OVIDUCAL LACTATE DEHYDROGENASE: HORMONAL INFLUENCES ON THE EXTRACELLULAR ISOENZYMES

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1. Introduction

Lactate dehydrogenase (E.C.1.1.1.27) exists in most mammalian tissues as five molecular forms. Each of these isoenzymes is a tetramer, and the multiplicity of the enzymes may be accounted for by the presence of two types of subunit (A and B), which are under the control of separate genetic loci. Hybridization between the different polypeptides allows for a sequence of heteromorphs whose subunit composition may be represented as A_4, A_3B, A_2B_2, AB_3, B_4 [1, 2].

Considerable interest has been evinced, recently, in the role of lactate dehydrogenase and its isoenzymes in early mammalian development. The developmental progressions of these multiple enzyme forms have contributed greatly to our understanding of differential gene control in early ontogeny [1–5], and it has become evident that the reactants of this enzyme (i.e. lactate and pyruvate) are of prime importance as energy sources during the initial stages of cell multiplication [6]. Furthermore, preimplantation mammalian ova exhibit extremely high levels of associated lactate dehydrogenase activity [6, 7].

An additional dimension to the established, major significance of this enzyme in early ontogeny, has been provided by the recent discovery that the extracellular fluid of the mammalian oviduct was a remarkably potent source of lactate dehydrogenase activity [8], and the implications of this finding led to the present investigation of hormonal influences on this oviducal enzyme.

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2. Methods

Mature female New Zealand white rabbits (approximately four kg body weight) were used in these experiments. After ligation of the oviduct, and transection at the utero-tubal junction, a polyethylene canula was secured to the tubal ostium, and led through the lateral abdominal wall to a polyethylene collection tube [9]. Fluid was removed by aspiration every twentyfour hours. In the second week after the operation, the test animals were injected with 10 mg progesterone in peanut oil, intramuscularly, every twentyfour hours for four consecutive days. The collection of the oviducal secretion was continued for several days after the completion of the course of hormone injections, and the samples were centrifuged at 100,000 *g* for 30 min, before analyses were undertaken.

Lactate dehydrogenase activities were assayed by measuring the rate of optical density decrease at 340 mμ resulting from the oxidation of reduced NAD in the presence of 0.0084 M sodium pyruvate and a sutable dilution of enzyme. The measurements were made on a Unicam recording spectrophotometer (Model SP800) at 30°C and pH 7.4 [3]. Protein determinations were performed spectrophotometrically with crystallized bovine serum albumin for standards [10].

As well, the supernatant fractions of these fluids were subjected to zone electrophoresis on vertical

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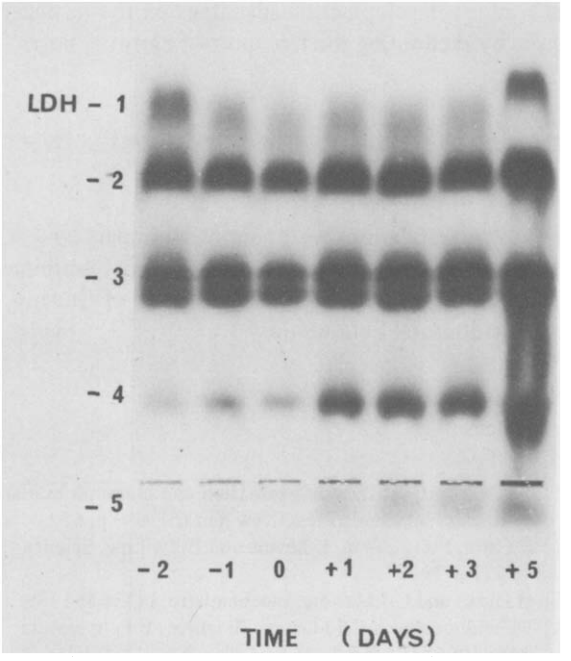

Fig. 1. Zymogram of lactate dehydrogenase activity in the oviducal secretion of the rabbit, before and after progesterone injections.

starch gels, with tris-glycine buffer (0.03 M, pH 9.0); and the regions of lactate dehydrogenase activity were demonstrated by a tetrazolium dye staining technique. The resulting zymograms were scanned in an integrating densitometer, and the percentage of B-type activity calculated. This methodology has been described in detail, elsewhere [3].

3. Results

The results of these analyses are summarized in fig. 1 and table 1.

In the initial period of collection, the zymograms display most of the lactate dehydrogenase in the LDH-2, and LDH-3 positions with a doublet of activity clearly resolved in the median isoenzyme region. On the day after injection of progesterone, and on consecutive days of this treatment, an increase in the specific activity of the lactate dehydrogenase in the fallopian tube secretion was observable, and at the same time, a shift in the emphasis of distribution of

Table 1

Oviducal lactate dehydrogenase. The influence of progesterone on the activities in the rabbit. Time is expressed in relation to the commencement of progesterone injections which continued for four days. Specific activity is expressed as international units of activity per mg protein. Results are the mean values for three animals.

Time (days)	Specific Activity	Percentage B-type
-2	0.32	65
-1	0.28	63
0	0.25	65
+1	0.44	60
+2	0.76	58
+3	0.87	58
+4	0.83	58
+5	1.4	52
+6	0.15	65
+7	0.10	65

multiple forms towards an increased A-type contribution (fig. 1, table 1). The highest values for activity occurred soon after the finalization of the hormone injections, and specific activity values then declined below pre-injection values. Neither, the addition of progesterone to oviduct fluid, nor the parallel collection on fluid from animals injected with peanut oil only, elicited comparable alterations of lactate dehydrogenase activity.

4. Discussion

These findings establish that progesterone treatment exerts an appreciable influence on the lactate dehydrogenase activity of the oviducal secretion; a result which appears to possess wide implications in relation to the biology of reproduction and early morphogenesis.

It is evident, for example, that the preimplantation ova are immersed in this oviducal fluid, and dependent upon this selective environment for normal growth and development [11]. In particular terms, the extraordinary magnitude of the lactate dehydrogenase activities constitutes a unique compositional feature of this ex-

tracellular secretion [8], and again, the principal reactants of this enzyme are established as critical energy sources during the initial stages of cell multiplication in the zygote [6]. Hence, physiological factors influencing the nature of the lactate dehydrogenase activity in the fallopian tube seem perforce, to bear significantly upon the developmental processes in early ontogeny. Progesterone, for its part, is recognized as a potent, natural luteoid with extensive involvements in mammalian reproduction, and whilst sex steroids have been shown to influence the intracellular activity of the lactate dehydrogenase in uterine previously [13, 14], the present results appear to provide the first demonstration of an hormonal regulation of the immediate extracellular environment of the preimplantation stages of mammalian development.

In relation to the changes induced in the oviducal lactate dehydrogenase by this hormone, it is interest to note the nature of the time-response correlationships. Although the initiation of progesterone injections of itself causes a modest elevation of enzyme activity, the most marked increase in total activity may be observed to follow closely the actual termination of the injection course (table 1). This time sequence suggests that it may be a metabolic product of progesterone, and not progesterone per se, which is the causative agent in the elevatory process, and this interpretation is supported by experiments with other steroids and other species [15].

Relating this sequence of events to the ovulatory process, it may be remarked that the release of the mammalian egg from the ovary to the oviduct is normally attended by a decrease in the peak levels of circulating progesterone [12]: hence, by analogy with the present results, a surge of lactate dehydrogenase activity in the oviducal fluid would be expected to coincide with the entry of the egg into the ampulla, and confer a developmental advantage on the fertilized zygote by facilitating the transport of essential nutrients.

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