Swine granulosa cells respond to follicle-stimulating hormone (FSH) and the insulin-like growth factor, IGF-I (somatomedin C), with synergistic increases in progesterone production. This facilitative interaction was not attributable to decreased catabolism of progesterone to 20α-hydroxyprogren-4-en-3-one, but rather to enhanced pregnenolone biosynthesis observed in response to provision of 25-hydroxycholesterol as exogenous sterol substrate. The latter evidence of increased functional cholesterol side-chain cleavage activity was accompanied by augmented incorporation of [3H]cholesterol into specific immunoisolated components of the cholesterol side-chain cleavage apparatus, viz. cytochrome P-450_cho and adrenodoxin.

The synergism between FSH and IGF-I could be sustained over 4 days of serum-free monolayer culture. Under these conditions, compactin, a competitive inhibitor of de novo endogenous cholesterol biosynthesis, suppressed stimulated progesterone production by ≈50%. However, synergism was not expressed at the levels of [14C]acetate incorporation into nonsaponifiable lipids or endogenous 3-hydroxy-3-methylglutaryl coenzyme A reductase activity per se. Conversely, exogenous sterol substrate provided in the form of low-density lipoprotein (LDL)-borne cholesterol increased the absolute magnitude of the combined actions of IGF-I and FSH by 3-6-fold. This increase in steroidogenesis in response to LDL was associated with enhanced surface binding, internalization, and degradation of [3H]iodo-LDL. In addition, when granulosa cells were incubated with [3H]cholesterol, FSH and IGF-I synergistically increased the intracellular accumulation of [3H]cholesterol and [3H]cholesteryl ester and the production of [3H]progesterone. Moreover, FSH and IGF-I coordinately increased the total mass of free and esterified cholesterol contained in granulosa cells.

We conclude that FSH and IGF-I can augment absolute rates of progesterone biosynthesis by granulosa cells by activating dual mechanisms: 1) stimulation of functional cholesterol side-chain cleavage activity and 2) enhancement of effective cellular uptake and utilization of low-density lipoprotein-borne sterol substrate.

Insulin-like growth factor I (IGF-I, somatomedin C) exerts potent differentiating effects on various epithelial tissues (1, 2). More recently, this growth factor has been found to significantly enhance basal rates of progesterone and estradiol biosynthesis in porcine ovarian (granulosa) cells (3, 4), and to facilitate FSH-stimulated progesterone production in both rat and pig granulosa cells (5, 6). These trophic actions of IGF-I are associated with specific, saturable, and high-affinity IGF-I receptors on granulosa cells (3, 7). Accordingly, granulosa cells provide a model in which to investigate the biochemical mechanisms subserving the role of IGF-I as a biological amplifier of hormonally stimulated steroidogenesis. Since processes regulating cellular sterol uptake and utilization represent plausible loci of hormone action in differentiating steroidogenic cells (8), we have examined the synergistic impact of IGF-I and FSH on cellular sterol metabolism in serum-free monolayer cultures of hormonally responsive swine granulosa cells.

**MATERIALS AND METHODS**

Culture fluid was from Gibco and estradiol and 25-hydroxycholesterol were from Sigma. Ovine FSH (NIH-oFSH-16) containing <0.04 unit NIH-LH-51 per mg was from the National Hormone and Pituitary Program (Baltimore, MD). The IGF-I (somatomedin C) used was prepared by recombinant DNA synthesis at Amgen Biologicals (Thousand Oaks, CA), and subsequent purity assessed by high-performance liquid chromatography, polyacrylamide gel electrophoresis, and N-terminal sequencing (4). This material differs from naturally occurring human IGF-I only in the substitution of ε methionine for threonine residue in position 59.

**Culture Conditions**—Granulosa cells were isolated from ovaries of immature swine (55-65 kg) by the method of Channing, as described earlier (3). Small and medium-sized follicles were aspirated aseptically with a 20-gauge needle, and the granulosa cells recovered by low-speed centrifugation. Approximately 2 or 6 × 10^6 viable cells, as determined by the exclusion of trypan blue, were plated in triplicate or quadruplicate culture dishes (2 or 6 cm diameter) with culture fluid consisting of Eagle’s minimum essential medium (GIBCO) buffered with bicarbonate. Cultures were incubated at 37 °C in an atmosphere of 5% CO₂ in air.

The abbreviations used are: IGF-I, insulin-like growth factor I; FSH, follicle-stimulating hormone; LDL, low-density lipoprotein; BSA, bovine serum albumin; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; SDS, sodium dodecyl sulfate.
of 95% air and 5% CO₂ with the addition of indicated hormone(s). Before initiating the lipoprotein studies, cultures were established for 48 h in medium supplemented with 1% fetal calf serum; thereafter, cultures were maintained in the absence of serum for an additional 40 h.

Lipoprotein Studies—Human low-density lipoprotein (LDL) was isolated by ultracentrifugation as described earlier at a density of 1.019–1.063 g/ml (9). Lipoproteins were iodinated by the method of MacFarlane using iodine monochloride, as described by Bilheimer et al. (10). After iodination, protein-bound iodide was separated by gel filtration, dialyzed exhaustively against serum-free culture medium, and stored at 4 °C for use within 2 weeks. The specific activity of the lipoprotein preparations ranged from 220 to 450 dpm/μg of protein, of which >97%–99% was precipitable with 20% trichloroacetic acid, and <3% was extractable as lipid with chloroform:methanol (2:1, vol/vol).

The binding of [125I]iodo-LDL to intact granulosa cells was assessed by incubation with increasing concentrations of [125I]iodo-LDL, or by incubation with a fixed quantity of [125I]iodo-LDL in the presence of increasing concentrations of unlabeled lipoprotein (9). After incubation at the indicated temperatures for designated intervals, cells were scraped from the culture dishes and washed by centrifugation eight times in phosphate-buffered saline containing 3 mg/ml bovine serum albumin (9). Subsequent saturation and displacement curves were analyzed by nonlinear, least-squares curve fitting to determine the apparent association or dissociation constants and the maximal binding capacity (11). Nonspecific binding was estimated by addition of 30-200-fold excess unlabeled competitor, as indicated.

To quantify internalization of LDL, granulosa cells were incubated with indicated concentrations of [125I]iodo-LDL for designated intervals at 37 °C, then chilled rapidly to 4 °C, washed three times in phosphate-buffered saline, exposed to heparin (30 mg/ml) for 60 min at 4 °C, and washed an additional six times at 4 °C in phosphate-buffered saline containing 3 mg/ml BSA (9). The amount of [125I]iodo-LDL released by heparin was considered bound to surface membranes, and that retained by the cell pellet, internalized.

In lipoprotein degradation studies, cultures were maintained at 37 °C (or at 4 °C in control replicates) in the presence of 3 mg/ml BSA and the indicated concentrations of human LDL. At designated intervals, the culture medium was removed and the dishes were scraped mechanically. Some medium was used for the immunoassay of progesterone as described earlier (3). The remaining medium was subjected to trichloroacetic acid precipitation (20%, vol/vol) in the presence of 10 mg/ml BSA). Preliminary experiments indicated that BSA concentrations of 3–30 mg/ml in association with 20% trichloroacetic acid precipitated >97% of [125I]iodo-LDL. The acid-soluble medium was treated further with hydrogen peroxide and extracted by aqueous:chloroform partitioning to remove free iodide (9).

To investigate the delivery and utilization of the cholesterol moiety of LDL, granulosa cells were exposed to [3H]cholesterol linoleate LDL. [3H]Cholesterol linoleate was incorporated into LDL by the method of Hough and Zilversmit (12) modified slightly as follows: 5 ml of human serum of density >1.063 was incubated with 125 μCi of [1,2,6,7-3H]cholesterol linoleate overnight at 37 °C. Then 2.5 mg of human LDL (d = 1.019–1.063 g/ml) was added to this mixture and the incubation continued for another 3 h. At the end of the incubation, the LDL fraction was isolated by ultracentrifugation and exhaustively dialyzed against 0.15 M NaCl containing 0.3 mM EDTA, pH 7.4, and sterilized by passage through a 0.22-μM Millipore filter (Millipore Corp., Bedford, MA). Greater than 98% of the 3H from labeled LDL was in the cholesterol ester, and the specific activity of the cholesterol ester quantitated by the method of Rudel and Morris (10) averaged 8600–7400 dpm/nmol. The labeled LDL migrated with human LDL on agarose-gel electrophoresis, and the 3H peak co-migrated with native human LDL. The labeled LDL contained 4.52 mg of protein/ml and 4.32 mg of total sterol/ml; the mass ratio of cholesterol ester:free cholesterol (mg/mg) was 1.8.

At designated intervals, cells were treated with heparin (30 mg/ml) for 1 h at 4 °C (9) and then washed as described above in the [125I]iodo-LDL binding studies. The intracellular accumulation of [3H]cholesterol and [3H]cholesterol ester was determined after chloroform:methanol (2:1) extraction, followed by one-dimensional thin-layer chromatography, as presented earlier (9, 14). [3H]ProGESTERONE accumulation in medium was measured by liquid scintillation counting of [3H]progesterone isolated by two-dimensional thin-layer chromatography (15). Total progesterone in the medium was quantitated by [125I]radioimmunoassay using reagents supplied by Diagnostic Products (Los Angeles, CA).

FIG. 1. A, dose-dependent facilitative interaction between IGF-I and FSH on total progesterone production by cultured swine granulosa cells. Granulosa cells were cultured in serum-free medium for 4 days in the absence or presence of a maximally effective concentration of FSH (200 ng/ml, with or without increasing doses of IGF-I (somatomedin C)). On day 4, the total content of progesterone in cells combined with medium was measured by radioimmunoassay. Data are means ± S.E., n = 4 independent determinations confirmed in three separate experiments. B, synergistic interaction between IGF-I and FSH on days 2 and 4 of serum-free monolayer culture, when granulosa cells were treated with FSH (200 ng/ml) and/or IGF-I (20 ng/ml).
methionine-free medium and cultured with cells were harvested by mechanical scraping, washed three times in 4 days in the absence or presence of IGF-I (20 ng/ml) and/or increasing concentrations of FSH (3-300 ng/ml). Data are otherwise as presented in the legend to Fig. 1.

Other Analytical Methods—Protein concentrations were determined by the method of Lowry or Bradford, and DNA by the procedure of Burton (6-18). [14C]Acetate incorporation into nonspontaneously lipids and microsomal HMG-CoA reductase activity were determined as described earlier (9). The cellular content of cholesterol and cholesteryl ester was measured by the microfluorimetric method of Heider and Boyett (19). This method was sensitive to 100 pg/tube free cholesterol and linear over the range of 0.1-300 ng/tube cholesterol.

Studies of Functional Cholesterol Side-chain Cleavage—Functional cholesterol side-chain cleavage activity in pig granulosa cells was estimated by measuring the production of pregnenolone over 4 h in the presence of trilostane (150 G) to block pregnenolone metabolism and a maximally effective concentration of 25-hydroxycholesterol (25 G/ml) as exogenous sterol substrate for cholesterol side-chain cleavage. After 4 h, the cells and media were harvested for the subsequent assay of total pregnenolone content. Data are otherwise as presented in the legend to Fig. 1.

Statistical Analyses—Untransformed data are presented as means ± S.E. Analysis of variance was employed to assess the statistical significance of overall treatment effects. The significance of any individual treatment was evaluated further by Duncan’s multiple range test with corrections for repeated measures (21). In addition, each experiment was performed at least three times using a separate batch of 300-400 ovaries to assess the generality and reproducibility of results.

RESULTS

Granulosa cells were cultured in serum-free medium for 4 days in the absence or presence of a maximally effective concentration of FSH (200 ng/ml), with or without increasing doses of IGF-I (1-100 ng/ml). The total content of progesterone contained in cells combined with medium was measured on day 4. As shown in Fig. 1, IGF-I alone stimulated total

TABLE I

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>Cholesterol side-chain cleavage constituent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cytochrome P-450&lt;sub&gt;om&lt;/sub&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>100 ± 13</td>
</tr>
<tr>
<td>FSH (200 ng/ml)</td>
<td>153 ± 2</td>
</tr>
<tr>
<td>IGF-I (30 ng/ml)</td>
<td>202 ± 2</td>
</tr>
<tr>
<td>FSH plus IGF-I</td>
<td>265 ± 1</td>
</tr>
</tbody>
</table>

balanced salt solution, and frozen for the later immunonosolation and quantitation of newly synthesized proteins. Cytochrome P-450<sub>om</sub> and adrenodoxin were immunosolated from labeled cell pellets and analyzed by SDS-polyacrylamide gel electrophoresis followed by autoradiography (20).
tion approximately 4-fold. The concerted effects of increasing progesterone production markedly with a 25-fold effect at a dose of IGF-I and/or FSH were synergistic dose of 100 ng/ml. FSH alone increased progesterone production in response to IGF-I effect alone).

Interaction was sustained over at least 4 days in serum-free monolayer culture when granulosa cells were treated with FSH (200 ng/ml) and/or IGF-I (20 ng/ml). Moreover, the absolute magnitude of progesterone production in response to the synergistic stimulation by FSH and IGF-I of progesterone production by swine granulosa cells. Ovarian cells were cultured in the absence or presence of compactin (25 μM), with or without FSH (200 ng/ml), IGF-I (20 ng/ml), or both hormones. Data are expressed as ng/10^7 cells/6 h. The subsequent organic extracts of cells combined with media were saponified and subjected to thin-layer chromatography to separate [14C]progesterone and [14C]cholesterol. Data are means ± S.E. for three to six separate determinations confirmed in two independent experiments.

**TABLE II**

<table>
<thead>
<tr>
<th>Condition</th>
<th>[14C]Acetate incorporation</th>
<th>Total progesterone produced*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Progesterone</td>
<td>Cholesterol</td>
</tr>
<tr>
<td></td>
<td>dpm/6 h/10^7 cells</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>760 ± 120</td>
<td>470 ± 60</td>
</tr>
<tr>
<td>FSH (200 ng/ml)</td>
<td>1,110 ± 170</td>
<td>420 ± 120</td>
</tr>
<tr>
<td>IGF-I (50 ng/ml)</td>
<td>10,100 ± 600</td>
<td>890 ± 170</td>
</tr>
<tr>
<td>FSH and IGF-I</td>
<td>4,500 ± 350⁵</td>
<td>480 ± 150</td>
</tr>
</tbody>
</table>

*Total progesterone production was assayed by 125I radioimmunoassay as ng/10^7 cells/6 h.

**Fig. 6.** Influence of compactin, a competitive inhibitor of de novo cholesterol synthesis, on the synergistic stimulation by FSH and IGF-I of progesterone production by swine granulosa cells. Ovarian cells were cultured in the absence or presence of compactin (25 μM), with or without FSH (200 ng/ml), IGF-I (20 ng/ml), or both hormones. Data are expressed as ng/10^7 cells/6 h. The subsequent organic extracts of cells combined with media were saponified and subjected to thin-layer chromatography to separate [14C]progesterone and [14C]cholesterol. Data are means ± S.E. for three to six separate determinations confirmed in two independent experiments.

**Fig. 5.** IGF-I and FSH synergistically enhance [3H]methionine incorporation into immunoprecipitable cholesterol side-chain cleavage constituents. Swine granulosa cells were cultured for 2 days in the presence of control solvent (lanes 1), FSH (200 ng/ml), IGF-I (30 ng/ml), or both hormones (lanes 4). A and B, SDS-polyacrylamide gel electrophoresis of the immunoisolated cytochrome P-450. C and D, SDS-polyacrylamide gel electrophoresis of the immunoisolated adrenodoxin protein. In each panel, the horizontal arrow denotes the location of the relevant cholesterol side-chain cleavage constituent. A and C provide data from immunoisolations performed from equal amounts of protein (75 μg of protein in B and 150 μg of protein in D). E gives the time course of synergistically enhanced progesterone production in the same cultures, from which the immunoisolations were performed on day 2.

FSH and IGF-I increased on day 4 compared to day 2 of culture. In contrast, basal and FSH-stimulated progesterone production actually decreased from days 2 to 4 in culture. The effect of IGF-I on the dose-response characteristics of FSH-stimulated progesterone production was examined (Fig. 2). In the presence of increasing doses of FSH, the addition of IGF-I (20 ng/ml) resulted in marked amplification of total progesterone production (p < 0.001 treatment effect). This synergistic interaction was not associated with any significant change in the half-maximally effective concentration (ED₅₀) of FSH required to stimulate progesterone production: viz. the ED₅₀ for FSH was 13 ng/ml (11–17) basally and 14 ng/ml
(13–15) in the presence of somatomedin C (n = 4 determinations). Rather, combined treatment with FSH and IGF-I increased the absolute maximal quantity of pregnestrone produced per 48 h. This increase in progesterone accumulation was not attributable to selective inhibition of progesterone’s catalysis to 20α-hydroxyprogren-4-ene-3-one (20α-dihydroprogesterone). As shown in Fig. 3, FSH alone, IGF-I alone, and the combination of FSH and IGF-I significantly stimulated accumulation of progesterone’s reduced metabolite, and this stimulation was synergistic in the presence of FSH and IGF-I.

To further examine the mechanisms subserving the facilitative interaction between FSH and IGF-I, granulosa cells were cultured for 48 h in the presence of FSH alone (200 ng/ml), IGF-I alone (20 ng/ml), or FSH and IGF-I. After this interval, culture fluid was replenished with medium containing trilostane (150 μM) to block pregnenolone metabolism and a maximally effective concentration of 25-hydroxycholesterol (25 μg/ml) to provide exogenous sterol substrate for the cholesterol side-chain cleavage enzyme. After 4 h, cells and media were harvested for the subsequent assay of total pregnenolone content. As shown in Fig. 4, FSH alone and IGF-I alone both significantly stimulated pregnenolone production from exogenous steroid substrate. Moreover, FSH and IGF-I resulted in synergistic enhancement of pregnenolone biosynthesis under these conditions (p < 0.001).

This enhancement of functional cholesterol side-chain cleavage activity was associated with increased incorporation of [35S]methionine into immunoprecipitable cholesterol side-chain cleavage constituents, viz., adrenodoxin and cytochrome P-450 (see Table I and Fig. 5, A and B). Increased incorporation of [35S]methionine into specific immunoprecipitated components of the cholesterol side-chain cleavage apparatus was observed whether immunoisolations were performed on the basis of equal amounts of total radioactivity incorporated into protein (Fig. 5, A and C) or equal amounts of total protein (Fig. 5, B and D). Time course studies indicated that this synergistic interaction between FSH and somatomedin C was most prominent on day 2. As anticipated, the increase in immunoisolated constituents of cholesterol side-chain cleavage apparatus was accompanied by a parallel increase in progesterone production (Fig. 5E).

The role of de novo cholesterol biosynthesis in supporting the synergism between FSH and IGF-I was tested by treating cultures with compactin, a competitive inhibitor of HMG-CoA reductase. In the presence of compactin (25 μM), stimulation of progesterone production by FSH, IGF-I, and FSH in conjunction with IGF-I was inhibited significantly (Fig. 6). Although the DNA content of compactin-treated cultures declined by ≈8%, progesterone production still decreased significantly when data were expressed in relation to DNA content in individual cultures. Moreover, inhibition by compactin was overcome by >85% by provision of exogenous mevalinolactone (20 mM) (data not shown). Since compactin may exert nonspecific cytotoxic effects in vitro, we also evaluated the influences of IGF-I and/or FSH on [14C]acetate incorporation into specific nonsaponifiable sterols (Table II) and on microsomal HMG-CoA reductase activity (Table III). Although IGF-I significantly stimulated both end points, the addition of FSH did not result in synergism. Accordingly, we infer that, although de novo cholesterol synthesis contributes utilizable sterol, it does not offer an intracellular mechanism for synergism.

The importance of the availability of sterol substrate for the cholesterol side-chain cleavage reaction was tested further by culturing granulosa cells in the presence or absence of exogenous low-density lipoprotein. In these experiments, granulosa cells were maintained for 4 days in the presence or absence of FSH (200 ng/ml), IGF-I (30 ng/ml), or both hormones, with or without addition of LDL (100 μg/ml). Progesterone production was measured and expressed as nanograms of steroid produced per 48 h per μg of DNA. Data are otherwise as presented in the legend to Fig. 6.

### Table III

| Hormonal treatment | HMG-CoA reductase activity
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.9 ± 3.1*</td>
</tr>
<tr>
<td>FSH (200 ng/ml)</td>
<td>3.5 ± 0.20*</td>
</tr>
<tr>
<td>IGF-I</td>
<td>31.0 ± 2.4*</td>
</tr>
<tr>
<td>FSH and IGF-I</td>
<td>12.5 ± 0.03*</td>
</tr>
</tbody>
</table>

*p* Differring superscripts denote significantly different means. Data are means ± S.E. for three separate experimental determinations.

FIG. 7. Stimulatory effect of exogenously supplied sterol in the form of human LDL on IGF-I and FSH-stimulated progesterone production. Swine granulosa cells were cultured in serum-free media for 4 days in the presence or absence of FSH (200 ng/ml), IGF-I (30 ng/ml), or both hormones, with or without the addition of a maximally effective concentration of human LDL (100 μg/ml). Progesterone production was measured and expressed as nanograms of sterol produced per 48 h per μg of DNA. Data are otherwise as presented in the legend to Fig. 6.
FSH and IGF-I Synergism

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Coordinate influence of IGF-I and FSH on [3H]sterol accumulation and [3H]progesterone production by cultured swine granulosa cells

Granulosa cells were cultured in serum-free medium in the absence or presence of IGF-I (30 ng/ml) and/or FSH (200 ng/ml) and exposed to [3H]cholesterol-labeled LDL (50 µg/ml) for 30 h. The cells and media were prepared for thin-layer chromatography to quantitate [3H]sterol accumulation and [3H]progesterone production. Data are means ± S.E., n = 4 separate determinations confirmed in two independent experiments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>[3H]Sterol accumulation</th>
<th>[3H]Cholesterol</th>
<th>[3H]Progesterone production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dpm/10^6 cells</td>
<td>dpm/10^6 cells</td>
<td>dpm/10^6 cells</td>
</tr>
<tr>
<td>Control</td>
<td>640 ± 110</td>
<td>340 ± 30</td>
<td>14,200 ± 1,340</td>
</tr>
<tr>
<td>FSH</td>
<td>1,080 ± 120</td>
<td>660 ± 60</td>
<td>21,800 ± 1,650</td>
</tr>
<tr>
<td>IGF-I</td>
<td>2,250 ± 80</td>
<td>1,300 ± 100</td>
<td>25,900 ± 1,520</td>
</tr>
<tr>
<td>IGF-I and FSH</td>
<td>3,140 ± 70*</td>
<td>2,150 ± 70*</td>
<td>32,200 ± 1,500*</td>
</tr>
</tbody>
</table>

*p < 0.05 versus IGF-I or FSH alone.

Discussion

We have used serum-free cultures of swine granulosa cells to appraise the mechanisms subserving the synergistic actions of IGF-I and FSH on the differentiation of ovarian cells in vitro. We have focused on the ability of these distinct regulatory hormones to influence progesterin biosynthesis and cellular cholesterol metabolism in a coordinate manner. Our observations indicate that the concerted effects of FSH and IGF-I impinge on several critical steps in cellular sterol homeostasis.

The supra-additive enhancement of progesterone biosynthesis by FSH and IGF-I was associated with increased production of progesterone as well as 20α-hydroxyprog-4-en-3-one by swine granulosa cells. This observation is in accord with that reported for cultured rat granulosa cells derived from diethylstilbestrol-treated hypophysectomized animals (22). Moreover, our results with 25-hydroxycholesterol as an exogenous soluble sterol substrate for cholesterol side-chain cleavage indicate that FSH and IGF-I also synergistically augment pregnenolone biosynthesis, presumptively by increasing functional cholesterol side-chain cleavage activity. Increased functional cholesterol side-chain cleavage activity was accompanied by enhanced [35S]methionine incorporation into two key immunosolated side-chain cleavage constituents, cytochrome P-450c and adrenodoxin. Although earlier observations have indicated that FSH alone and IGF-I alone can increase cholesterol side-chain cleavage activity (20, 23), the present observations provide the first evidence for combined actions of these two regulatory hormones on this critical biochemical step in the steroidogenic pathway.
The absolute magnitude of the synergistic effect of FSH and IGF-I on progesterin biosynthesis by swine granulosa cells was reduced by compactin, an inhibitor of HMG-CoA reductase activity. We have shown that this antifungal compound effectively (by >90%) inhibits de novo cholesterol biosynthesis and HMG-CoA reductase activity in these cells (24). However, a supra-additive interaction between IGF-I and FSH persisted in the presence of compactin, albeit at a lower absolute level of progesterone production. Moreover, the effects of IGF-I and FSH were not expressed to a synergistic degree on [14C]acetate incorporation into nonsoapifiable lipids or on microsomal HMG-CoA reductase activity. These results indicate that the essential nature of the synergism between IGF-I and FSH is not dependent upon the de novo synthesis of sterol substrate in granulosa cells. Conversely, provision of exogenous sterol substrate in the form of low density lipoprotein-borne cholesterol markedly amplified the absolute magnitude of the synergism between FSH and IGF-I. This effect was associated with increased surface binding of [125I]iodo-LDL and with enhanced rates of internalization and cellular degradation of iodinated lipoprotein. Enhanced delivery of lipoprotein-borne cholesterol to the steroidogenic pathway was corroborated by measurements of [3H]cholesterol uptake and [3H]progesterone secretion by swine granulosa cells exposed to LDL reconstituted with [3H]cholesterol ester. Moreover, these facilitative effects on radiolabeled cholesterol disposal were also accompanied by increases in the total mass of free and esterified cholesterol contained in ovarian cells.

We observed that endogenous steroid deprivation (treatment with compactin) or exogenous steroid delivery (provision of LDL-borne cholesterol) significantly influenced absolute rates of progesterone production without altering the essential property of synergism. Thus, sterol substrate economy significantly modulates the total amount of progesterone synthesized, but does not alter the occurrence of steroidogenic synergism, as defined by a supra-additive stimulatory interaction between IGF-I and FSH. This interesting observation suggests that the fundamental mechanism subserving synergism between these two trophic hormones is not a mechanism for the basic facilitative interaction between IGF-I and FSH. Instead, the essential nature of the synergism between IGF-I and FSH was expressed in LDL-borne cholesterol provides a means to increase sterol availability for use in the activated steroidogenic pathway. In addition, the remarkable enhancement in LDL uptake induced by the two hormones seems to result in saturation of the LDL degradation system in these cells. We would suggest that these coordinate effects of IGF-I and FSH are both sterol delivery and utilization would account for the profound (>100-fold) increase in total progesterone biosynthesis achieved by these two trophic hormones.

In summary, we have observed that cultured swine granulosa cells respond synergistically to the co-administration of IGF-I and FSH with enhanced biosynthesis of pregnenolone, progesterone, and 20α-hydroxypreg-4-en-3-one. IGF-I and FSH increase functional cholesterol side-chain cleavage activity in a supra-additive fashion and enhance delivery of exogenous lipoprotein-borne cholesterol to intracellular pools that contribute to progesterone biosynthesis. These observations permit us to suggest that these two physiological effector hormones operate coordinately to enhance both the availability and the effective utilization sterol substrate by ovarian cells. Accordingly, this work offers a basis for future studies on the molecular biology of hormonally regulated sterol homeostasis in ovarian cells.

Acknowledgments—We thank Chris McNett for the skilful preparation of the manuscript; Dr. Jerome F. Strauss III for his critical comments and for preparing the [3H]cholesterol linoleate-labeled LDL; Paula P. Azimi for the artwork; the Gwaltney-Smithfield Packing Corp. for providing swine ovaries; Dr. Michael L. Johnson for the nonlinear curve-fitting program; Dr. Joseph Lerner and Michael Cronin for helpful discussions; and L. Ravi for assistance in immunolocations.

REFERENCES