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Lactate Dehydrogenase Activity in Oviducts from Transgenic Mice

Amelia L. Dean, Kathleen A. Farley, Kelly W. Glass, T.E. Wagner** and Marjorie A. Jones

Departments of Chemistry and Biological Sciences
Illinois State University
Normal IL. 61761
(309) 438-2366

**Edison Center, Ohio University
Athens, OH 45701

ABSTRACT

Lactate dehydrogenase (LDH) activity in mouse oviducal homogenates was compared between litter mates expressing either human growth hormone (hGH) or human beta Interferon genes. Within a group of litter mates, the presence of the exogenous human gene had no apparent effect on LDH activity. The results are surprising since the transgenics receiving the hGH gene are substantially larger than their litter mates.

INTRODUCTION

The use of transgenic animals in basic research is rapidly expanding due to our increased understanding of the molecular biology of genetic material transfer. However there are currently few reports exploring the potential enzymic changes in the reproductive tract of animals receiving genes especially those which might not normally be evaluated in the reproductive tract. It is conceivable that transgenics may exhibit effects on regulation, directly or indirectly, of genes which are essential in reproduction in either the male or female animal. It has been reported that expression of growth hormone genes in mice is associated with reproductive disorders (Bartke et al., 1988). To further probe this question we have studied the effects of two different transgenic models on a specific enzyme, lactate dehydrogenase (LDH: EC 1.1.1.27), in the female oviduct. From other studies (Farley et al., 1990) it was suggested that LDH is an important

enzyme in the oviduct of mice. Early embryos use both pyruvate and lactate, in preference to glucose, for metabolic energy (Georgiev et al., 1970). LDH catalyzes the interconversion of pyruvate and lactate and, therefore, may have an important role in the regulation of the ratio of these energy sources available for the embryo.

Regulation of the amounts and activities of LDH is certainly of interest in successful embryo development and oviducal transport. In our studies, therefore, transgenic mice were tested in order to determine if the new gene affected LDH activity. Two transgenic models were used: human growth hormone (hGH) and human beta interferon (Interferon).

MATERIALS AND METHODS

Materials

Pyruvate, nicotinamide adenine dinucleotide reduced form (Grade III) (NADH), and Bovine Albumin Serum (BSA, Fraction V) were purchased from Sigma Chemical Co. (St. Louis, MO). All water for reagents was doubly distilled and subjected to a Millipore filter system (Corning mega-pure three liter automatic). Kreb's Ringer bicarbonate buffer was made from 6.9 g/L NaCl, 0.35 g/L KCl, 0.16 g/L KH_2PO_4, 0.14 g/L $MgSO_4$, 0.90 g/L glucose, 2.1 g/L $NaHCO_3$, and 0.18 g/L $CaCl_2$ (pH 7.4). All other chemicals were obtained from standard suppliers.

Mice

Transgenic and nontransgenic litter mates (control mice) were obtained from the colonies of Dr. Thomas E. Wagner, Ohio University, Athens, Ohio and housed in the animal facilities at Illinois State University until used. Transgenic mice used in this study were produced by the methods of Wagner et al., (1981). Transgenic strains used were metallothionine promoter driven transgenic human growth hormone(hGH) and Beta Interferon. All tissues were obtained from transgenic mice (+ Interferon or + hGH) or non-transgenic litter mates (-Interferon or - hGH) of approximately 8 months of age. The animals were sacrificed by cervical dislocation, followed by rapid removal of the oviducts. The oviducts were trimmed of fat, weighed, and kept on ice. They were then placed in 1 mL of Kreb's Ringer bicarbonate buffer in a microcentrifuge tube (1.5 mL polypropylene tube, Tekmar Co., Cincinnati, Ohio) and homogenized using a Brinkmann Polytron (probe size PTA 7K) on a setting of 3 for 30 seconds. All steps were done as rapidly as possible on ice.

Measurement Of Oviducal Activity

All oviducts were analyzed spectrophotometrically at 340 nm using the method of Reeves and Fimognari (1963) . In brief this involved: 1.8 mL of Kreb's Ringer bicarbonate buffer, 0.1 mL of 3.3 mg/mL of NADH, 0.1 mL of 3.9 mg/mL of pyruvate, and 0.2 mL of oviducal homogenate placed in a quartz cuvet and inserted into a Beckman DU-40 spectrophotometer set at 340 nm. A change in

absorbance was monitored for 2 minutes. As a control, a rate was determined for each oviduct using identical conditions but omitting the pyruvate. In all cases, this rate was negligible.

Protein Assay

Protein from oviducal homogenates was evaluated by the method of Lowry et al., (1951) using BSA as a standard.

Specific Activity Calculations

The units of LDH which catalysed the oxidation of umoles of NADH per minute were determined by dividing the rate (change in absorbance at 340 nm per minute) by the extinction coefficient which is 6.2. The specific activity of LDH was calculated by dividing the umoles of NADH oxidized per min per mg of homogenate protein. Statistics were performed using analysis of variance. Data were considered to be significantly different at $p < 0.05$.

RESULTS

The reproductive tracts, specifically the ovaries, of the transgenic mice were observed to be grossly different from those of the controls in the same group as well as different from those of the ICR strain we currently work with in the laboratory. Location of the ovaries proved especially difficult from the hGH transgenics because the tissues were embedded in a large layer of fat. In addition, we noted that the ovaries of the transgenic mice were approximately one half the size of the control strain and appeared to be inactive. The corpora lutea and follicles which should normally be present were visually absent from the hGH transgenic strain.

Individual oviducts from transgenic and normal mice were weighed and recorded (Table 1). These wet weights show that statistically there is no difference between the weights of oviducts from normal and the Interferon transgenic litter mates or between normal and the hGH transgenic litter mates. Furthermore, there was no statistical difference between the two groups of mice in this measurement.

The amount of protein per oviduct was estimated using the method of Lowry et al., (1951) relative to a BSA standard. The total amount of protein (ug) per oviduct was found to be statistically different between the transgenic hGH group and the oviducts from the ± Interferon group, but not different from the hGH litter mates (Table 1). This suggests that the hGH gene does not change the ratio between the wet weight of a tissue and the protein content of that tissue.

However, when the data were normalized as ug protein per mg oviduct wet weight the values within each group were not significantly different from each other. The value for the transgenics expressing the Interferon gene appeared to be significantly smaller than the value for the controls from hGH group but not the transgenic litter mates expressing the hGH gene. However, no significant differences were observed between these two groups when only total protein per oviduct was calculated. Finally specific activities of the lactate dehydrogenase

were calculated and reported in Table 2 as umoles of product formed/min/mg protein. The specific activities of control and transgenic interferon litter mates were found to be statistically similar as were the control and transgenic hGH litter mates. However, there was a difference in specific activities between these two groups. The group ± Interferon had approximately 30% more activity than did the group ± hGH. This was an unexpected difference because weights of oviducts among the groups were not significantly different.

DISCUSSION

These enzyme experiments were performed in oviducal tissue in order to determine if LDH activity is modulated in transgenic mice relative to control litter mates. From other studies (Patterson and Masters, 1972; Farley et al., 1990) it has been demonstrated that the specific activities of oviducts, at all reproductive stages studied, are considerably higher than the values found in uterine tissues. In addition, the specific activities change in response to the reproductive state of the animal (Farley et al.,1990; Nieder and Corder, 1983). Since embryos are considerably dependent upon the lactate and pyruvate in their environment (Georgiev et al., 1970), especially in the oviduct, LDH is considered to have some effect on successful reproduction. In this study, LDH was easily detected in the transgenic and normal mouse individual oviducts. The specific activities of LDH in mouse oviducts (Table 2) show that among litter mates there was no difference; however, between groups there clearly was a difference. These results are quite surprising in view of the facts that the wet weight of oviducts between groups and within the same group, with and without the foreign gene, were not significantly different. In addition, the hGH mice were much larger (total body weight) than either their litter mates or the ± Interferon group (Bartke, et al., 1988). Total protein per oviduct does show a difference between the groups. These data suggest that the specific activities of enzymes are probably the best way to compare results between groups, tissues, and other variables when using animals to study enzymic reactions.

The results from the transgenics receiving the hGH gene are rather surprising especially in view of the fact that the animals are substantially larger than their litter mates. We had anticipated that the reproductive organs would reflect some change in size. Shea et al.(1987) report that, in transgenic mice expressing growth hormone genes, some organs, such as spleen, kidney, and liver, increased in size in proportion to the increase in body weight. However, they did not evalute reproductive tissues. Our data have implications for the reproductive capabilities of transgenic versus normal litter mates. Since there are few changes in the size of the reproductive organs, the apparent amount of proteins, or the activity of one specific enzyme, LDH, one would anticipate few reproductive problems with these transgenic mice. This is, however, not always the case since Bartke et al. (1988) report a decrease in the fertility of female hGH transgenics. Transgenic animals produce potentially fertile eggs, mate, and yet fail to become pregnant. Bartke et al. (1988) attribute the infertility to luteal deficiency. Possible abnormal oviduct function could also contribute to reproductive failure. Our lack of differences in the specific activities of LDH, however, argues against this activity being involved with the sterility of

transgenics. In addition, we suggest that when using transgenic mice it is important to carefully compare litter mates of mice used in the investigation as it may be difficult to compare data between groups.

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Table 1. Wet weights and protein values of mouse oviducts*

GROUP	N	μMOLES/MIN/mg PROTEIN (Mean±SD)
- Interferon	5	0.320±0.001a
+ Interferon	10	0.299±0.046a
- hGH	7	0.227±0.058b
+ hGH	7	0.191±0.025b

N = number of animals

values with the same letter are not significantly different ($p<0.05$)

Table 2. Specific activies of LDH in mouse oviducts

GROUP	N	WET WEIGHT(mg) OVIDUCT	TOTAL PROTEIN OVIDUCT (μg)	μg PROTEIN mg OVIDUCT
- Interferon	10	3.40±0.84[a]	230±29.6[b]	72.8±27.1[de]
+ Inteferon	20	3.85±1.27[a]	233±76.8[b]	60.1±17.1[d]
- hGH	14	3.64±1.69[a]	281±158[bc]	80.6±47.5[e]
+ hGH	14	4.14±12.3[a]	322±88.3[c]	80.5±19.7[de]

* MEAN ± SD

N = number ofoviducts

values with the same letter are not significantly different ($p<0.05$)