HORMONAL INFLUENCES ON THE OVIDUCAL LACTATE DEHYDROGENASE AND ITS ISOENZYMES

G. VANITHAKUMARI, P. GOVINDARAJULU AND N. SRINIVASAN

Department of Endocrinology,
P.G. Institute of Basic Medical Sciences,
University of Madras, Taramani, Madras-600 042

(Received on April 16, 1980)

Summary: Changes in the specific activities and distribution of Lactate dehydrogenase (LDH) isoenzymes have been studied in the oviduct of intact, ovariectomized and ovariectomized-hormone treated rats. Total LDH and M-subunits were higher in the oviduct during estrus compared to early diesturs phase. Ovariectomy lowered the total activity as well as the proportion of H and M-subunits of LDH, with a total loss of LDH. Treatment of ovariectomized rats with progesterone (2 *mg*/100 *g*. body wt/rat/day) and estradiol-17β (0.5 μg/100 *g* body wt/rat/day) for 7 consecutive days induced marked augmentations in the total activities of this oviducal enzyme. Further, there was a shift in the isoenzyme patterns towards M-type, especially LDH_5. Prolactin (400 μg/100 *g* body wt/rat/day) depressed the total LDH activity and the distribution of isoenzymes. While LDH_4 and LDH_5 were very low, LDH_1—LDH_3, inclusive, were absent in the oviduct of prolactin-treated group. None of the hormonal replacements could stimulate the LDH_1 – subunit. The significance of these changes has been discussed in relation to metabolic activities in the tissue and hormonal influences.

Key words: oviduct, lactate dehydrogenase, LDH isoenzymes, estrus, diestrus, ovariectomy, estradiol-17β, progesterone, prolactin

INTRODUCTION

Lactate dehydrogenase and its isoenzymes are intimately involved in mammalian development and reproduction (17,4). Lactate and pyruvate, the two reactants of this enzyme, are especially important as energy sources during early growth and cell multiplication of the mammalian embryo (1,9). Remarkably high specific activities of this enzyme has been reported in association with preimplantation of ova (3.9), uterine tissue and fluid of rat (5,8), rabbit (11), and human (6,18); with the ovary (14,15) and with the extracellular secretion of the oviduct of the rabbit, mouse, guinea pig and also oviducal tissue of rat (7,10,14,16).

As oviducal fluid was shown to be very rich source of this enzyme by the above workers, it was considered to be of value to define the nature of this enzyme in the conjacent oviducal tissue, and to establish the type of changes incurred during the normal oestrus cycle, ovariectomy and after replacement therapy with steroids and prolactin to the ovariectomized rats.

MATERIALS AND METHODS

Animals :

Female rats of Wistar strain (approximate body weight 190 g) were maintained in temperature and humidity controlled individual cages under a lighting schedule of 6.00 – 20.00 h. The rats had free access to standard rat diet and water. Vaginal smears were examined daily to establish the phase of the cycle, and all rats were taken through three normal cycles before use. Several series were bilaterally ovariectomized at definite periods prior to the injection of steroids and prolactin. Estradiol-17β and Progesterone in 1,2-propanediol solution were injected intramuscularly at levels of 0.5 μg and 2 mg/100 g body wt/rat/day, respectively, daily for 7 days, the controls received the vehicle as such; the daily volume of each mixture was under 0.2 ml. Prolactin (ovine) in 0.01N NaOH was also administered at the dose level of 400 μg/100 g body wt/rat/day for 7 consecutive days to a third group of ovariectomized animals. Controls received vehicle only.

The ovariectomized animals were sacrificed by swift decapitation 19-24 h following the last injection of specific hormones and the intact animals at specific phases of the oestrus cycle. The oviducts were rapidly excised, oviducal fluids removed by flushing with saline, blotted and after determining the wet weight, were homogenized in 2 ml of 0.5M phosphate buffer (pH 7.4) and centrifuged at 10,000 x g for 30 min. The supernatants were either used directly or stored at –20°C until required.

Total LDH was analyzed in a DU-2 Spectrophotometer by following the absorbance change at 440 nm due to the reduction in NAD^+ (12) and the LDH unitage expressed in terms of m.I.U. of Pyruvate formed/min/mg protein. LDH isoenzymes were determined by zone electrophoresis on polyacrylamide gels (2) with tris-glycine buffer (pH 8.4) and the gels being quantitated by microdensitometry. The subunit composition of the enzymes were calculated. Portions of each of the filtrate were analyzed for protein by the method of Lowry *et al.* (13). Statistical significance of the respective differences was ascertained by analysis of variance.

RESULTS

The specific activities of LDH and the percentage of M-type contribution of this enzyme activity in the oviduct of normal, ovariectomized and ovariectomized- hormone treated groups are summarized in Tables I and II. The specific activity showed a peak at estrus and declined sharply ($p<.001$) to the diestrus stage. Major alterations were

seen in M-subunits of LDH, which declined from 86% (estrus) to 76% at diestrus. A decrease in LDH_1 to LDH_3 with concomittant increase in LDH_4 and LDH_5 at estrus compared to diestrus phase was observed.

TABLE I : Total LDH activity in the oviduct of normal, ovariectomized and ovariectomized rats treated with steroids and prolactin.@

S.No	Groups	No. of rats	Oviduct wt. (mg)	Total LDH activity (m. I.U. of pyruvate formed/ min/mg protein)	Protein (µg/100 mg wet tissue)
1.	Estrus	12	31.46±1.89[b]	2650.45±118.46[b]	5845.46±191.24[b]
2.	Diestrus	15	24.65±1.45	2056.77±234.25	4651.77±123.56
3.	Ovariectomized (OVX)	15	14.35±1.11[c]	1245.46±109.12[c]	3260.24±170.34[c]
4.	OVX + Estradiol-17β	14	20.45±0.83**	1750.56± 78.80**	3980.45±143.44***
5.	OVX + Progesterone	15	14.45±1.98	2078.23±303.58***	3334.55± 83.45*
6.	OVX + Prolactin	14	17.45±1.24	350.31± 25.44***	3341.44±246.54

@ : Mean ± S.E.M. b : $p<0.001$ vs. all other stages; c : $p<0.001$ vs. intact groups.
*$p<0.05$; **$p<0.01$; ***$p<0.001$ vs. ovariectomized group.

Following ovariectomy, the total LDH activity decreased 50% to that of intact controls ($p<.001$). There was a total loss of LDH_1 and a significant decrease of LDH_5. The percentage of M sub-units fell to 68% with a shift in the LDH isoenzyme pattern to LDH_2 and LDH_3. Treatment with estradiol-17β caused definite increase in oviducal total enzymes but the isoenzymes, LDH_1 to LDH_3 were decreased and concomittantly LDH_5 was elevated significantly. Administration of progesterone led to a sharp increase in total LDH enzyme activity, which was comparable to the diestrus group values. However, LDH_5 was significantly lower than that of estradiol injected rats. It may be seen that progesterone stimulated LDH_2 markedly without much alteration in LDH_1 and the percentage of M sub-units was comparable to estradiol- treated groups.

Treatment with prolactin caused a 30% decline in the total LDH activity in the oviduct of ovariectomized rats. There was a total absence of LDH_1 to LDH_3 and a drastic reduction in LDH_4 and LDH_5 and consequently the percentage of M as well as H sub-units were very low.

DISCUSSION

The present investigations suggest that the oviducal tissue has a rich source of LDH enzyme and hormones have a definite influence over the different sub-units of this

TABLE II : Percentage distribution of LDH isoenzymes in normal ovariectomized and ovariectomized rats treated with steroids and prolactin.@

S.No. Groups	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5	% M type
1. Estrus (12)	1.41±0.20	3.84±0.06b	13.81±0.88b	28.24±2.24b	56.34±3.48b	86.38±3.20b
2. Diestrus (15)	1.66±0.18	5.48±0.82	18.15±1.24	26.30±1.23	46.48±2.36	75.08±2.35
3. Ovariectomized (OVX) (15)	absent	9.77±0.91c	29.77±1.43c	32.25±2.04c	29.08±1.90c	68.05±2.33c
4. OVX+Estradiol-17B (14)	absent	5.90±0.12***	23.20±1.24**	34.20±1.88	36.00±2.00**	72.00±2.40
5. OVX+Progesterone (15)	absent	16.40±2.32***	22.40±1.18***	28.20±1.06	30.40±2.60	75.60±1.84**
6. OVX+Prolactin (14)	absent	absent	absent	6.84±0.34b	9.40±0.92b	15.00±0.12b

@ : Mean ± S.E.M. No. of animals given in parenthesis.

**p<0.01 : ***p<0.001; *p<0.05 vs. ovariectomized group. b : p<0.001 vs. all other stages. c : p<0.001 vs intact gorups.

enzyme. The LDH pattern seen in the present studies are similar to that observed in rat oviducal secretions (10,14). The peak LDH activity with increased M sub-units during estrus compared to early diestrus may reveal a correlation between the LDH M-type activity and circulating oestrogens (19). It has been demonstrated by these workers that ovarian production of oestrogens in the rat is maximal at late diestrus and early proestrus, low in estrus and absent in early diestrus. The change in the percentage of M sub-units in the oviduct would accord with this pattern of oestrogen production. Patterson and Masters (14) have reported a peak activity of LDH and its M sub-unit during proestrus with a sharp decline at diestrus in oviducal tissue and fluid, as evident in the present studies. Similar responses have been observed in the rat and rabbit uterus (11,18).

Galbraith *et al.* (7) have suggested that the increase in the proportion of H sub-units, seen at early diestrus, may enable the uterus to maintain TCA cycle activity in a fully functional state. This, in turn, may enable the uterus to derive the maximum energy from glucose metabolism, and assist the catabolism of uterine protein which appears to accompany the involution of uterus at this period of the cycle. Such a reasoning may be applicable to the oviduct also as the LDH patterns reflected similar fluctuations (Table II).

The alterations in the specific activities and relative expression of M and H -type LDH in the oviduct after ovariectomy confirm the hormone dependency of the enzyme. The specific activity (total) declined sharply with a total loss of LDH_1 and decrease in LDH_4 and LDH_5 due to estradiol and progesterone deprivation. The steroid replacements to these ovariectomized rats not only prevented the post castration decline in the LDH activity but actually restored the total activity of this enzyme to near normal levels and further encouraged the M-type sub-unit distribution. However, estradiol-17β exerted a directional influence on the LDH enzyme preferentially stimulating the production of M sub-units, better than progesterone. On the other hand, progesterone appeared to stimulate the H sub-units also. Similar trends have been observed in the rat and human uterus (7,6).

Contrary to the influences of estradiol and progesterone, prolactin not only reduced total LDH activity but also suppressed the H and M-type sub-unit distributions. Thus, prolactin seems to have an inhibitory influence on the glycolytic pathway. The exact mechanism by which such changes were brought about is not known and further studies are warranted in this respect.

REFERENCES

1 Brinster, R.L. Lactic dehydrogenase activity in preimplantation rat embryo. *Nature, Lond.*, **214** : 1246–1247, 1967.

2. Dietz, A.A., T. Lubrano and H.M. Rubin-Stein. Disc electrophoresis of lactate dehydrogenase isoenzymes. *Clin. Chim. Acta.*, **27** : 225–232, 1970.

3. Epstein, C.J., L. Kwok and S. Smith. The source of lactate dehydrogenase in preimplantation mouse embryos. *FEBS Lett.*, **13** : 45–48, 1971.
4. Fieldhouse, E. and C.J. Masters. Development redistributions of porcine lactate dehydrogenase. *Biochem. biophys. Acta.*, **118** : 538–548, 1966.
5. Filho, H.M. Lactic and malic dehydrogenase isozymes in mammalian uterus. *Rev. bras. Pesqui. med. biol.*; **6** : 245–248, 1973.
6. Fottrell, P.F., C.M. Spellman and E. O Dwyer. Lactate dehydrogenase isoenzymes in human reproduction. *Clin. Chim. Acta.*, **26** : 584–585, 1960.
7. Galbraith, H., D.A. Robb and P.J. Heald. Changes in the proportion of 'H' and 'M' subunits of lactate dehydrogenase in rat uterus during an oestrus cycle. *Biochim. biophys. Acta.*, **201** : 391–393, 1970.
8. Gershbein, L.L. and K.G. Raikoff. Uterine total lactic dehydrogenase and isozymes of rats administered steroids. *Enzyme.*, **23** : 64–69, 1978.
9. Gibson, C. and C.J. Masters. On the lactate dehydrogenase of preimplantation mouse ova. *FEBS Lett.*, **7** : 277–279, 1970.
10. Gibson, C. and C.J. Masters. Oviducal lactate dehydrogenase. *J. Reprod. Fert.*, **22** : 157–159, 1970.
11. Goodfriend, T.L. and N.O. Kaplan. Effects of hormone administration on lactic dehydrogenase. *J. biol. Chem.*, **239** : 130–135, 1964.
12. King, J. Practical clinical enzymology. D. Van. Norstrand Co., London, 1965.
13. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. Protein measurement with folin phenol reagent. *J. Biochem.*, **193** : 265–275, 1951.
14. Patterson, C. and C.J. Masters. Lactate dehydrogenase isoenzymes and the reproductive cycle in the rat. *J. Reprod. Fert.*, **30** : 425–431, 1972.
15. Poznakhirkina, N.A., O.L. Serov and L.I. Korochkin. A study on lactate dehydrogenase isozymes in rat ova. *Biochem. Genet.*, **13** : 65–72, 1975.
16. Vanithakumari, G., N. Srinivasan and P. Govindarajulu. Influence of sex steroids and prolactin on the metabolic activities of oviduct. *J. Steroid. biochem.*, **9** : 851, 1978.
17. Wiggert, B.O. and C.A. Villee. Multiple molecular forms of malic and lactic dehydrogenase during development. *J. biol. chem.*, **239** : 444–451, 1964.
18. Wilson, E.W. The effect of oestradiol-17β on enzymes concerned with metabolism of carbohydrate in human endometrium *in vitro*. *J. Endocr.*, **44** : 63–68, 1969.
19. Yoshinaga, K., R.A. Hawkins and J.F. Stocker. Estrogen secretion by the rat ovary *in vivo* during the estrous cycle and pregnancy. *Endocrinology*, **85** : 103–112, 1969.