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RELATIONSHIP BETWEEN GLYCOLYSIS AND STEROIDOGENESIS IN CULTURED GRAAFIAN FOLLICLES STIMULATED BY LH OR PROSTAGLANDIN E_2

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SUMMARY

Graafian follicles isolated from 3-month-old rats during early pro-oestrus (8:00–12:00 a.m.) and placed in organ culture responded to luteinizing hormone (LH)(NIH-LH-S18; 5 μg/ml) with increased release of lactate and steroids (progesterone, androstenedione and oestradiol-17β) into the medium during 6 h incubations; addition of prostaglandin E_2 (PGE_2; 10 μg/ml) to the medium likewise enhanced aerobic glycolysis and progesterone accumulation in the medium. Cyanoketone (10^{-4} M) prevented the steroidogenic effects of LH and PGE_2, and reduced the basal rate of follicular steroidogenesis, without impeding the stimulatory action of either LH or PGE_2 on aerobic glycolysis. Aminoglutethimide (10^{-3} M) also suppressed basal as well as LH- or PGE_2-induced steroidogenesis, but did not impair the glycolytic effect of LH; the effect of PGE_2 on glycolysis, however, was reduced or abolished. Iodoacetate ($2{\cdot}5 \times 10^{-5}$ M) prevented the LH effect on glycolysis and significantly reduced, yet did not abolish, the steroidogenic action of LH. It is concluded that the hormonal stimulation of follicular glycolysis and steroidogenesis involves divergent pathways, rather than a single sequence of events. However, the glycolytic effect reinforces the steroidogenic action of LH, perhaps by supplying NADPH needed for steroid hydroxylation.

INTRODUCTION

Luteinizing hormone (LH) was shown to stimulate steroidogenesis in isolated preovulatory follicles of the rabbit[1] and the rat[2, 3] and to increase the rate of aerobic glycolysis[4, 5] and of glucose oxidation[6] by prepubertal rat ovaries *in vitro*. Prostaglandin E_2 (PGE_2) was also reported to stimulate progesterone production[3, 7], glycolysis [8] and oxidation of D-[6-^{14}C] glucose[6] in rat ovaries *in vitro*.

It has been shown that the initial interaction of LH or PGE_2 with ovarian tissue leads to stimulation of adenylate cyclase [for reviews see 3 and 9]. It is possible that enhanced steroidogenesis and glycolysis represent parallel phenomena initiated by increased ovarian cyclic AMP formation. Alternatively, they may constitute successive links in a single causal chain. Thus it has been suggested that the steroidogenic effect of ACTH may be dependent on replenishment of reduced cofactors through glycolytic mechanisms[10]; conversely, it was proposed that stimulation of glycolytic activity in the luteinized ovary by LH may be a sequel to increased steroidogenesis[11].

In the present study, we employed selective inhibitors of steroid synthesis and of aerobic glycolysis in order to analyze the relationship between these two responses to LH or PGE_2, namely steroidogenesis and glycolysis, in cultured rat Graafian follicles.

MATERIALS AND METHODS

Chemicals. Ovine LH (NIH-LH-S18), kindly supplied by the National Institutes of Arthritis and Metabolic Diseases, Bethesda, Md., was dissolved in saline (1 mg/ml). PGE_2, kindly made available by the Ono Pharmaceutical Co., Osaka, was dissolved in absolute ethanol (10 mg/ml) and diluted with 9 ml 2×10^{-3} M Na_2CO_3. Iodacetic acid (1·86 mg/ml) was dissolved in saline. Cyanoketone (2α-cyano-4,4,17α-trimethyl-5-androst-5-en-17β-ol-3-one), a gift of Dr. Potts of the Sterling–Winthrop Research Institute, was dissolved in absolute ethanol (2 mg/ml). Aminoglutethimide (Elipten, Ciba–Geigy AG, Basel) was dissolved in saline (20 mg/ml). Lactate dehydrogenase was the product of Sigma Chemical Co., St. Louis, Mo. [1,2,6,7-^{3}H]-progesterone (92 Ci/mmol), [1,2-^{3}H]-androstenedione (48 Ci/mmol), and [6,7-^{3}H]-estradiol (49 Ci/mmol) were purchased from New England Nuclear, Boston, Mass.

Animals. Three-month-old Wistar derived rats of the deparmental colony were housed in air-conditioned quarters, illuminated between the hours 5:00 and 19:00. Pelleted food (Ralston Purina Co., St. Louis, Mo.) and water were offered without restriction. Only animals that had shown at least

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two normal 4-day cycles, as determined by daily vaginal smears, were used.

Culture of follicles. Animals were killed by cervical dislocation between 8:00 and 12:30 on the day of pro-oestrus. The endogenous gonadotrophin surge in our colony begins at about 14:30 and reaches its peak at 17:00–18:00[12]. The follicles were excised and incubated as previously described[13], except that the osmolarity of the medium was 280 mosmol, fungizone was omitted, and the fetal calf serum used was dialyzed (Gibco). Inhibitors were added to the medium, in the appropriate treatment groups, immediately after isolation of the follicles, and LH (5 μg/ml) or PGE_2 (10 μg/ml) were added 30 min after starting the incubation. The follicles were cultured for an additional 6- or 12-h period, as specified. When inhibitors were not used, LH or PGE_2 were added to the medium immediately after isolation of the follicles.

Determination of lactic acid. At the end of incubations, lactic acid production was determined in aliquots of the medium by an enzymatic method[14], using a micromodification previously described[15].

Radioimmunoassay (RIA) of steroid hormones. The rate of steroid accumulation in the culture medium was measured by RIA. The general procedure used for RIA, and the determinations of progesterone, have been described[2]. Androst-4-ene-3,17-dione (androstenedione) and estradiol-17β were extracted from the medium with 5 vol. ether, and aliquots of the ether extract were taken for RIA using antisera raised with bovine serum albumin conjugates of androstenedione-7-carboxyethylthioether and estradiol-17β-6-carboxymethyloxime[16]. There was good agreement[16] between values obtained by RIA applied to unfractionated extracts of the culture media and those obtained by using extracts purified by paper chromatography (progesterone) or by Sephadex LH-20 chromatography (androstenedione and estradiol).

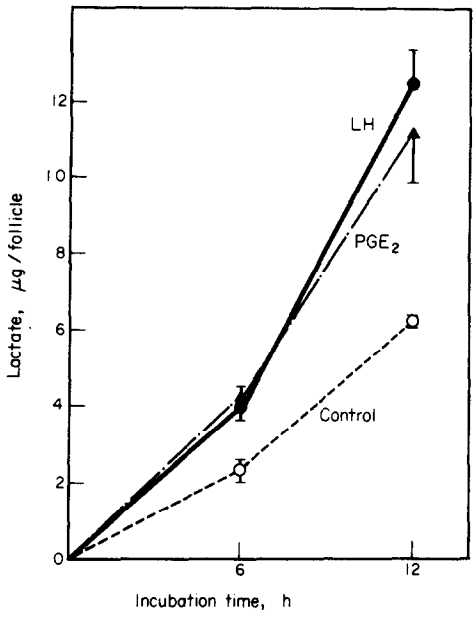

Fig. 1. Effect of LH (5 μg/ml) and prostaglandin E_2 (PGE_2; 10 μg/ml) on aerobic glycolysis in cultured Graafian follicles. Shown is the amount of lactate accumulated in the medium[15], divided by the number of follicles (5–6) in the culture dish. Vertical brackets, SEM (n = 4).

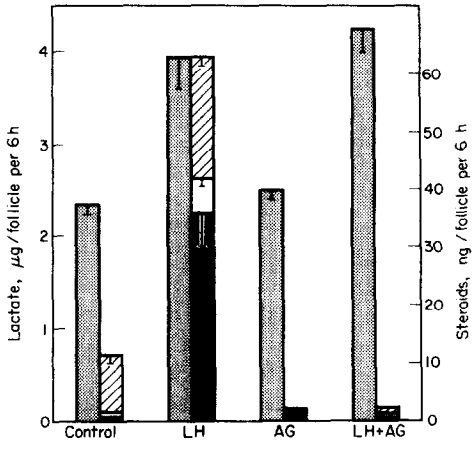

Fig. 2. Stimulation by LH (5 μg/ml) of lactate release from cultured Graafian follicles during inhibition of steroidogenesis by cyanoketone (10^{-4} M). ▨, lactate; ■, progesterone; □, androst-4-ene-3,17-dione; ▨, oestradiol-17β. Steroids were determined by radioimmunoassay. Vertical brackets, SEM (n = 8–9).

RESULTS

Hormonal stimulation of lactate production. Incubation of follicles with LH (5 μg/ml) stimulated the rate of lactate accumulation in the medium measured after 6 and 12 h by 66% and 100%, respectively (Fig. 1). PGE_2 (10 μg/ml) likewise increased the rate of release of lactate into the medium by 75% (6 h) and 81% (12 h) compared to that in control incubations (Fig. 1).

Glycolysis during inhibition of steroidogenesis. Addition of LH (5 μg/ml) to the medium stimulated the release of progesterone, androstenedione and estradiol-17β, as well as that of lactate, by cultured follicles during 6 h incubations (Fig. 2 and 3). Cyanoketone (10^{-4} M; Fig. 2) or 10^{-3} M aminoglutethimide (Fig. 3) prevented the LH-induced rise in the secretion of the three steroids measured and reduced the basal level of steroid secretion. Suppression of steroidogenesis by either compound had no significant effect on LH-induced stimulation of lactate production nor on the basal rate of lactate accumulation.

Addition of PGE_2 (10 μg/ml) to the medium stimulated the release of follicular progesterone and lactate during 12 h incubations (Fig. 4 and 5). Cyanoketone (10^{-4} M; Fig. 4) or 10^{-3} M aminoglutethimide (Fig. 5) prevented this effect of PGE_2

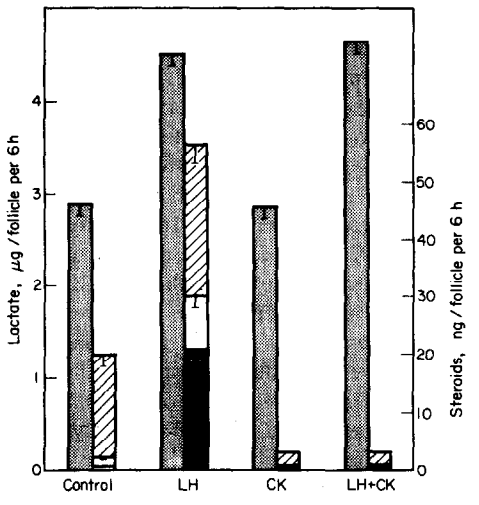

Fig. 3. Stimulation by LH (5 μg/ml) of lactate release from cultured Graafian follicles during inhibition of steroidogenesis by aminoglutethimide (10^{-3} M). ▨, lactate; ■, progesterone; □, androst-4-ene-3,17-dione; ▨, oestradiol-17β. Vertical brackets, SEM (N = 5–6).

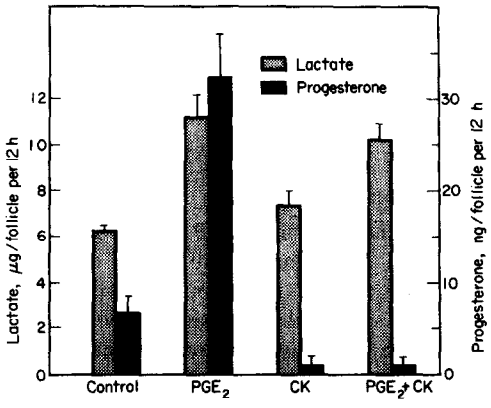

Fig. 4. Stimulation by PGE_2 (10 μg/ml) of lactate release from cultured follicles during inhibition of progesterone synthesis by cyanoketone (10^{-4} M). Vertical brackets, SEM (N = 4).

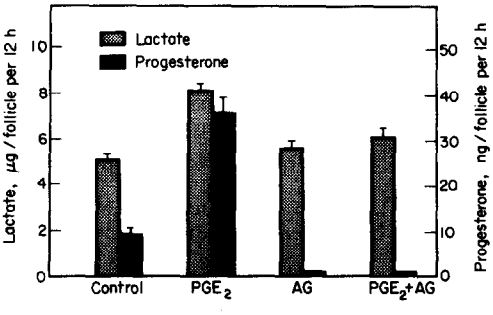

Fig. 5. Effect of aminoglutethimide (10^{-3} M) on PGE_2-induced release of lactate and progesterone from cultured follicles. PGE_2 concentration 10 μg/ml. Vertical brackets, SEM (N = 4).

and reduced the basal rate of progesterone accumulation.

Suppression of steroidogenesis by cyanoketone had no significant effect on the PGE_2-induced rise in lactate accumulation. By contrast, aminoglutethimide markedly inhibited or abolished the glycolytic response to PGE_2 (Fig. 5).

Steroidogenesis during inhibition of glycolysis. Addition of iodacetate ($2{\cdot}5 \times 10^{-5}$ M) abolished the stimulatory effect of LH (5 μg/ml) on lactate production. The effect of LH on progesterone accumulation was significantly reduced at this concentration of the inhibitor (Fig. 6), but progesterone was still released at ten times the basal rate. At higher concentrations of iodoacetate, both lactate and progesterone accumulation progressively declined: at 10^{-4} M iodacetate, lactate accumulation in the medium became undetectable (<1 μg/follicle) and the LH effect on progesterone release was abolished. These effects of 10^{-4} M iodacetate were not overcome by enrichment of the medium with 5 or 25 mM pyruvate.

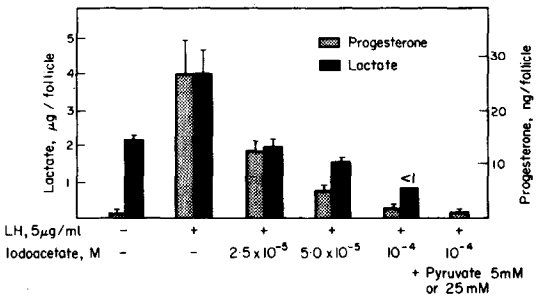

Fig. 6. Effect of iodoacetate on LH-induced release of lactate and progesterone from cultured Graafian follicles. Follicles (5–6 per culture dish) wer incubated for 6 h. Vertical brackets, SEM (n = 3–4).

DISCUSSION

The results described show that isolated Graafian follicles, like intact ovaries from prepubertal rats (see Introduction), respond to LH as well as PGE_2 under aerobic conditions *in vitro* with increased lactic acid production. Parallel observations on follicles isolated from prepubertal rats treated with pregnant mare serum gonadotrophin yielded similar results[17, 18]. The cellular mechanism underlying the glycolytic effect of LH has been analyzed in some detail, and appears to involve enhancement of the transport of glucose across the cell membrane, activation of glycogen phosphorylase and activation of phosphofructokinase (for review, see[19]). Yet the functional significance of this effect of LH- or of the analogous effect of PGE_2- has not been elucidated.

In the present study it was shown that the stimulatory action of LH on lactic acid production by cultured preovulatory follicles was retained when the production of progesterone, androstenedione and oestradiol-17β was prevented by

addition to the medium of either cyanoketone, an inhibitor of 3β-hydroxysteroid dehydrogenase[20], or of aminoglutethimide, an inhibitor of cholesterol 20α-hydroxylase[21]. These results imply that the glycolytic response of the follicle to LH is not secondary to an effect of the hormone on steroidogenesis [cf. 11]. This conclusion is reinforced by the finding that PGE_2, too, was able to stimulate follicular glycolysis during inhibition of steroidogenesis by cyanoketone. Aminoglutehimide, however, significantly inhibited the action of PGE_2 on both lactic acid production and steroidogenesis. The mechanism by which this inhibitor affects exclusively PG-induced glycolysis is not understood, but is likely to be divorced from the effect of aminoglutethimide on steroidogenesis.

It is of interest to compare these findings with the results of similar studies on isolated adrenals. It has been reported[22, 23] that ACTH increased lactic acid production by intact mouse adrenals *in vitro*; that addition of corticosterone to the medium also stimulated adrenal glycolysis, although to a lesser extent than ACTH; that addition of aminoglutethimide to mouse adrenals decreased the production of lactic acid and reduced, but did not abolish, the glycolytic response to ACTH. These authors concluded that "ACTH-induced glycolysis of intact mouse adrenal glands *in vitro* is mediated to a significant extent, but not exclusively, by the glycolytic action of increased levels of tissue corticosterone". The glycolytic response of intact mouse adrenals differs, however, from that of a mouse adrenal cell culture studied by Kowal[24]. Aminoglutethimide inhibited steroid production, but not glycolysis, in this adrenal cell culture. The cultured adrenal cells were not capable of synthesizing the naturally occurring, glycolytically active steroid end-product, corticosterone, a fact which serves to explain the discrepancy between the intact mouse adrenals and the adrenal cell culture. In the rat follicle, on the other hand, no part of the glycoltic effect of LH appears to be steroid-mediated, at least under the *in vitro* conditions used in the present experiments.

The possibility that the stimulation of ovarian carbohydrate metabolism by LH yields increasing intracellular levels of NADPH, which is essential for steroidogenesis, cannot be excluded. Experiments of Armstrong and co-workers[4, 25] make it unlikely that LH selectively stimulates glucose utilization via the pentose phosphate pathway, which was one of the important ingredients of the original hypothesis of Haynes and Berthet[10] regarding trophic hormone action on the adrenals. It has, however, recently been reported that pyruvate can be directly oxidized by rat adrenal mitochondria, thereby producing sufficient intramitochondrial NADPH to support steroid-hydroxylation processes[26, 27]. It is therefore possible that ACTH-stimulated glycolysis can augment adrenal steroidogenesis through this mechanism. Whether ovarian mitochondria can also oxidize pyruvate directly has not been studied, but it has been shown that LH increases not only lactic acid release from the isolated prepubertal rat ovary but also ovarian pyruvate levels[28].

Elimination of the LH-effect on follicular lactate production *in vitro* by addition of iodacetate at a moderate concentration ($2{\cdot}5 \times 10^{-5}$ M) significantly reduced the effect of the hormone on progesterone release. However, even under these conditions LH caused a 10-fold stimulation of progesterone release. These findings suggest that replenishment of NADPH by LH-induced glycolysis may contribute to the steroidogenic action of LH, but does not entirely account for this LH-effect. Only at higher iodacetate concentration (10^{-4} M) was the LH effect on progesterone production abolished. At this concentration, however, iodoacetate probably no longer acted selectively on the Embden-Meyerhoff pathway, since supplementation of the medium with pyruvate failed to restore the LH-effect: damage to respiration and depletion of adenine nucleotides may ensue at such a high concentration of this inhibitor[29].

LH and PGE_2 are able to induce the oocytic maturation division in cultured follicles during inhibition of both the steroidogenic and glycolytic effects of these hormones [3; Tsafriri, Lieberman, Ahren and Lindner, in preparation; Lieberman, Tsafriri, Bauminger, Collins, Ahren and Lindner, in preparation]. It is known that LH increases the cyclic AMP level in Graafian follicles of the rat within seconds[3, 9], and that PGE_2 has a similar effect[6]. Thus cyclic AMP may act as the intracellular trigger for several effects common to LH and PGE_2, such as the stimulation of glycolysis, steroidogenesis and the initiation of ovum maturation; yet the pathways leading to these terminal events appear to diverge at an early stage rather than to form a single causal sequence.

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