THE INFLUENCE OF PROGESTERONE ON THE LACTATE DEHYDROGENASE ISOENZYMES OF THE RAT REPRODUCTIVE TRACT

Carla PATTERSON and C.J.MASTERS
Department of Biochemistry, University of Queensland, St. Lucia, 4067, Brisbane, Australia

Received 16 October 1970

1. Introduction

In most vertebrate species, lactate dehydrogenase (LDH) exists in five molecular forms of subunit composition A_4 (LDH-5), A_3B (LDH-4), A_2B_2 (LDH-3), AB_3 (LDH-2) and B_4 (LDH-1); with A and B being different polypeptides under the control of separate genetic loci [1–3].

Following the demonstration of extraordinary levels of LDH activity in the extracellular secretion of the mammalian oviduct [4], the indications of the significant role occupied by this enzyme in reproduction and early ontogeny [5], and the evidence of hormonal augmentation of this enzyme [6], it was considered pertinent to define the influence of progesterone in the whole reproductive tract i.e. ovary, fallopian tube, uterine horns and uterus, as well as the associated fluids. The present communication describes these findings.

2. Methods

Mature female rats of the locally bred Wistar strain were separated into groups according to the stage of their estrus cycle (proestrus, estrus, metestrus, diestrus). This was determined by means of vaginal smears. Animals in estrus were injected intramuscularly with 0.2 mg progesterone on each of four consecutive days – the animals having been tested for normal periodicity, previously. At least 6 rats were sampled on every day during the sequence studied. The rats were killed by decapitation at appropriate times in relation to the sequence of progesterone treatment, and the reproductive tract was removed and dissected into ovary, fallopian tube and uterus. Extracellular secretions were collected from the fallopian tube and uterus by flushing with minimal quantities of tris buffer, pH 8.0, 0.01 M. The tissues were then homogenized in the same buffer, and both homogenates and fluids centrifuged at 50,000 *g* and 5° for one hr.

LDH activity was determined by measuring the rate of optical density decrease at 340 nm and 30° resulting from the oxidation of NADH in the presence of 0.0084 M pyruvate as substrate and an appropriate quantity of enzyme [7]. Protein was estimated by the method of Lowry et al. with recrystallized bovine serum albumin as a standard [8]. Electrophoretic patterns of isoenzyme distribution were established by vertical starch gel electrophoresis and tetrazolium coupled staining followed by scanning in an integrating densitometer in order to calculate the relative contributions of A- and B-type activity [9].

3. Results

The activities of lactate dehydrogenase in the tissues and fluids of the rat reproductive tract are summarized in table 1; with a normal cycle as control, and with the response to progesterone treatment.

Following the commencement of progesterone injections, most tissue activities show a tendency towards slightly lower values. It is noticeable however that the fallopian tube shows a marked increase in activity, some days after the progesterone treatment had finished, and that the fallopian and uterine fluids also increased in specific activity following the progesterone injections. Associated with the more notable augmentations of activity in the last days of the ex-

Table 1

Lactate dehydrogenase activities in the rat reproductive tract under the influence of progesterone. Time is expressed in relation to the commencement of progesterone injections which continued for four days. Activity if expressed as international units of activity per mg protein. Results are the mean values for six animals.

Time	Stage of cycle	Ovary	Fallopian tube	Uterine tube	Fallopian secretion	Uterine secretion
Normal cycle	Metoestrus	0.95	1.6	0.9	1.0	0.6
	Dioestrus	1.7	0.75	1.0	1.4	0.8
	Proestrus	1.8	1.8	1.2	2.4	0.7
	Oestrus	1.2	1.5	1.9	1.1	0.9
+ 1 day	Oestrus	0.85	1.8	1.9	1.7	1.0
+ 2	Oestrus	1.1	1.5	1.8	1.8	0.8
+ 3	Metoestrus	0.75	1.7	1.1	2.0	0.6
+ 6	Metoestrus	0.6	1.5	1.1	1.6	1.5
+ 7	Metoestrus	0.8	4.4	0.8	2.6	2.0

perimental period were marked shifts towards a decreased A-type content in the fallopian tube, and a corresponding increase in the A-type percentage of the fallopian tube fluid. These shifts of subunit type were of the order of 15% in each of these cases.

4. Discussion

Progesterone is a potent natural luteoid, with extensive involvements in mammalian reproduction. It has been reported recently that the injection of this hormone into rabbits induces a marked augmentation of the specific activity of lactate dehydrogenase in the oviducal secretion; and this finding appears to hold the promise of wide significance in terms of the energy requirements of preimplantation ova, the metabolic interconversions of the reproductive hormones, and the biochemistry of mammalian ontogeny [5]. The present results serve to extend the delineation of these hormone–isoenzyme relationships, both by the provision of comparative mammalian data, and by the inclusion of the responses of the separate, component tissues in the reproductive tract in addition to those of the oviducal fluid.

Although vertebrate animals differ markedly in the details of their reproductive patterns, it is evident from the present data (table 1), that the increased LDH activity of the fallopian secretion caused by progesterone treatment is not a characteristic which is restricted to the type of mammal exhibiting induced ovulation (e.g. the rabbit), but also occurs in this example of a polyestrous spontaneous ovulator (a type of reproductive behaviour which is far more wide-spread among the higher mammals). Furthermore, the peculiar delayed nature of the enzyme augmentation in the oviducal fluid which was observed with the rabbit is again evident in the fallopian fluid of the rat, as is the common occurrence of a shift towards increased A-type LDH activity at this time (table 1, [6]). These facts, then, generalize the suggestions that the causative agent in these processes may not be progesterone, per se, but rather a metabolic product of this hormone; and that this hormonal augmentation of LDH activity may lead to a developmental advantage for the zygote [5].

In addition, the present information would seem to comment significantly on the localization of the hormonal response in this situation, and on the probable derivation of much of the extraordinary activity levels of LDH in the extracellular secretion of the mammalian reproductive tract. The notable increase in enzyme activity (and shift of subunit expression in the fallopian tube on day 7, coupled with the associated increase and type shift in the LDH of the oviducal secretion) points to the fallopian tube as the region of the reproductive tract which is most heavily influenced by this hormone. In this context, it may be remarked that this localization of a hormonally directed enzyme response is in distinct contrast to much of the

present emphasis in reproductive physiology, which is more often directed towards the uterus or ovary as target tissues. Furthermore, the sequence of events described above may be viewed as consistent with the explanation that progesterone treatment results in an increased synthesis of LDH in the rat fallopian tube, with an accompanying alteration of the membrane permeability in this region of the reproductive system, allowing increased release of the tissue enzyme into the oviducal fluid. While this suggested mechanism correlates well with histochemical studies of this process [10], the precise definition of this biochemistry awaits additional investigation.

Acknowledgements

These investigations were supported in part by grants from the Australian Research Grants Committee.

References

[1] T.Wieland and G.Pfleiderer, in: Advances in Enzymology, Vol. 25, ed. F.F.Nord (Interscience, New York, 1963) p. 329.
[2] C.L.Markert, in: Cytodifferentiation and Macromolecular Synthesis (Academic Press, New York, 1963) p. 65.
[3] R.Cahn, N.O.Kaplan, L.Levine and E.Zwilling, Science 136 (1962) 962.
[4] Carla Gibson and C.J.Masters, J. Reprod. Fertility 22 (1970) 157.
[5] P.Georgiev, R.S.Holmes and C.J.Masters, Biochim. Biophys. Acta, in press.
[6] P.Georgiev, Carla Gibson, R.S.Holmes and C.J.Masters, FEBS Letters 6 (1970) 238.
[7] F.Wroblewski and J.S.La Due, Proc. Soc. Exptl. Biol. N.Y. 91 (1957) 231.
[8] O.H.Lowry, N.J.Rosebrough, A.K.Farr and R.J.Randall, J. Biol. Chem. 193 (1951) 265.
[9] M.Hinks and C.J.Masters, Biochemistry 3 (1964) 1789.
[10] C.J.Masters, unpublished data.