54	2.4 ± (2.5)	13.6
47	30.42 ± 8.69	36.93 ± 20.59	2.22 ± (2.3)	16.6
470	31.51 ± 10.38	44.26 ± 32.17	2.70 ± 2.29	16.4

Table 3. Comparison of concentrations of metabolites before and after a perfusion with PRL

µmol/g	Before perfusion $n = 5$	$p <$	After perfusion (PRL) $n = 8$
Glucose	0.96 ± 0.41	0.02	1.62 ± 0.46
Lactate	18.02 ± 2.63	0.05	12.48 ± 5.20
Pyruvate	0.32 ± 0.27	0.001	not detectable
ATP	0.32 ± 0.05	0.001	0.12 ± 0.05
ADP	0.95 ± 0.08	0.001	0.16 ± 0.10
AMP	0.63 ± 0.07	0.001	0.82 ± 0.06
ATP/ADP	0.33	ns	0.77

ns = not significant

Discussion

The results demonstrate an influence of PRL on the energy metabolism of perfused corpus luteum bearing ovaries. It is also interesting to compare the tissue concentrations of glucose, lactate, pyruvate, ATP and ADP after perfusion with PRL (Table 3) with those obtained from previous perfusion experiments in which PRL was not used (Stähler et al. 1971). Ovaries perfused with PRL showed significantly higher concentrations of glucose (2.47 ± 0.51 µmol/g, $p < 0.01$) and pyruvate (0.11 ± 0.02 µmol/g, $p < 0.001$) and significantly lower concentrations of lactate (2.90 ± 0.80 µmol/g, $p < 0.001$). The levels of ATP (1.90 ± 0.20 µmol/g) and ADP (0.84 ± 0.12 µmol/g) were significantly higher ($p < 0.001$) and AMP was not measured.

We found the most impressive change induced by PRL to be the increase of anaerobic energy production from glucose by 40.5%, while the aerobic metabolism of glucose increased by only 0.5%. During the steady state, the total energy generated was 14.5 J/g/60 min, a value similar to that reported previously (Stähler et al. 1971).

Increased energy production was shown in a corpus luteum of pregnancy (27.2 J/g/60 min). Inactive ovaries showed values of only about 10.5 J/g/60 min. In contrast to the described increase in anaerobic metabolism with PRL, our previous investigations in human ovaries showed a 220% increase in energy production by aerobic metabolism with stimulation by Human Menopausal Gonadotropin (hMG) and Human Chorionic Gonadotropin (hCG) (Stähler et al. 1974), the increase in aerobic glucose metabolism being only 7%.

The activation of anaerobic glucose metabolism is shown by the increase of the lactate/pyruvate ratio from 12 (steady state) to 16 (with PRL). The CO_2/glucose ratio behaved in a similar fashion, increasing from 3.2 (steady state) to 4.4–4.9. The results also showed an inhibition of oxidative phosphorylation under the influence of PRL. ATP was not further regenerated from ADP by oxidative phosphorylation. It was shown that during PRL perfusion, tissue ATP and ADP decreased and AMP increased. The ATP/ADP ratio was 2.2 before perfusion with PRL and only 0.8 afterwards. The fact that oxygen uptake remained unchanged and aerobic energy generation increased by 0.25% indicates that the stimulation of anaerobic processes is not caused by tissue hypoxia.

The lactate/pyruvate ratio was 11.9 in hour 1 and 16.6 during perfusion with 47 ng/ml PRL. This ratio remained at this level with 470 ng/ml of PRL. A steadily increasing hypoxia would lead to a linear rise in lactate/pyruvate ratio along with a decrease in pH. During PRL perfusion, the glucose uptake was higher than that which could be explained by aerobic and anaerobic glucose metabolisation. An increase in pyruvate oxidation did not occur because an increase in CO_2 and ATP production could not be seen. Even pyruvate tissue concentration would be expected to be higher.

A probable explanation is an increased incorporation of glucose via acetyl co-enzyme A in long-chain fatty acids and cholesterol with a possible activation of cholesterol and steroid metabolism. In this context, McNatty et al. (1976) working with mouse ovaries described a positive relation between PRL dosage and progesterone secretion; they also showed that PRL was needed to maintain progesterone secretion in cultured human granulosa cells (McNatty et al. 1977). Behrman et al. (1970) also showed that PRL was needed to maintain an adequate precursor pool for the synthesis of progesterone. Finally, Armstrong et al. (1969, 1970) demonstrated in rat corpora lutea that PRL stimulated the incorporation of 14-C-acetate in long-chain fatty acids as well as in free and estered cholesterol. These effects were not seen in ovaries not perfused with PRL (Stähler et al. 1971) or in those stimulated by hCG/hMG (Stähler et al. 1974). During these conditions oxidative phosphorylation is apparently not blocked, and energy is generated by aerobic glucose metabolism. As anaerobic energy metabolism evolved earlier than aerobic metabolism, PRL would seem to be the "older" hormone in phylogenetic terms.

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