

INFLUENCE OF SEX STEROIDS AND PROLACTIN ON RAT MAMMARY GLAND

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Summary: The effect of sex steroids and prolactin on rat mammary glands were studied. Estradiol-17 β alone was found to increase the protein concentration significantly. Progesterone increased LDH and G-6-PD activities whereas Estradiol and Prolactin decreased LDH activity. G-6-PD activity was increased by estradiol and prolactin. Glycogen concentration was decreased by prolactin alone. Further, these hormones increased the percentage of total 'M' subunits and decreased total 'H' subunits of LDH. In general, sex steroids and prolactin increased glycogen utilisation and prepared the Mammary Gland for anaerobic metabolism.

Key words: mammary gland LDH G-6-PD estradiol-17 β progesterone prolactin

INTRODUCTION

Steroid and peptide hormones play an important role in the regulation of metabolic activities in mammary gland (11, 1, 2). Estrogens and progesterone induced an increase in the activity of the enzymes involved in carbohydrate metabolism in rats during midlactation (11). An increase in Lactate dehydrogenase (LDH) isoenzyme fractions 4 and 5 was observed during pregnancy (15). Prolactin enhanced the incorporation of ³H-Leucine into protein in mouse mammary explants (1) and also increased LDH activity with insulin and cortisol (9). In the mammary gland of hypophysectomized rats, prolactin was found to increase Glucose-6-phosphate dehydrogenase (G-6-PD) activity (8). Multi-fold increase in glycogen content of the mammary gland was evident in ovariectomized and adrenalectomized rats (11).

In the present investigation, the alterations in the glycogen and enzymes involved in glycolytic and pentose phosphate pathways in the mammary gland with reference to Estradiol-17 β , progesterone and prolactin have been studied.

MATERIALS AND METHODS

Animals: Albino, virgin female rats of Wistar strain (90-100 days old and 100-125 g body weight) exhibiting regular 4 day cycles were housed in a well ventilated

animal house. The rats were fed with commercial rat food pellets (Hindustan Lever Ltd., Bombay, India) and water *ad libitum*.

The animals were subjected to bilateral ovariectomy following the techniques of Zarrow *et al.* (17) and fourteen days later they were divided into four groups :

- Group I* : Control (ovariectomized) rats treated with vehicle only.
- Group II* : Ovariectomized rats treated with Progesterone (2 mg/100 g b.w./rat/5 days intramuscularly).
- Group III* : Ovariectomized rats treated with Estradiol-17 β (5 μ g/100 g b.w./rat/5 days intramuscularly).
- Group IV* : Ovariectomized rats treated with prolactin (ovine) (400 μ g/100 g b.w./rat/8 days intramuscularly).

Estradiol-17 β and progesterone were dissolved in propane-1,2-diol. Prolactin (ovine) was dissolved in 0.01N NaOH. All the chemicals and reagents used were of analytical grade. Estradiol-17 β and Progesterone were obtained from BDH (England) and ovine prolactin was a gift from NIH (USA).

Methods : The animals were sacrificed by cervical dislocation twenty four hours after the last injection of specific hormones as scheduled for treatments. The mammary glands were removed following the procedure of Zarrow *et al.* (17) and were immediately blotted and weighed accurately on a torsion balance to the nearest milligram. The tissue was homogenised in ice-cold 0.05M Tris buffer in a Potter-Elvehjem homogeniser for 7 to 10 min. The whole homogenates were centrifuged for 20 min at 4°C in a refrigerated centrifuge. The supernatant was used for estimation of total protein and enzymes. Protein content of the tissue was estimated by the method of Lowry *et al.* (12). LDH and Glucose-6-phosphate dehydrogenase (G-6-PD) activities were estimated by the method of King (10). LDH-isoenzymes were separated on 5.5% Polyacrylamide gel Electrophoresis following the method of Dietz *et al.* (3). Glycogen was estimated by the method of Hassid and Abraham (5). The percentage distribution of the isoenzymes were determined by the intensity of stained isoenzymes scanned in Joyce-Loebl densitometer. The data were analysed statistically (13) using the Students 't' test. Values for p of 0.05 or less were considered significant.

RESULTS

The influence of Progesterone, Estradiol and Prolactin administration on the protein concentration, glucose-6-phosphate dehydrogenase, LDH activities and glycogen

concentration in the rat mammary gland is presented in Table I. Progesterone and prolactin did not influence the protein concentration appreciably but estradiol triggered an increase ($p < 0.005$). All the three hormones were found to increase the G-6-PD activity but prolactin had a profound influence. Progesterone alone was able to increase ($p < 0.005$) LDH activity. The decrease in LDH activity brought about by prolactin is quite marked ($p < 0.001$). The glycogen concentration was not significantly altered by progesterone and estradiol treatments. However, prolactin depleted the glycogen concentration ($p < 0.025$).

TABLE I: Effect of sex steroids and prolactin on the mammary gland.

Parameters	CONTROL (OVARIECTOMIZED)	Progesterone treatment	Estradiol-17 β treatment	Prolactin treatment
Protein (mg/100 mg tissue)	2.26 \pm 0.26	2.75 \pm 0.11 (t=1.736)	3.20 \pm 0.09** (t=3.416)	3.04 \pm 0.36 (t=1.756)
Glucose-6-phosphate dehydrogenase (μ moles/min/mg protein)	41.27 \pm 8.20	153.79 \pm 12.44*** (t=7.552)	130.50 \pm 11.85*** (t=6.192)	426.04 \pm 16.73*** (t=20.652)
Lactate dehydrogenase (mIU/min/mg protein)	1312.00 \pm 123.81	2988.62 \pm 488.20** (t=3.320)	889.26 \pm 91.89* (t=2.742)	477.51 \pm 55.65*** (t=6.140)
Glycogen (μ g/100 mg tissue)	61.46 \pm 6.37	59.03 \pm 3.93 (t=0.325)	54.67 \pm 2.39 (t=0.998)	41.35 \pm 5.13* (t=2.459)

Each value is Mean \pm S.E.M. of 10 experiments.

*** $p < 0.001$

** $p < 0.005$

* $p < 0.025$

The percentage distribution of LDH isoenzymes under various hormone treatment schedule is presented in Table II. In ovariectomized animals the 'M' subunits of LDH isoenzymes are predominant over 'H' subunits. The most significant alterations were found in LDH-1, 2, 3 and 5 isoenzymes. Irrespective of the hormone treatment the same pattern of increased 'M' subunits was always maintained. All the three hormones increased LDH-5 isoenzyme and decreased LDH-1 and 2. LDH-5 was found to be maximum due to progesterone and LDH-4 was found to be maximum due to estradiol treatment. The decrease in LDH-1 due to prolactin was not as much as observed in progesterone and estradiol treated groups.

TABLE II : Effect of sex steroids and prolactin on percentage distribution of LDH-ISOENZYME pattern of the mammary gland.

LDH-ISOENZYMES	CONTROL (OVARIECTOMIZED)	Progesterone treatment	Estradiol-17 β treatment	Prolactin treatment
LDH-1 (HHHH)	4.35 \pm 0.58	0.21 \pm 0.02*** (t=7.134)	0.83 \pm 0.12*** (t=6.047)	2.27 \pm 0.13* (t=3.499)
LDH-2 (HHHM)	17.65 \pm 2.35	2.30 \pm 0.56*** (t=6.354)	9.17 \pm 0.75* (t=3.438)	7.21 \pm 1.12** (t=4.010)
LDH-3 (HHMM)	29.41 \pm 4.12	9.44 \pm 3.88** (t=3.529)	25.42 \pm 3.18 (t=0.767)	14.54 \pm 1.66* (t=3.348)
LDH-4 (HMMM)	30.15 \pm 3.12	21.68 \pm 2.77 (t=2.030)	38.75 \pm 2.52 (t=2.144)	29.29 \pm 2.11 (t=0.228)
LDH-5 (MMMM)	18.44 \pm 1.89	66.58 \pm 5.89*** (t=7.782)	25.83 \pm 1.78@ (t=2.839)	46.19 \pm 3.99*** (t=6.285)
Total 'H' Subunits	40.00 \pm 1.95	13.50 \pm 5.29** (t=3.871)	30.00 \pm 1.85* (t=3.237)	23.00 \pm 2.91** (t=4.419)
Total 'M' Subunits	60.00 \pm 2.12	86.50 \pm 6.51*** (t=4.700)	70.00 \pm 1.22** (t=3.720)	77.00 \pm 3.21*** (t=4.853)

Each value is Mean \pm S.E.M. of 5 experiments

*** p < 0.001

** p < 0.005

* p < 0.01

@ p < 0.025

DISCUSSION

The mammary gland is influenced by multifarious hormones and therefore, it is natural that the metabolic activities in the mammary gland will be influenced accordingly. In this respect quite a number of investigations have been oriented on the influence of pregnancy, lactation (15) and tumor implantation (14) on the metabolic activities of the mammary gland. However, these studies do not specifically suggest the hormone and biochemical interaction in the mammary tissue. The physiological status implied in such studies are due to the influence of more than one hormone and therefore, the information obtained mostly reveal the combined hormonal influence over the metabolic activities in the mammary tissue. In pregnancy, progesterone and estradiol are elevated and in lactation, prolactin is elevated on and off. In such circumstances the alterations in metabolic activities in the mammary gland is mostly due to such hormonal factors.

In the present study, to have a specific idea on the hormone-biochemical interrelationship in the mammary gland, the ovariectomized rats have been selected and the individual hormonal influence has been studied on the mammary gland.

Estradiol administration alone was able to increase the protein concentration. Progesterone and prolactin did not appreciably alter the protein concentration in the mammary gland. It has been suggested that prolactin induces the incorporation of amino acids for protein synthesis (1). However, in the present study such an influence was not evident as prolactin did not trigger an increase in protein concentration. The obvious differences in the results obtained may be due to differences in experimental approach, while the present study have been carried *in vivo*. Anderson *et al.* (1) have based their observation of the results obtained on *in vitro* in mice. Estradiol normally induced cellular proliferation in target organs (4). The increase observed in protein concentration due to Estradiol may be due to cellular proliferation.

The synthesis and utilisation of glycogen due to the hormonal influence, suggest that glycogen is depleted by prolactin treatment. Estradiol and progesterone treated groups did not alter the glycogen concentration significantly.

In general, progesterone was observed to encourage LDH and G-6-PD activities. However, Estradiol and Prolactin induced an increase only in G-6-PD activity, where prolactin had a quite significant influence than estradiol. The trend of events suggest that progesterone favours glycogen utilisation through glycolytic as well as HMP shunt pathways, whereas estradiol and prolactin through HMP shunt pathway only.

Estradiol was found to increase NADPH generation through HMP shunt pathway in rat mammary gland (6). This increased NADPH may be utilised for the conversion of pyruvate to phosphoenolpyruvate, which could then be used for the resynthesis of glucose and the ultimate synthesis of glycogen (7). In this respect normally the glycogen store would increase. However, in the present study did not bring about such an increase. The reason for the same may be due to the decreased availability of pyruvate because of the decrease in LDH activity. In the resynthesis of glycogen not only NADPH but also pyruvate and phosphoenolpyruvate are required (7). The same is applicable with respect to prolactin as well. However, progesterone appears to have a different influence over the carbohydrate metabolism in the mammary tissue. In spite of this increased glycolytic and HMP shunt pathway enzymes, the glycogen store was not depleted significantly. This may be due to the increased availability of NADPH through HMP shunt and pyruvate through glycolytic pathways, and the subsequent resynthesis of glycogen.

Prolactin had brought about a decrease in LDH activity and an increase in G-6-PD activity compared to other hormones. In experimental study it was shown that the decrease in prolactin through bromocryptine, increased pyruvate and lactate availability which serve as substrates for LDH activity (16). Therefore, the increased availability of prolactin is normally expected to bring about the opposite picture. This was favoured to be reflected in the present studies.

In pregnancy and lactation, increased G-6-PD and LDH activity was reported (15). This is not comparable to the results obtained in the present investigation. In pregnancy, the increase in other hormones such as progesterone, estradiol and prolactin may have combined influence over the mammary gland. It appears that progesterone may be dominant over the other hormones in reflecting its influence on LDH activity, during pregnancy. In lactation there is no observation of consistent prevalent increase of prolactin. The prolactin availability mostly depends upon the frequency of suckling and even in such cases oxytocin is the factor of influence than prolactin. In postpartum lactation period, the ovarian hormones may also contribute their influence on mammary gland.

Regarding LDH isoenzyme pattern, the ovariectomized rat mammary gland tissue suggest the prevalence of anaerobic type of metabolism as shown by the increased LDH-4 and 5 subunits over LDH-1 and 2. Treatment with progesterone, estradiol or prolactin does not influence this trend. However, a favourable and specific influence on LDH-5 was reflected by progesterone and prolactin. Estradiol was favouring more of LDH-4. Comparatively the ratio between 'M' and 'H' subunits suggested the specific progesterone induced increase in 'M' subunits than other hormones. Ofcourse, prolactin followed progesterone closely.

Thus, while taking to consideration of the individual hormonal influence on the mammary gland tissue with reference to carbohydrate metabolism, the present study suggest that progesterone triggers both HMP shunt and glycolytic pathways for the provision of energy. Prolactin and Estradiol prefer HMP shunt pathway alone for such purpose. All these three hormones influence the tissue to favour anaerobic trend of metabolism which was reflected more in the case of progesterone.

REFERENCES

1. Anderson, Larry, D. and Jame A. Rilemma. Effects of hormones on protein and amino acid metabolism in Mammary Gland explants of mice. *Biochem. J.*, **158** : 355-359, 1976.
2. Betts, S.A. and John Mayer. Regulation of enzyme turnover during tissue differentiation : Studies on 6-phosphogluconate dehydrogenase in rabbit mammary gland in organ culture. *Biochim. Biophys. Acta*, **496** : 302-311, 1977.

3. Dietz, A.A., T. Lubrano and H.M. Rubinstein. Disc Electrophoresis of Lactate dehydrogenase isoenzymes. *Clin. Chim. Acta*, **27** : 225-232, 1970.
4. Ghoneim, S.M. Histologic and ultrastructural studies of resting and lactating rat Mammary tissue. Influence of exogenous hormone stimulation. *Diss. Abst. Int. B*, **32** : 5276-5277, 1972.
5. Hassid, W.Z. and S. Abraham. I. Determination of glycogen and starch. In : *Methods in Enzymology*, Vol. III Edited by Colowick, S.P. and N.C. Kaplan, Academic Press, New York and London, p. 34-36, 1957.
6. Hilf, R., I. Michel and C. Bell. Dose responses of R 3230 AC Mammary tumor and Mammary tissue to estrogen : enzymes, nucleic acids and lipids. *Cancer Res.*, **26** : 865-870, 1966.
7. Hollmann, S. The pentose phosphate cycle. In: Non-glycolytic pathways of metabolism of glucose, Academic Press, New York and London, 1964.
8. Jones, E.A. Changes in the enzyme pattern of the mammary gland of the lactating rat after hypophysectomy and weaning. *Biochem. J.*, **103** : 1420-1423, 1967.
9. Jones, E.A. and I.A. Forsyth. Increases in the enzyme activity of mouse mammary explants induced by prolactin. *J. Endocr.*, **43** : XI, 1969.
10. King, J. Practical Clinical Enzymology (D. Van Norstrand Co., London), 1965.
11. Korsrud, G.O. and R.L. Baldwin. Effects of Adrenalectomy, Adrenalectomy-Ovariectomy and Cortisol and Estrogen Therapies upon enzyme activities in Lactating rat Mammary glands. *Can. J. Biochem.*, **50** : 366-376, 1972.
12. Lowry, O.H., N.S. Rosebrough, A.L. Farr and R.J. Randall. Protein measurement with Folin Phenol Reagent. *J. Biol. Chem.*, **193** : 265-275, 1951.
13. Ostle, B. Statistics in Research, Oxford and IBH, New Delhi, 1966.
14. Richard, A.H. and R. Hilf. Effect of Estrogen administration on G-6-PD and LDH isoenzymes in Rodent Mammary tumors and normal mammary glands. *Cancer Res.*, **32** : 611-616, 1971.
15. Richard, A.H. and R. Hilf. Influence of pregnancy, Lactation and Involution on Glucose-6-phosphate dehydrogenase and Lactate dehydrogenase isoenzymes in the rat Mammary gland *Endocrinology*, **91** : 287-295, 1972.
16. Robinson, A.M. and D.H. Williamson. Comparison of Glucose metabolism in the lactating Mammary gland of the rat *in vivo* and *in vitro*. Effect of starvation. prolactin or insulin deficiency. *Biochem. J.*, **164** : 153-159, 1977.
17. Zarrow, M.X., J.M. Yochim and J.L. McCarthy. *Experimental Endocrinology* - A source book of Basic Techniques, Academic Press, New York and London, p- 39-40, 1964.