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Gonadotropin Modulation of 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase Activity in Desensitized Luteinized Rat Ovary†

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ABSTRACT: These studies were done to examine the effect of gonadotropin on rat luteal 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase activity (the rate-limiting step in cholesterol biosynthesis) in ovaries of pregnant mare's serum gonadotropin (PMSG)–human chorionic gonadotropin (hCG) primed rats. Administration of hCG stimulated HMG CoA reductase activity in a time- and dose-dependent manner: significant increases were noted within 4 h, with maximum effects (30–40-fold increases) seen 24 h after hCG (25 IU) administration. This effect was specific in that only LH, of several hormones tested, was as effective as hCG in stimulating HMG CoA reductase activity, and no change in the activity of either liver microsomal HMG CoA reductase or luteal microsomal NADPH-cytochrome *c* reductase was seen after hCG. The gonadotropin-induced increase in HMG CoA reductase activity seemed to be due to a net increase in enzyme activity, not to a change in the phosphorylated/dephosphorylated state of the enzyme. Pretreatment of animals with aminoglutethimide, an inhibitor of the conversion of cholesterol to steroid (pregnenolone), prevented the hCG-induced rise in HMG CoA reductase activity, whereas treatment with 4-aminopyrazolo[3,4-*d*]pyrimidine (4-APP), which depletes cellular cholesterol content, led to striking increases in enzyme activity. However, the combined effects of 4-APP and hCG were additive, suggesting that the stimulating effect of hCG on HMG CoA reductase activity is not entirely due to a depletion of cellular sterol content of luteinized ovaries. Similarly, cholesteryl ester and cholesterol syntheses as measured by [^{14}C]acetate conversion were also increased by hCG and 4-APP treatment. Furthermore, hCG compared to 4-APP was the preferred stimulator of cholesteryl ester, and combined treatments resulted in synergistic action. In summary, hCG stimulates HMG CoA reductase activity in luteinized ovaries. This effect appears to be tissue, hormone, and enzyme specific and not entirely as a function of a reduction in plasma or tissue cholesterol concentrations. Thus, gonadotropin regulation of ovarian function does not seem to be limited to steroidogenesis but also involves profound effects on cellular cholesterol metabolism.

Several recent reports have raised the possibility that disturbances in intracellular sterol metabolism may be involved in the process by which rat luteal cells become "desensitized" to gonadotropin stimulation. Thus, there is mounting evidence that rat luteal cells preferentially utilize high density lipoprotein (HDL) derived cholesterol for steroidogenesis (Azhar & Menon, 1981; Azhar et al., 1981; McNamara et al., 1981; Schuler et al., 1981a,b; Brout et al., 1982; Gwynne & Strauss, 1982), and it appears that a substantial fall in intracellular cholesteryl ester content occurs early in the development of human chorionic gonadotropin (hCG) induced desensitization (Azhar et al., 1983). Furthermore, it has been shown that the decline in steroidogenesis seen in luteinized ovaries can be overcome, at least early in the process, by the addition of HDL. The present study was undertaken in an effort to define further the changes in sterol metabolism that take place in hCG-desensitized luteal cells and has focused on defining the effects of hCG on HMG CoA reductase activity, the rate-limiting enzyme in cholesterol biosynthesis. The results to be presented include that HMG CoA reductase activity is increased approximately 30–40-fold in hCG-desensitized luteal cells. This effect is tissue, enzyme, and hormone specific and does not appear to be a simple function of the intracellular depletion of cholesteryl ester that is associated with hCG-induced desensitization.

Experimental Procedures

Materials

Purified human chorionic gonadotropin (CR-121; biopotency 13 450 IU/mg) was kindly provided by Dr. R. E. Canfield, College of Physicians and Surgeons of Columbia University, New York, NY, through the Center for Population Research of the National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD. Ovine luteinizing hormone (o-LH NIAMDD-OLH-22; biopotency 9 units/mg) and rat prolactin (NIAMDD-rat prolactin-B-2; biopotency 20 IU/mg) were gifts from Pituitary Hormone Distribution Program, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, MD. The following chemicals were supplied by Sigma Chemical Co., St. Louis, MO: DL-3-hydroxy-3-methylglutaryl-CoA; DL-mevalonic acid lactone; cytochrome *c*; glucose-6-phosphate dehydrogenase (240 units/mg of protein; product no. G-4134); cholesterol; cholesteryl oleate;

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$NADP^+$. 4-Aminopyrazolo[3,4-*d*]pyrimidine was a product of Aldrich Chemical Co., Milwaukee, WI. Aminoglutethimide (Elipten) was supplied by Ciba-Geigy. Silica gel G (Redi-Plate TM) and silica gel LK5DF thin-layer plates were obtained from Fischer Scientific Co., Pittsburgh, PA, and Whatman Chemical Separation Inc., Clifton, NJ, respectively. DL-3-Hydroxy-3-methyl[3-^{14}C]glutaryl coenzyme A (specific activity = 50.3–55.1 mCi/mmol), DL-[^{3}H]mevalonic acid (specific activity = 2–10 Ci/mmol), and [1,2-^{3}H]cholesterol (specific activity = 40–60 Ci/mmol) were purchased from New England Nuclear, Boston, MA. [U-^{14}C]Acetic acid sodium salt (specific activity = 90–100 mCi/mmol) was obtained from Research Products International Corp., Mount Prospect, IL.

Methods

Animals and Hormonal Treatment. Female Sprague-Dawley rats, 22–24 days old, were employed in the present studies. Highly luteinized ovaries from these rats were obtained by following a regimen originally described by Parlow (1961), as modified by Tan & Robinson (1977). Rats were injected subcutaneously with 50 IU of pregnant mare's serum gonadotropin (PMSG) followed 65 h later with 25 IU of hCG (Sigma). Day 0 was taken as the day of hCG injection. Where required, rats were injected (SC) again with hCG (25 IU, unless otherwise stated) between 0900 and 1000 h on day 5 (post-hCG), and animals were killed after 1, 1.5, 4, 12, or 24 h. Control animals received the vehicle. In some cases, rats were also injected (SC) with o-LH (50 μg), o-FSH (50 μg), or r-prolactin (100 μg) on day 5 (post-hCG), and rats were killed 12 h later. Whole ovaries from 0.9% NaCl or hormone-injected rats were used for the measurement of HMG CoA reductase, and NADPH cytochrome *c* reductase, [^{14}C]acetate incorporation into cholesterol and cholesteryl ester, or cholesterol and cholesteryl ester content.

Assay of HMG CoA Reductase Activity. Groups of two luteinized ovaries were minced and homogenized in 4 mL of buffer A (100 mM potassium phosphate buffer, pH 7.4, 10 mM ethylenediaminetetraacetic acid (EDTA), 5 mM dithiothreitol, and 0.25 M sucrose). The homogenates were first centrifuged at 10000*g* for 20 min to sediment unbroken cells, nuclei, and mitochondria. The supernatant fraction in each case was next centrifuged at 105000*g* for 1 h to sediment microsomes. Pelleted microsomal fraction was washed 2 times in buffer A and finally resuspended in the same buffer. The resulting microsomal fraction was used for HMG CoA reductase activity determination. HMG CoA reductase activity was determined by a slightly modified procedure of Balasubramaniam et al. (1977). Briefly, incubation mixture, in a final volume of 0.2 mL, contained 100 mM potassium phosphate, pH 7.5, 2.5 mM $NADP^+$, 20 mM glucose 6-phosphate; 0.7 units of glucose-6-phosphate dehydrogenase, 10 mM Na_2EDTA, 5 mM dithiothreitol, 200 μM DL-3-hydroxy-3-methyl[3-^{14}C]glutaryl-CoA (4500 dpm nmol), and suitable aliquots of microsomal protein (10–100 μg). Following preincubation of the reaction mixture at 37 °C for 10 min, the reaction was initiated by the addition of DL-3-hydroxy-3-methyl[3-^{14}C]glutaryl to a final concentration of 200 μM (Schuler et al., 1979). After further incubation at 37 °C usually for 20 min, the reaction was terminated by addition of 30 μL of 5 N HCl. To assure lactonization of [^{14}C]-mevalonic acid, tubes were again left at 37 °C for 30 min. The resulting [^{14}C]mevalonolactone was extracted and separated by thin-layer chromatography (silica gel G) as described by Kayden et al. (1976). The areas corresponding to [^{14}C]-mevalonolactone were located by exposure to iodine vapor, and spots were scraped into scintillation vials and counted for radioactivity determination in a Beckman LS 9000 liquid scintillation spectrometer after the addition of Beta Phase (West Chem Products); the recovery of the product which averaged 70–80% was monitored by addition of DL-[5-^{3}H]-mevalonic acid (4×10^4 dpm).

The enzyme activity is expressed as picomoles of mevalonic acid formed per minute per milligram of protein. All assays were carried out under optimal time and protein concentration. In control groups activity was linear for 90 min (with 100 μg of protein) and up to 500 μg of protein (using 20-min incubation). Similarly, in hCG-treated groups the linear rate of activity was observed up to 60 min of incubation (10 μg of protein) and 40 μg of protein (20-min incubation period) per incubation.

Measurement of Phosphorylation and Dephosphorylation State of HMG CoA Reductase. Because HMG CoA reductase in several systems has been shown to exist in phospho (inactive) and dephospho (active) forms (Brown et al., 1979; Ingebritsen et al., 1979; Beg & Brewer, 1981), we also examined the relative activity of two forms of enzyme in luteal tissue. Phosphorylated and dephosphorylated states of luteal enzyme were determined by measuring enzyme activity in microsomal preparations isolated in the presence of 50 mM NaCl or 50 mM NaF (Brown et al., 1979). Sodium fluoride, an inhibitor of phosphoprotein phosphatase, blocks the conversion of inactive enzyme to the active form, which appears to proceed through the action of phosphatase. Two luteinized ovaries from each group were homogenized in 4 mL of medium containing 0.3 M sucrose, 10 mM NaEDTA, 10 mM 2-mercaptoethanol, and either 50 mM NaCl or 50 mM NaF. Each homogenate was then processed for isolation of a microsomal pellet as described above. The pellet in each case was resuspended in suitable aliquots of buffer B (20 mM imidazole/chloride, pH 7.4, and 5 mM dithiothreitol) and use for enzyme assay as described above.

Measurement of Rat Liver HMG CoA Reductase Activity. Enzyme activity was measured as described by Brown et al. (1979). Specific activity was expressed as picomoles of [^{14}C]mevalonic acid formed per minute per milligram of protein.

Assay of NADPH-Cytochrome c Reductase. Rat luteal microsomal NADPH-cytochrome *c* reductase activity was measured by the procedure of Bramley & Ryan (1978). The millimolar extinction difference ($cm^{-1} \cdot mM^{-1}$) of 21.1 between reduced, and oxidized cytochrome *c* at 550 nm (Omura & Takesue, 1970) was used to calculate enzyme activity.

Aminoglutethimide (Elipten) Treatment. Aminoglutethimide, the inhibitor of cholesterol side-chain cleavage enzyme, was used to block steroidogenesis in luteinized rat ovary. Groups of rats were injected intraperitoneally (day 5 post-hCG) with the drug at a dose of 60 mg/kg body weight every 4 h for up to 12 h (Andersen & Dietschy, 1981; Schuler et al., 1981b). Isolated luteinized ovaries were then used for the measurement of HMG CoA reductase activity quantitation of cholesteryl ester and cholesterol content.

4-Aminopyrazolo[3,4-d]pyrimidine (4-APP) Treatment. To lower blood cholesterol levels, the rats were injected intraperitoneally with 4-APP (15 mg/kg body weight) (Azhar & Menon, 1981) in phosphate-buffered saline, pH 3.0, between 0900 and 1000 h on days 3, 4, and 5 (post-hCG). Control animals received the vehicle, and all the animals were sacrificed on day 6. This treatment lowered blood cholesterol levels from 45.5 ± 1.9 (control) to 5.3 ± 0.6 mg/dL (4-APP treated). To study combined effects of 4-APP and hCG, in some cases

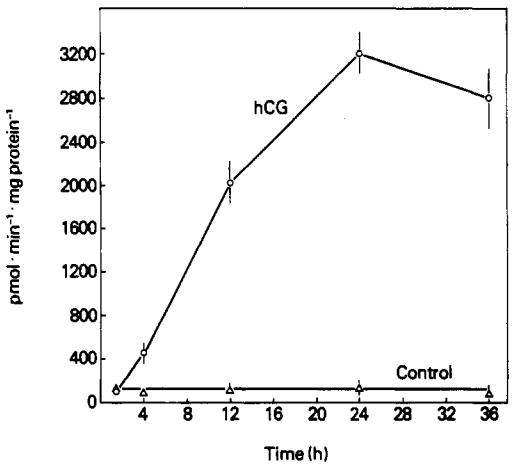

FIGURE 1: Time-dependent effect of hCG administration on stimulation of HMG CoA reductase activity. Results are mean ● SE of three separate experiments. Other details were the same as described under Experimental Procedures.

4-APP-treated rats were also injected with 25 IU of hCG on day 5 and animals were killed 24 h later.

[^{14}C]*Acetate Incorporation into Luteal Cholesterol and Cholesteryl Ester.* The effect of gonadotropin treatment on [^{14}C]acetate incorporation into luteal cholesterol and cholesteryl ester was assessed as follows. Ovarian slices (~2-mm thickness) from one ovary were incubated in a final volume of 1 mL of medium 199 containing 0.1% bovine serum albumin and 2 mM acetate (5 μCi of [^{14}C]acetate) at 37 °C in the atmosphere of 5% CO_2/95% O_2 for 4 h. After incubation, slices were washed extensively with medium 199 containing 2 mM unlabeled sodium acetate to remove any extracellular radioactivity. Total lipids from slices were extracted with chloroform–methanol by the method of Bligh & Dyer (1959) as modified by Billah et al. (1981). Briefly, slices were homogenized in 2.4 mL of 0.9% NaCl, followed by successive addition and mixing of 9 mL of chloroform/methanol (1/2) containing 2 × 10^4 dpm of [^3H]cholesterol (to monitor recovery), 3 mL of chloroform, and 3 mL of 2 M KCl containing 5 mM EDTA. Following the removal of upper aqueous phase, the organic phase was washed 4 times with 1/1 mixture of methanol and 0.9% NaCl. Finally, the organic phase was dried under N_2 and the residue redissolved in chloroform and spotted on Whatman LK5DF linear-K plates. The spotted plates were subjected to two developments in one dimension as described by Bitman et al. (1981). Chloroform/methanol/acetic acid (98/2/1) was used as a solvent for the first development (up to 17 cm), and hexane/ethyl ether/acetic acid (96/4/0.2) was used as a solvent for the second development (up to the top of the plate). Areas corresponding to cholesterol and cholesteryl esters were localized by exposure to iodine vapor, scraped, and counted for radioactivity in a Beckman LS 9000 liquid scintillation spectrometer after the addition of Beta-Max.

Miscellaneous Analytical Procedures. Ovarian-free cholesterol and cholesteryl esters were extracted and separated by the silicic acid/Celite column chromatography procedure of Brown et al. (1975) as described previously (Azhar & Menon, 1981; Azhar et al., 1983; Verschoor-Klootwyk et al., 1983). Isolated cholesteryl esters, after saponification in alcoholic KOH and free cholesterol, were quantitated by the micromethod of Glick et al. (1964). The cholesterol content of plasma samples was determined enzymatically by a Reagents Kit supplied by Sigma. The protein content of the microsomal fraction was determined by a modification of the procedure of Lowry et al. (1951) as described by Markwell et al. (1978).

Table I: Effect of Administration of Various Hormones on Mean (±SE) HMG CoA Reductase Activity in Luteinized Rat Ovary[a]

hormone	HMG CoA reductase activity [pmol·min^{-1}·(mg of protein)$^{-1}$] (±SE)
control	85 ± 21
hCG (2 μg, 25 IU)	1977 ± 170
o-LH (50 μg)	1773 ± 246
o-FSH (50 μg)	360 ± 21
r-prolactin (100 μg)	99 ± 19
growth hormone (50 μg)	110 ± 32

[a] Groups of three to six rats were injected subcutaneously with indicated dose of various hormones. Rats were killed 12 h later, and HMG CoA reductase activity was measured in isolated luteal microsomal fractions.

Table II: Effect of hCG Treatment on Microsomal HMG CoA Reductase Activity in Rat Liver and Luteinized Ovary

treatment	activity [pmol·min^{-1}·(mg of protein)$^{-1}$] (±SE)	
	liver	luteinized ovary
control	359 ± 70	132 ± 24
hCG (25 IU, 24 h)	343 ± 29	3216 ± 350

Table III: Effect of hCG Treatment on Microsomal NADPH-Cytochrome *c* Reductase and HMG CoA Reductase in Luteinized Rat Ovary

treatment	NADPH-cytochrome *c* reductase [pmol·min^{-1}·(mg of protein)$^{-1}$] (±SE)	HMG CoA reductase [pmol·min^{-1}·(mg of protein)$^{-1}$] (±SE)
control	55 ± 3	183 ± 37
hCg (25 IU, 24 h)	51 ± 2	3757 ± 491

Results

Time- and Dose-Dependent Effect of hCG Administration on HMG CoA Reductase Activity. Results shown in Figure 1 demonstrate the ability of hCG (25 IU) to increase HMG CoA reductase activity of luteinized rat ovaries as a function of time. An increase in HMG CoA reductase activity was noted as early as 4 h following injection of hCG, and maximum stimulation (~30–40-fold) was seen at 24 h. Enzyme activity, however, was slightly lower at 36-h post-hCG treatment. In contrast, enzyme activity in saline-treated controls remained essentially unchanged during the entire experimental period.

The increase in HMG CoA reductase activity in response to various amounts of hCG is shown in Figure 2. A 10-fold increase in enzyme activity was seen with the lowest amount of hCG used (0.2 unit), and maximum activation (35–40-fold) was reached at a dose of approximately 10 IU of hCG.

Specificity of Gonadotropin Effect. Several other hormones were tested for their ability to stimulate HMG CoA reductase activity, but the data in Table I indicate that only o-LH (50 μg) effectively enhanced enzyme activity. The maximum LH effect was observed approximately 12 h after administration, and the extent of stimulation was comparable to that of hCG. It can be seen that o-FSH treatment resulted in only a slight increase in enzyme activity, while r-prolactin or growth hormone were without effect.

Further evidence for the specificity of the gonadotropin-induced rise in HMG CoA reductase of luteinized ovaries was the inability of hCG to induce any change either in liver HMG CoA reductase activity or in luteinized ovary microsomal NADPH-cytochrome *c* reductase activity (Tables II and III).

Phosphorylation–Dephosphorylation State of HMG CoA Reductase. Since HMG CoA reductase activity has been

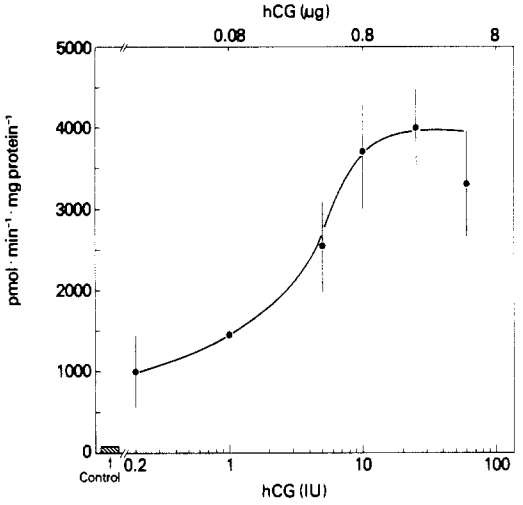

FIGURE 2: Dose-dependent effect of hCG administration on HMG CoA reductase activity. Results are mean ± SE of three separate experiments. Groups of three rats were injected with the indicated dose of hCG for 20 h. Other details were the same as described under Experimental Procedures.

Table IV: Effect of NaF on Mean (±SE) Microsonal HMG CoA Reductase Activity from Luteinized Ovary of Saline or hCG-Injected Animals

	HMG CoA reductase activity [pmol·min^{-1}·(mg of protein)$^{-1}$][a]	
treatment	NaF (50 mM)	NaCl (50 mM)
saline	34 ± 8	114 ± 5.6
hCG	345 ± 53	1249 ± 114.0

[a] Results are mean ± SE of four separate experiments. For these experiments rats were treated with hCG (25 IU) for only a short time (6 h).

shown to exist in both phospho (inactive) and dephospho (active) forms (Beg & Brewer, 1981; Brown et al., 1979; Ingebritsen et al., 1979), we attempted to see if the hCG-induced stimulation in enzyme activity was due to a change in the phosphorylated state of the enzyme. In order to examine this question, we isolated luteal microsomes in the presence of sodium fluoride; i.e., a treatment which blocks conversion of the inactive (phospho) to the active (dephospho) form of HMG CoA reductase, or in the absence of NaF. The results appear in Table IV and indicate that HMG CoA reductase activity was reduced by about 70% when microsomes were isolated in the presence of NaF, and this was true when the animals had been injected with either saline or hCG. However, although smaller in absolute magnitude, the relative ability of hCG to elevate HMG CoA reductase activity was not reduced when microsomes were isolated in the presence of NaF. Specifically, hCG produced an approximate 10-fold elevation in enzyme activity when microsomes were isolated in either NaCl or NaF. These results indicate that 70% of the luteal microsomal enzyme exists in the inactive (phospho) form under normal conditions, and the hCG-induced rise in HMG CoA reductase activity is due to a net increase in total amount of enzyme protein.

Effect of Aminoglutethimide and 4-Aminopyrazolo[3,4-d]pyrimidine (4-APP) Treatments. The rise in HMG CoA reductase activity in luteinized ovaries is associated with a decrease in both progesterone secretion and cellular sterol content. In order to evaluate the role played by the suppression of steroidogenesis in the increased level of enzyme activity, we studied the effect of aminoglutethimide, a potent inhibitor of progesterone secretion. The results of these experiments are seen in Table V. Table III again demonstrates the approximate 20-fold increase in HMG CoA reductase activity seen following hCG administration. In marked contrast, enzyme activity did not increase following aminoglutethimide. These data indicate that AMG-induced inhibition of progesterone synthesis and secretion did not lead to an alteration in HMG CoA reductase activity. However, aminoglutethimide treatment blocked the hCG-induced rise in enzyme activity (Table V).

Table V: Effect of Aminoglutethimide, 4-APP, and hCG Treatment on Choesteryl Ester and Cholesterol Content and HMG CoA Reductase Activity in Luteinized Rat Ovary

treatments	HMG CoA reductase [pmol·min^{-1}·(mg of protein)$^{-1}$] (±SE)	cholesteryl esters (μg/ovary) (±SE)	free cholesterol (μg/ovary) (±SE)
condition A			
control	91 ± 23	1068 ± 92	454 ± 28
hCG (25 IU, 24 h)	1700 ± 261	151 ± 20	381 ± 26
aminogluteth-imide[a]	59 ± 7	2041 ± 300	407 ± 42
aminogluteth-imide + hCG	155 ± 55	1732 ± 198	642 ± 96
condition B			
control	80 ± 7	911 ± 43	403 ± 23
hCG (25 IU, 24 h)	2233 ± 599	147 ± 5	420 ± 14
4-APP[b]	1422 ± 247	116 ± 9	272 ± 29
4-APP + hCG	5895 ± 874	122 ± 16	233 ± 44

[a] 60 mg/kg body weight every 4 h for 12 h. [b] 15 mg/kg body weight for 3 days.

The data in Table V illustrate the effects of hCG and aminoglutethimide on cellular cholesteryl ester and cholesterol content. It is clear from these results that the two treatments have totally different effects on cellular sterol content. In agreement with previously published results (Azhar et al., 1983), hCG caused a substantial fall in cholesteryl ester levels (Table V). However, aminoglutethimide treatment either alone or in combination with hCG resulted in an increase in cholesteryl ester levels. In contrast, drug treatment had no significant effect on free cholesterol content in luteinized ovary. Furthermore, combined treatments of aminoglutethimide and hCG produced a slight but significant increase in cholesterol levels.

The data in Table V could be interpreted to mean that the hCG-induced rise in HMG CoA reductase activity is a simple consequence of the depletion of cellular steryl ester content of the luteinized ovary. If this were the case, 4-APP treatment should lead to similar changes in cellular sterol content and HMG CoA reductase activity.

The data illustrated in Table V indicate that this is at least partially true. Thus, the data also demonstrate the cholesteryl ester content of luteinized ovaries was markedly reduced following administration of either hCG or 4-APP. In contrast, combined treatments of 4-APP and hCG did not reduced cholesteryl ester levels any further. Furthermore, the results shown also demonstrate that this was associated in both cases with a striking increase in HMG CoA reductase activity.

If the hCG-induced increase in HMG CoA reductase activity were simply secondary to a depletion of ovarian cholesteryl ester content, no further increment in HMG CoA reductase activity should occur when hCG was given in conjunction with 4-APP. However, the results in Table V clearly demonstrate that this was not the case, and the combination of hCG and 4-APP led to an increase in activity of HMG CoA reductase which was more than 3 times the level seen with

Table VI: Effect of 4-APP and/or hCG Treatment on [^{14}C]Acetate Incorporation into Cholesteryl Esters and Cholesterol of Luteinized Rat Ovary

treatments	radioactivity (dpm/mg of content) (±SE)	
	cholesterol	cholesteryl esters
control	17922 ± 2086	7273 ± 1192
hCG (25 IU, 4 h)	94906 ± 14018	288176 ± 43658
4-APP (15 mg/kg BW, 3 days)	82296 ± 7420	84830 ± 8632
hCG + 4-APP	99544 ± 9989	828635 ± 136006

either hCG or 4-APP given alone. Thus, it seems reasonable to suggest that the increase in HMG CoA reductase activity associated with hCG administration cannot be viewed as being a simple function of a reduction in ovarian cholesteryl ester content.

We next examined the effect of 4-APP and/or hCG on [^{14}C]acetate incorporation into cholesterol and cholesteryl esters. Results presented in Table VI demonstrate that hCG or 4-APP treatment enhanced [^{14}C]acetate conversion into cholesterol. However, combined treatments of 4-APP and hCG failed to produce any synergistic effect on cholesterol synthesis. In contrast, these two agents showed a differential effect on [^{14}C]acetate incorporation into cholesteryl ester. As can be seen, hCG was much more effective compared to 4-APP in stimulating [^{14}C]acetate conversion into cholesteryl ester. Furthermore, like the rise in HMG CoA reductase, combined treatments with hCG and 4-APP produced a synergistic increase in cholesteryl ester synthesis. These experiments further support the notion that the hCG-induced rise in enzyme activity was not simply due to depletion of sterol esters.

Discussion

In the present studies, we have attempted to study regulation of HMG CoA reductase during gonadotropin-induced desensitization of steroidogenesis response. Results of previous studies have demonstrated changes in HMG CoA reductase activity in ovaries from pregnant rabbits (Kovanen et al., 1978) and pseudopregnant rats (Schuler et al., 1979). However, in contrast to the rapid and massive stimulation of the enzyme (30–35-fold) observed in the present studies, HMG CoA reductase activity in rabbit ovary did not reach peak levels until 4–6 days after LH-induced ovulation or start of pregnancy (Kovanen et al., 1978). Similarly, in PMSG–hCG-primed rats, significant increases in enzyme activity (approximately 4-fold) were not reported until 2 days after initiation of pseudopregnancy (Schuler et al., 1979). Since both pregnancy and pseudopregnancy are characterized by an increase in progesterone synthesis and cholesteryl ester content, the increase in ovarian HMG CoA reductase activity was thought to be secondary to this phenomenon. However, the current results have clearly indicated that ovarian HMG CoA reductase activity increased dramatically in response to hCG, at a time when steroidogenesis has been more than 90% suppressed (Azhar et al., 1983). Further support for the lack of any constant or direct relationship between steroidogenesis and ovarian HMG CoA reductase activity can be derived for the fact that aminoglutethimide inhibition of progesterone synthesis had no stimulatory effect on enzyme activity. However, combined treatment of aminoglutethimide–hCG prevented the hormone-stimulated rise in enzyme activity. The exact mechanism by which aminoglutethimide prevented the hCG-stimulated rise of HMG CoA reductase is not clear at the present time. It is possible that increased utilization of stored cholesterol for pregnenolone production in response to hCG may in turn induce de novo cholesterol synthesis through the stimulation of HMG CoA reductase to meet increased demand for cholesterol. By inhibition of the pregnenolone production, aminoglutethimide decreased the demand for stored cholesterol and thus prevented the rise of HMG CoA reductase activity. However, studies dealing with time-related changes in progesterone response and HMG CoA reductase levels do not fully support this conclusion. For example, HMG CoA reductase is increased at times when cellular progesterone production was suppressed to about 90%. These results further point to a dissociation between extent of steroidogenesis and levels of enzyme activity. The latter results, however, do not rule out the possibility of feedback signals originating between cholesterol and progesterone.

Although HMG CoA reductase activity appears to be easily disassociated from the ability of the ovary to synthesize and secrete progesterone, the relationship between luteal cell sterol content and enzyme activity is more complicated. It is apparent that the increase in HMG CoA reductase seen in ovaries from desensitized rats is associated with a marked decline in luteal cell sterol content. Furthermore, HMG CoA reductase activity markedly increased when intracellular cholesteryl ester content was reduced by administration of 4-APP. On the other hand, we think it possible for several reasons that the elevation in HMG CoA reductase activity of luteinized ovaries is not a simple consequence of cellular cholesterol depletion. In the first place, HMG CoA reductase activity increased dramatically in response to the administration of hCG plus 4-APP, reaching levels which were significantly greater than those seen following treatment with either agent alone. Indeed, HMG CoA reductase activity of rats treated with hCG plus 4-APP was greater than the additive effect of either when they were administered alone, despite the fact that combined treatment with 4-APP plus hCG did not reduce cholesteryl ester levels more than either agent by itself. Furthermore, Freeman & Ascoli (1982) have demonstrated that the depletion of cholesteryl ester stores observed in hCG-desensitized cultured Leydig tumor cells did not lead to any change in HMG CoA reductase activity. Finally, the increase in HMG CoA reductase activity seen in ovaries from pregnant rabbits and pseudopregnant rats was associated with an increase in intracellular sterol content. Thus, a complex relationship seems to exist between the intracellular sterol content of steroidogenic cells and HMG CoA reductase activity.

In conclusion, the results we have presented demonstrate that there is a prompt and striking increase in luteal cell HMG CoA reductase activity early in the process of desensitization. This response to hCG is hormone, tissue, and enzyme specific, and the increase in HMG CoA reductase activity is due to a net increase in total amount of enzyme. The increase in enzyme activity in luteal cells cannot be explained on the basis of the suppression of steroidogenesis that occurs in luteinized ovaries, does not appear to be a simple consequence of depleted intracellular sterol content, and may, at least in part, represent a specific response to hCG. The role played in the process of desensitization by this change in activity of the key enzyme-regulating cholesterol biosynthesis remains to be clarified.

Registry No. HMG CoA, 9028-35-7; PMSG, 9002-70-4; hCG, 9002-61-3; LH, 9002-67-9; cholesterol, 57-88-5.

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Direct Transfer of Reduced Nicotinamide Adenine Dinucleotide from Glyceraldehyde-3-phosphate Dehydrogenase to Liver Alcohol Dehydrogenase†

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ABSTRACT: The reduction of benzaldehyde and *p*-nitrobenzaldehyde by NADH, catalyzed by horse liver alcohol dehydrogenase (LADH), has been found to be faster when NADH is bound to glyceraldehyde-3-phosphate dehydrogenase (GPDH) than with free NADH. The rate of reduction of aldehyde substrate with GPDH–NADH follows a Michaelian concentration dependence on GPDH–NADH. The reaction velocity is independent of GPDH concentration when [GPDH] > $[NADH]_{total}$. The K_m for GPDH–NADH is higher than that for free NADH. The reaction velocities in the presence of excess GPDH over NADH cannot be accounted for on the basis of the free NADH concentration arising from dissociation of the GPDH–NADH complex. These observations suggest that transfer of NADH from GPDH to LADH proceeds through the initial formation of a GPDH–NADH–LADH complex. Arguments for a direct enzyme–coenzyme–enzyme transfer mechanism are substantiated and quantitated both by steady-state kinetic studies and by determinations of all of the appropriate enzyme–coenzyme equilibrium dissociation constants. In contrast, over a similar concentration range, the complex lactate dehydrogenase (LDH)–NADH is not a substrate for the LADH-catalyzed reductions. Likewise, the LADH–NADH complex is not a substrate for the LDH-catalyzed reduction of pyruvate.

In a previous paper from this laboratory, Weber & Bernhard (1982) demonstrated that 1,3-diphosphoglycerate is transferred between glyceraldehyde-3-phosphate dehydrogenase (GPDH)[1] and phosphoglycerate kinase (PGK) via direct enzyme–enzyme complex formation. The question arose immediately as to the generality or frequency of occurrence of such direct transfer steps in glycolysis, and in other pathways involving globular cytosolic enzymes which do not form multienzyme complexes.

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[1] Abbreviations: GPDH, glyceraldehyde-3-phosphate dehydrogenase; PGK, phosphoglycerate kinase; LADH, liver alcohol dehydrogenase; LDH, lactate dehydrogenase; EDTA, ethylenediaminetetraacetic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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