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Increased Glycolysis in Immature Rat Ovaries Using Endogenous Gonadotropins Secreted in Response to Exogenous Luteinizing Hormone-Releasing Hormone[1] (38919)

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(Introduced by R. L. Clancy)

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Many of the physiological responses to gonadotropins require the availability of the hormone for several hours in order to gain expression (1–3). Changes in the rate of glycolysis, measured by *in vitro* accumulation of lactic acid, or utilization of glucose, in ovaries from immature rats have been shown to be a dose related response to many gonadotropins including luteinizing hormone (LH) and follicle stimulating hormone (FSH) (4). Ahren, Hamberger and Perklev (5) reported that a 1 min *in vivo* exposure to LH was sufficient to obtain the response. Furthermore, these authors suggested that increased plasma LH which came from the animal's own pituitary might cause an increase in glycolysis which would persist long after the hormone concentration had returned to normal. These findings suggested that glycolysis might be a useful biologic response for the study of altered concentrations of LH and FSH induced by the administration of luteinizing hormone-releasing hormone (LH-RH).

Material and Methods. Immature (23–25 day) female rats of the Holtzman strain were used. They were kept 10–12 per cage on cedar shavings with free access to Purina laboratory chow and tap water. The room was temperature (24 ± 2°) and light (0600–2000 hr) controlled. Increases in endogenous gonadotropin concentrations were produced by injections of synthetic LH-RH. This material, prepared by Dr. John Stewart of the University of Colorado School of Medicine, was dissolved in saline and stored frozen. After complete anesthetization with urethane (150 mg/100 g body weight) a 25 gauge needle, with syringe attached, was inserted and allowed to remain in a tail vein. A 0.1 ml bolus of saline containing the LH-RH was injected every 10, 20 or 30 min. The animals were killed by decapitation 10 min after the last injection and the blood from the trunk collected in small centrifuge tubes. After clotting for 30 min at room temperature, and overnight at 4°, the serum was separated and either assayed for FSH and LH immediately or stored at −17°. The ovaries were removed a few seconds after killing, cleaned of extraneous tissue, weighed on a torsion balance, and placed in 10 ml Erlenmeyer flasks containing 1 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) and 5.5 m*M* glucose. The flasks were gassed with 95% O_2 and 5% CO_2, sealed, and incubated for 2 hr in a Dubnoff shaker at 37°. At the end of the incubation the fluid was removed and assayed for lactic acid content using the enzymatic method (6). The results were expressed as μg of lactic acid per mg of ovary formed per 2 hr.

LH and FSH in the serum were measured by double antibody radioimmunoassays using kits supplied by the NIAMDD-Rat Pituitary Program. The results were calculated by the method of Rodbard, Bridson and Rayford (7) and expressed in terms of the standards (RP-1) supplied with the kits.

Results and Discussion. Previous experience (2) had indicated that the rate of glycolysis in immature ovaries was increased to a maximum within 60 min after *in vivo* exposure to exogenous gonadotropin and remained elevated for at least 4 hr. However, increased endogenous gonadotropin, produced by a single intravenous injection of 20, 100 or 200 ng of LH-RH, had no effect upon the rate of lactic acid production when

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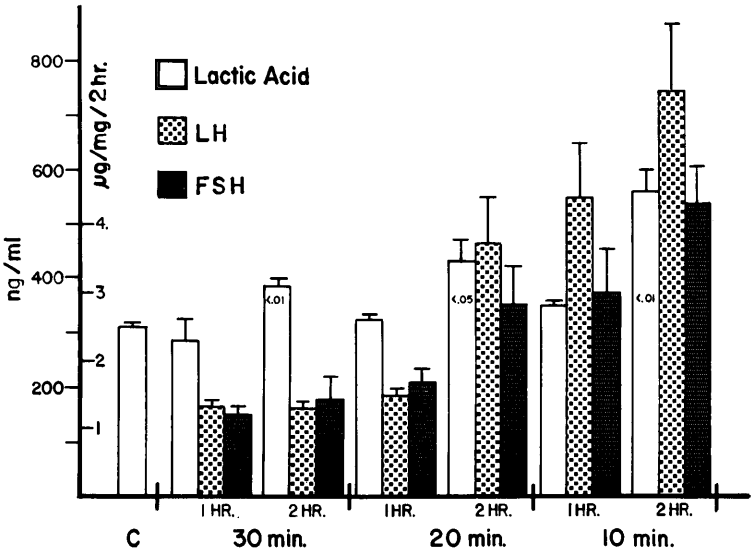

FIG. 1. Serum LH and FSH in animals killed 70 min (1 hr) or 130 min (2 hr) after the first of several 20 ng injections of LH-RH. The interval between doses is indicated below each set of bars. The mean levels of gonadotropins in controls were 42.5 ± 2.0 ng/ml for LH and 155 ± 9.0 ng/ml for FSH. The lactic acid produced by ovaries from these animals during a 2-hr incubation *in vitro* is shown by the open bars. The indicated level of significance is relative to the controls at the left. Standard errors for groups of five animals are shown by vertical lines.

measured 2 hr later. This failure to respond suggested that either the level of gonadotropin was not high enough or that the elevated level was not sustained long enough. The next studies used multiple injections of LH-RH so that both of these factors could be controlled.

The results shown in Fig. 1 demonstrate that ovarian glycolysis can be increased by endogenous gonadotropins. However only ovaries from animals treated for 2 hr had a rate significantly higher than that for the controls. When measured after 2 hr glycolysis was not significantly higher in animals treated every 20 min than in those treated every 30 min but a dose of LH-RH every 10 min did produce a higher rate ($P < 0.05$).

When given every 30 min LH-RH caused a 390% increase in serum LH when compared to controls but FSH was not significantly altered (Fig. 1). LH-RH injected every 20 min for an hour (4 doses) produced the same LH and FSH levels as did the 30-min injections, but during the second hour (7 doses total) there was a 150% increase in LH ($P < 0.01$) and a 67% increase in FSH ($P > 0.05$). An injection every 10 min for an hour (7 doses) produced a serum LH level of 549 ± 99 ng/ml which was more than 10 times the value for controls; FSH was increased 2.4 times that of controls. Continuation of the injections for another six doses caused a further increase in both gonadotropins but the difference from the 1 hr group was statistically insignificant ($P > 0.05$).

A review of Fig. 1 reveals that an increase in the rate of ovarian glycolysis requires exposure to hormone for a distinct period of time but that after this lag the rate is a function of the serum concentration of gonadotropin.

In a second series particular attention was given to the lag period for stimulation of glycolysis. Animals were injected every 10 min with 10 ng of LH-RH and groups were killed 6 min after each dose to determine the effect on serum LH and FSH; previous experience indicated that 10 ng produced a very moderate increase in gonadotropin and that a peak for LH in the serum was reached in about 6 min. Two hours after the first injection ovaries from animals treated with either one, three, five or seven doses of

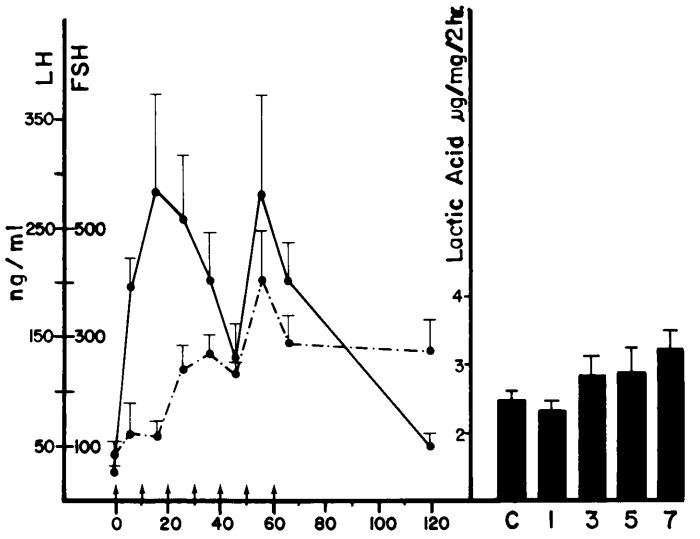

FIG. 2. Serum LH (solid line) and FSH (dashed line) measured in groups of five rats killed 6 min after intravenous injection of 10 ng of LH-RH. Each dose of releasing hormone is indicated by an arrow. The last injection was given at 60 min. The ovaries were removed from animals treated with 1, 3, 5 or 7 doses of LH-RH 2 hr after the first injection and incubated *in vitro* for 2 hr; the lactic acid production rate by these ovaries is indicated in the bar graph. Only animals treated with seven doses of LH-RH had a significantly increased rate of glycolysis.

LH-RH were incubated to determine their rate of lactic acid production.

The left part of Fig. 2 shows the LH and FSH concentrations after each injection of LH-RH. The LH value shown at 120 min is that measured in animals getting seven injections but it was not different from the value measured in animals getting one, three or five doses; i.e. LH had returned to the level found in starting controls before the ovaries were removed for incubation regardless of how many doses of LH-RH had been given. The FSH level at 2 hr was 230% higher ($P < 0.01$) than in controls but the concentration in animals getting seven doses of LH-RH was not significantly higher than in animals with fewer injections. The pattern of changes in gonadotropins clearly indicates that each dose of LH-RH did not have the same effect upon the pituitary.

The rates of glycolysis in ovaries removed 2 hr after the first injection of LH-RH, or saline, are shown in the right part of Fig. 2. The only animals which showed an increased rate were those treated with seven doses of LH-RH. Animals given three or five doses had slightly, though not significantly, higher rates than those given one injection or saline. We interpret these findings to mean that even rather modest increases in serum gonadotropins are sufficient to stimulate glycolysis provided they are maintained for about an hour.

Blake and Sawyer (8) reported that urethane injected intraperitoneally could alter ovarian function by a direct action. The possibility of the anesthetic agent altering glycolysis was investigated by comparing the rates of lactic acid production by ovaries from untreated controls with those from animals given urethane or urethane plus intravenous injections of saline or LH-RH. The rates were: 2.7 ± 0.11 µg/mg/2 hr for for the untreated controls; 2.68 ± 0.17 2 hr after urethane; 2.35 ± 0.16 after 13 injections of saline; and 3.56 ± 0.21 after 13 injections of LH-RH. The only group with a rate different from that of the untreated control was that treated with LH-RH.

Why do the ovaries require so much more exposure to endogenous than to exogenous gonadotropin to produce an effect upon glycolysis? Removal of immature rat ovaries one minute after an intravenous injection of bovine LH produced more than a 100% increase in the rate of lactic acid production

(5). In contrast, none of the ovaries exposed to increased amounts of endogenous hormone for less than an hour showed any increase in glycolysis. Certainly first consideration for this difference should be given to the doses of hormones involved. Ahren *et al.* (5) injected 100 μg LH/100 g body weight which is several orders of magnitude beyond the level possible to produce with LH-RH. Therefore the results obtained with the present study may be more realistic in terms of a physiological response. There may also be the problem of qualitative differences in the hormones used. Not only must we consider the difference between bovine and rat LH, but also the possible heterogeneity of rat LH secreted at various times after the injection of LH-RH. We have relied upon immunoreactivity for our assays and we cannot be sure that all of the hormone released is biologically the same. Until this problem is resolved we cannot be sure that comparisons between exogenous and endogenous hormonal effects are valid.

From the available evidence it appears that the increase in ovarian glycolysis induced by elevated concentrations of gonadotropins requires a distinct threshold of time and that sudden and brief changes have no physiologically important effects. Consequently, when considering serum levels of gonadotropins in the normal animal the length of time of increased concentrations appears equally important to the absolute amounts. Furthermore, apparent quantitative excesses of hormone for a biologic response, such as the proestrus surge of LH associated with ovulation, may be the consequences of a mechanism which insures sufficient time of elevated levels.

Summary. The rate of glycolysis, as indicated by *in vitro* lactic acid production, in ovaries from immature rats was stimulated by raising serum concentrations of endogenous LH and FSH by intravenous injections of luteinizing hormone-releasing hormone. The rate was shown to be a function of the serum concentration of gonadotropin but the response required the presence of elevated hormone levels for about 1 hr. These data are offered as further evidence for the lack of physiological importance of brief changes in pituitary function.

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