HORMONAL EFFECTS ON THE RAT GONADAL LACTATE DEHYDROGENASES

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Received 17 May 1971

1. Introduction

Additional lactate dehydrogenase (LDH) isoenzymes (EC 1.1.1.27) beyond the five molecular forms* normally found in animal tissues have been reported in homogenates of testis and sperm from a number of adult animal species [1–3]. A single additional LDH isoenzyme, referred to as 'LDH-X', with an electrophoretic mobility between H_2M_2 and HM_3 has been reported in testicular extracts of rabbit [4], mouse [5], and rat [6, 7]. In testicular extracts of the male rat, which follows a seasonal sexual cycle, two additional LDH isoenzymes, one with an electrophoretic mobility between H_3M and H_2M_2 and the other between H_2M_2 and HM_3 have been reported [8]. Since the gonadal-specific LDH isoenzymes appear in this animal only during times when spermatogenesis occurs, it has been suggested that a third gene, responsible for the synthesis of the additional polypeptide subunit, uniquely present in gonadal LDH isoenzymes, may be activated by a hormonal factor [8]. Recently, it has been reported that the activity of the sperm acrosomal enzyme, hyaluronidase, disappears after hypophysectomy in the rat, and replacement therapy with the anterior pituitary gonadotrophins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) or the testicular hormone, testosterone, restores the enzyme activity to normal levels [9].

We have observed similar changes with the two rat gonadal-specific LDH isoenzymes. These enzymes, which appear in the developing testis cells on about day 35 after birth, disappear in the hypophysectomized animal in about 21 days after hypophysectomy. Replacement therapy with either FSH and LH or with testosterone completely prevents the loss of enzyme activity. Thus, the rat gonadal LDH isoenzymes, as hyaluronidase, [9] can be used as biochemical markers for the development of spermatogenesis and for a study of hormone-dependent transitions in the spermatogenic event.

* These five LDH isoenzymes, which are tetramers of the polypeptide subunits designated 'H' and 'M' and thus correspond to the tetramers, H_4, H_3M, H_2M_2, HM_3 and M_4, have different electrophoretic mobilities and their syntheses are under the control of two separate genetic loci.

2. Materials and methods

Wistar rats which were 45–50 days old at the onset of the experiment and weighed between 190–250 g were used. Purina laboratory chow and water were given ad libitum throughout the experimental period. Animals were placed in four groups, designated A, B, C and D of 12 animals each, corresponding to normal-untreated, hypophysectomized-saline-treated, hypophysectomized-gonadotrophin-treated and hypophysectomized-testosterone-treated. All hormones were given as daily subcutaneous injections in the following amounts: LH 25 μg/100 g body weight, FSH 15 μg/100 g body weight, and testosterone 1 mg/100 g body weight. The gonadotrophins FSH (NIH-FSH-P-1) and LH (NIH-LH-B_7) were kindly supplied by the Endocrinology Study Section, NIAMD. Surgical hypophysectomies of animals in groups B, C and D were performed by the parapharyngeal approach [10]. Following autopsy, the completeness of hypophysectomy was established by histological examination of serial sections of the hypophysical fossa. Only animals which had no pituitary remnants were used in this study.

At 7, 14 and 21 days following hypophysectomy and saline, FSH and LH, or testerone treatment, a single testis of one animal from each group was rapidly excised and freed of epididymis and surrounding tissue. Each testis was cut into pieces, rinsed several times with 0.15 M NaCl and homogenized in 0.32 M sucrose (1:3 w/v) in a tight-fitting Potter-Elvehjem homogenizer with teflon pestle at 4° for 2 min at 200 rpm. Extracts were separated by centrifugation at 20,000 *g* for 20 min at 4° and an aliquot of the supernatant subjected to cellulose acetate electrophoresis (Millipore) at pH 8.4 in 0.1 M tris-barbital buffer. A voltage gradient of 10 V/cm was applied for 35 min. The separated LDH isoenzymes were made visible by formazan formation using either L-acetate or DL-α-hydroxybutyrate as substrate [11]. The other testis from the same animal was fixed in Bouin's solution, sectioned at 10 μM and stained with hematoxylin for histological examination. Mature rat sperm was collected in the following way: the epididymis was removed and freed of surrounding connective tissue. A small razor blade incision was made in the cauda and the exposed area placed in a petri dish containing 0.15 M saline. Upon application of very gentle finger pressure to the epididymis above the incision, the sperm flowed freely into the saline. Sperm thus collected from six animals was pooled, centrifuged for 20 min at 20,000 *g*, the cells resuspended, washed with saline and recentrifuged. Washing and centrifugation was repeated ten times to ensure complete removal of epididymal fluid. The washed sperms were suspended in 0.32 M sucrose (1:3 w/v) containing 1% triton X-100 and sonicated for 30 sec at 20 kHz in a Model LS-75 Branson Sonifier. The emulsion was centrifuged for 30 min at 20,000 *g* and an aliquot of the supernatant subjected to electrophoresis as described above for the testicular extracts.

3. Results and discussion

Homogenates of normal sexually mature rat testis (group A) exhibit two additional LDH isoenzymes, one with an electrophoretic mobility intermediate between M_3H and M_4 (G_2) and another migrating towards the anode close to HM_3 (G_1) as seen in fig. 1. In B, the electrophoretic pattern from the testicular homogenate of an animal which has been hypophysectomized and injected with saline for 21 days is visualized (group B). In this electrophoretogram, it can be seen that the two gonadal-specific LDH isoenzymes, which appear normally at about day 35 in the Wistar strain of rats used in these experiments, have completely disappeared, whereas the overall distribution of the other five LDH isoenzymes remains about the same. This observed disappearance of the G_1 and G_2 LDH isoenzymes seems to be progressive with time, since the electrophoretogram performed on testicular homogenates of animals on day 7 after hypophysectomy possesses both gonadal-specific LDH isoenzymes, whereas on day 14 after hypophysectomy, the amount of stainable gonadal LDH is considerably diminished. By day 21, there is no evidence of the presence of the two gonadal LDH isoenzymes. Histologically, in group B on day 7, although degenerative changes are already evident, numerous sperms are still retained in the seminiferous tubules. By day 14, considerable involution of the germinal epithelium and sperm fragmentation has occurred, whereas on day 21 following hypophysectomy, the germinal epithelium is extremely involuted and sperm is completely absent. It has been suggested that the decrease in the activity of the mouse testicular 'LDH-X' isoenzyme following hypophysectomy is due partly to the loss of maturing spermatids as well as the regression of the seminiferous tubular epithelium to the primary spermatocyte stage [12]. That the two rat gonadal-specific LDH isoenzymes are carried by sperm is indicated by the electrophoretic visualization of only G_1 and G_2 in sonicated homogenates of washed sperm obtained from the epididymis.

Electrophoretic patterns from testicular homogenates of animals in group C, which have been treated with gonadotrophins, or animals in group D, treated with testosterone, are identical after day 7, 17 or 21 following hypophysectomy. Such electrophoretograms, visualized after day 21, are also shown in fig. 1. Electrophoretogram C is from the testicular homogenate of an animal sacrificed 21 days after hypophysectomy, but which was given replacement therapy with FSH and LH beginning on day 2 after hypophysectomy. Similarly, electrophoretogram labelled D is from the testicular homogenate of an animal sacrificed 21 days after hypophysectomy but which was given replacement therapy with testosterone starting on day 2 after hypophysectomy. Histologically, the testis from animals in groups C and D 21 days

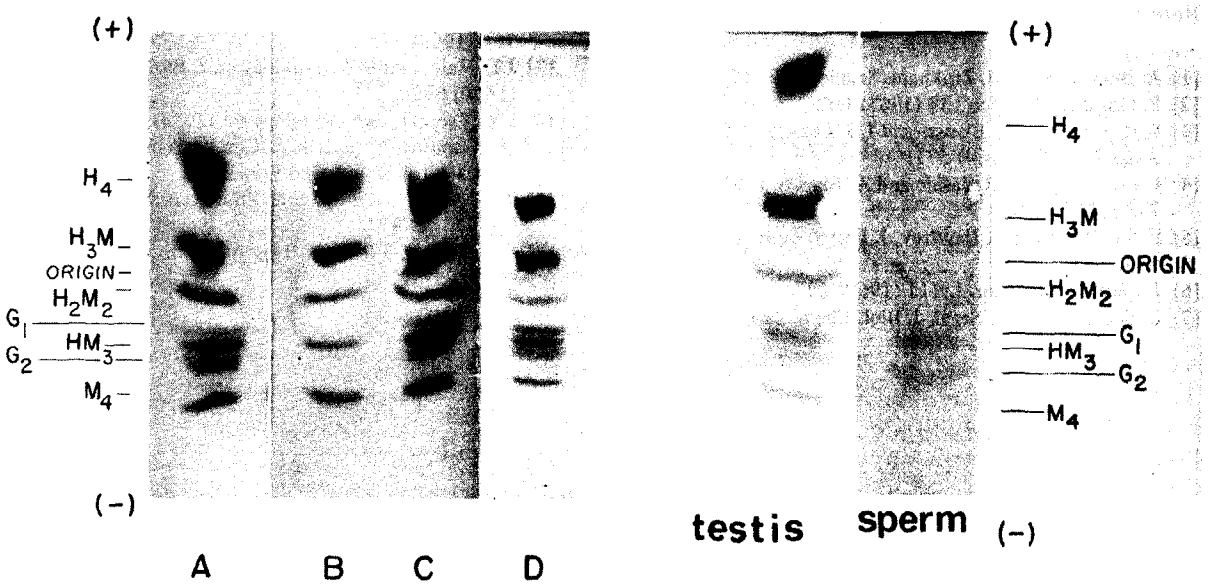

Fig. 1. Cellulose acetate electrophoretograms of rat gonadal LDH isoenzymes in homogenates of mature rat testes on day 21 after onset of experiment (see text for details). (A) normal untreated animal, (B) hypophysectomized saline-treated, (C) hypophysectomized gonadotrophin-treated, and (D) hypophysectomized-testerosterone-treated. Position of the gonadal-specific LDH isoenzymes is indicated by G_1 and G_2.

after hypophysectomy and replacement therapy with either FSH and LH or testosterone show no degenerative changes and numbers of sperm are retained in the seminiferous tubules. From experiments performed at different times after hypophysectomy, it appears that the activity of the gonadal LDH isoenzymes can be fully maintained by gonadotrophin or testosterone treatment in hypophysectomized animals providing replacement therapy is initiated prior to day 14 after hypophysectomy. If replacement therapy is begun after day 14, the gonadal LDH isoenzymes disappear at about day 21 and the electrophoretogram is similar to that observed in animals in group A. In the case of hyaluronidase, deprivation of gonadotrophins for just a 2 day interval between hypophysectomy and hormone replacement therapy is sufficient to prevent the attainment of normal enzyme levels for an additional 30–35 days [9]. Attempts to initiate the earlier appearance of the gonadal-specific LDH isoenzymes in immature rats (2 days post partum) with daily injections of either testosterone or FSH and LH were unsuccessful.

Similar experiments with hyaluronidase were likewise unsuccessful [9], suggesting that incubation of at least these two enzyme systems occurs at a definite time in the development of spermatogenesis and cannot be advanced by hormonal stimulation. Two other enzymes involved in sperm metabolism, β-glucuronidase and acid phosphatase have recently been studied in the rat following hypophysectomy [13]. The β-glucuronidase activity which normally decreases with sperm age and acid phosphatase which normally increases with sperm age revert to the immature type of activity following hypophysectomy.

For the gonadal LDH isoenzymes, it is interesting to speculate that there may occur a 'turning on', by the pituitary gonadotrophins or the testicular testosterone, of the gene responsible for the synthesis of the gonadal-specific polypeptide subunit, which is presumably repressed in the immature rat testis.

Acknowledgements

We thank Miss Betsy Dickinson and Mrs. Carolyn Steckler for Technical assistance and Grants SM-05084 (MBW) and FR-05374 (ADW) for financial support.

References

[1] A. Blanco and W.H. Zinkham, Science 139 (1963) 601.
[2] E. Goldberg, Science 139 (1963) 602.
[3] W.H. Zinkham, W. Blanco and L.J. Clowry, Ann. N.Y. Acad. Sci. 121 (1964) 571.
[4] L.J. Battallino, F.R. Jaime and A. Blanco, J. Biol. Chem. 243 (1968) 5185.
[5] E. Goldberg and C. Hawtrey, J. Exptl. Zool. 164 (1967) 309.
[6] J. Clausen, Biochem. J. 111 (1969) 207.
[7] L. Schatz and H.L. Segal, J. Biol. Chem. 244 (1969) 4393.
[8] A. Blanco, M. Gutierrez and C.G. De Henquin, Science 164 (1969) 835.
[9] J.L. Males and R.W. Turkington, J. Biol. Chem. 245 (1970) 6329.
[10] J.W. Everett, Endocrinology 54 (1954) 685.
[11] W. Gerhardt, J. Clausen, E. Christensen and J. Riisheda, Acta Neurol. Scand. 304 (1963) 85.
[12] A.W. Blackshaw and J.S.H. Elkington, Biol. Reproduction 2 (1970) 268.
[13] J.L. Males and R.W. Turkington, Endocrinology 88 (1971) 579.