Mechanism of Action of Gonadotropin

I. EVIDENCE FOR GONADOTROPIN-INDUCED MODIFICATIONS OF OVARIAN NUCLEAR BASIC AND ACIDIC PROTEIN BIOSYNTHESIS, PHOSPHORYLATION, AND ACETYLATION*

(Received for publication, May 5, 1972)

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SUMMARY

The time course of chorionic gonadotropin stimulation of nuclear basic and acidic protein biosynthesis, phosphorylation, and acetylation as well as DNA biosynthesis in ovaries of immature rats has been investigated. After injection of radioactive precursor and chorionic gonadotropin into immature rats, ovarian histone fractions F1, F2a1, F2a2, F2b, and F3 were isolated by selective acid extraction. Acidic protein fractions AP1 and AP2 were extracted with 4 M CsCl at pH 11.6 and 14, and were separated from DNA by equilibrium density centrifugation. Histones, acidic proteins, and DNA were purified by Sephadex chromatography. Chorionic gonadotropin was demonstrated to accelerate the incorporation of [3H]lysine into nuclear acidic protein fraction AP1 and into histones F2a1, F2a2, and F3 within 10 min following a single injection of the hormone. Increased rates of phosphorylation of histones F1 and F2a1 and acidic proteins AP1 and AP2, as well as increased rates of acetylation of histone F2a1 and acidic protein AP2, were also observed within 10 min following gonadotropin injection. Stimulation of DNA, histones F1 and F2b and acidic protein AP2 biosynthesis lagged and was not observed until 30 to 180 min after hormone administration. A similarly delayed response to hormonal stimulation was observed when phosphorylation and acetylation of histones F2a2 and F3 were studied. Actinomycin D and cycloheximide administration abolished gonadotropin-stimulated biosynthesis but not phosphorylation and acetylation of histones and acidic proteins. This indicates that hormonally stimulated modification of nuclear proteins by acetylation and phosphorylation was not entirely dependent on protein synthesis. It is suggested that these results would be compatible with the idea that modification of ovarian nuclear protein metabolism is an early event in the action of chorionic gonadotropin.

In addition to steroidogenesis, gonadotropins control various metabolic processes in the ovary including, most fundamentally, ovarian differentiation, growth, and function. The mechanisms whereby gonadotropins control these processes are not well understood. It has been shown that the biological action of gonadotropins in the ovary of prepubertal rats is preceded by a stimulation of rapidly labeled nuclear RNA synthesis in vivo (1, 2) and of DNA-dependent RNA polymerase activity assayed in preparations of isolated nuclei (3). Any attempt to elucidate the primary sites of action of gonadotropins must necessarily focus on the sequence of molecular events prior and ultimately leading to increased RNA synthesis.

Nuclear basic and acidic proteins have been implicated in the regulation of nuclear RNA synthesis. Histones, for example, repress DNA-directed RNA synthesis in vitro (4, 5), but the relatively limited number of histones (6) and their regular distribution in diverse cell types make it unlikely that the precision and selectivity required for the control of transcription in the chromosomes of differentiated tissues are primarily due to histone concentration and distribution. Auxiliary mechanisms have been proposed through which histones could acquire a limited degree of specificity necessary for the activation or deactivation of specific genetic mechanisms. Among these mechanisms are the well established enzymatic chemical modifications of histone molecules by acetylation (7) or phosphorylation (8), and it is widely accepted that these structural modifications may, at least to a certain degree, decrease their repressor activity and account for their specific and selective role in controlling DNA transcription in different cell types (9-12). In contrast to the suppressing activity of histones on DNA-dependent RNA polymerase activity (13), acidic nuclear phosphoproteins seem to increase the rate of transcription from a DNA template in the presence of histones (9). Of even greater significance, the distribution of acidic nuclear proteins is apparently tissue-specific (14, 15). It is thought that their interaction with nuclear DNA provides tissue-specific regulation of RNA synthesis.

If, as suggested (4, 16-19), regulation of tissue-specific nuclear RNA synthesis is one of the major effects in the intracellular action of growth-promoting and developmental hormones, one would expect changes in metabolism of regulatory chromosomal proteins to occur at times of hormonally induced modification of gene expression. The experiments to be described deal with the effects of administration to immature female rats of HCG on ovarian nuclear basic and acidic protein
biosynthesis and their enzymatic chemical modification. Evidence will be presented to show that HCG causes increases in biosynthesis, phosphorylation, and acetylation of various ovarian nuclear protein fractions shortly after its administration. It is conceivable that these early changes in chromosomal protein metabolism proceed or represent an integral part of the induction process of RNA synthesis.

**EXPERIMENTAL PROCEDURE**

Chemicals—Cesium chloride, optical grade, and sucrose were obtained from Schwarz/Mann, Orangeburg, N. Y. All other biochemical reagents were purchased from Sigma Chemical Company, St. Louis, Mo. The following radioactive chemicals were supplied by Amersham/Searle Corporation, Arlington Heights, Ill.: Sodium 

\(^{14}C\)acetate (specific activity 50 mCi per mmole), \(^{3}H\)thymidine (specific activity 26 Ci per mmole), \(^{3}H\)lysine (specific activity 4.6 Ci per mmole), and carrier-free \(^{32}P\)orthophosphate acid. The \(^{32}P\)orthophosphate acid was neutralized with 0.2 \(\times\) NaHCO\(_3\) before use. A.P.L. (human chorionic gonadotropin) from Ayerst Laboratories, New York, N. Y., was used for ovarian stimulation.

Animals and Isotope Administration Procedures—Immature female rats, 20 days old, weighing 45 to 50 g, were obtained from Holtzman Company, Madison, Wis. Each animal received an intraperitoneal injection of 0.2 ml of 0.9% sodium chloride solution per 100 g of body weight containing the following quantities of isotopes: 0.1 mCi of \(^{3}H\)orthophosphoric acid, 40 \(\mu\)Ci of \(^{3}H\)lysine, 40 \(\mu\)Ci of \(^{14}C\)acetate. All animals were killed 1 hour after injection of the isotopes. HCG was administered at various times before death as described in the text. The ovaries were removed, dissected free of adipose and connective tissue, and used for the isolation of nuclei.

Isolation of Nuclei—All subsequent steps in the isolation of nuclei and nuclear proteins were performed in the cold room at 2°C. The ovaries were homogenized in 10 volumes of 0.32 M sucrose-3 mM CaCl\(_2\)-0.05 mM Tris, pH 7.2, with a Teflon-to-glass homogenizer. The homogenate was filtered through gauze and again homogenized with three strokes in all glass conical homogenizer with a pestle clearance of 3 \(\times\) 10\(^{-3}\) inches at 1,750 rpm. The homogenate was centrifuged at 1,100 \(\times\) g for 20 min. The crude nuclear pellet was resuspended in 10 volumes of 2.1 M sucrose-3 mM CaCl\(_2\)-0.05 mM Tris, pH 7.2, and the nuclei were sedimented by centrifugation at 105,000 \(\times\) g for 1\(\frac{1}{2}\) hours. The purified nuclei were suspended by gentle homogenization with a glass homogenizer in 20 volumes of 0.14 M NaCl-0.01 mM sodium citrate. The nuclei were recovered by centrifugation at 2,000 \(\times\) g for 5 min. This step was repeated once. The resultant nuclear residue was used for the extraction of histones, DNA, and acidic proteins.

**Extraction of Histones, Nuclear Acidic Proteins, and DNA—** Histones F1, F2a, F2b, and F3 were isolated from the purified nuclear fractions by selective acid extraction according to the slightly modified method of Hnilica et al. (20) which we have described in a previous publication (21). Histone F2a was separated by acetone precipitation into subfractions F2a1 and F2a2 according to the method of Johns (22). After extraction of the histones, the nuclear acidic protein fractions API and AP2, and DNA were isolated from the acid-extracted nuclear residue by a modified procedure of Benjamin and Gellhorn (23) which we have described in detail (24). Isolation of acidic proteins API and AP2 and DNA by this method was achieved by selective extraction of the nuclear residue with 4 M CsCl at pH 11.6 and 14, respectively, and subsequent separation of the acidic proteins and DNA by equilibrium density centrifugation.

**Column Chromatography of Histones, Acidic Proteins, and DNA**—All column chromatographic procedures have been described previously (21, 24). Histones were purified by chromatography on Sephadex-carboxymethylcellulose C-50. Elution of histones was carried out with a linear gradient between a solution of 0.005 \(\times\) HCl-0.5 M NaCl and 0.01 \(\times\) HCl-0.5 M NaCl. The fractions containing the histones were dialyzed for 20 hours against 0.005 \(\times\) HCl and concentrated by lyophilization to solutions containing about 3% protein. The solutions obtained were used for the determination of radioactivity and polyacrylamide gel electrophoresis. Acidic proteins API and AP2 were purified by gel filtration on Sephadex G-200. Elution of the proteins was carried out with 0.01 \(\times\) NaOH-8 M urea-2 M EDTA-2 M mercaptoethanol. The fractions containing protein were combined, dialyzed for 18 hours against 0.01 \(\times\) NaOH-2 M mercaptoethanol, followed by dialysis against deionized water for 5 hours and lyophilization. The lyophilized fractions were used for determination of radioactivity and acrylamide gel electrophoresis. The nuclear acidic protein fractions API and AP2 prepared in this fashion were free of contaminating DNA and RNA as determined by the diphenylamine or orcinol reaction.

DNA obtained after equilibrium density centrifugation in 4 M CsCl contained 0.5 to 0.7% contaminating protein. To remove the protein, we added 2 mg of DNA dissolved in 1 ml of eluent to a column (20 \(\times\) 1 cm) of Sephadex G-200 packed in 1 M NaCl-0.01 M Tris buffer, pH 8.5. This buffer was also used for elution. Under these conditions, DNA was not retained on the column and was eluted with the void volume. The fraction containing DNA was dialyzed for 20 hours against 0.01 \(\times\) NaOH. The DNA recovered contained less than 0.01% protein and less than 0.1% RNA, as determined by the Lowry or orcinol reaction.

Histone and nonhistone acidic protein were determined according to the standard method of Lowry et al. (25) with calf thymus F1-histone sulfate as reference substance. DNA was determined with diphenylamine, following the procedure of Burton (26) with calf thymus DNA (Sigma; type I) as standard. RNA was assayed with the orcinol reaction (27).

**Polyacrylamide Disc Electrophoresis—** Polyacrylamide gel disc electrophoresis was performed with the polyacrylamide gel electrophoresis unit and diffusion destainer from Hoefer Scientific Instruments, San Francisco, Calif. All chemicals used for electrophoresis were supplied by Canaco, Rockville, Md. The procedure used for disc electrophoresis of histone was described in detail in a previous publication (21). Gel electrophoresis of acidic nuclear proteins was done by the procedure of Teng et al. (15).

**Measurement of Radioactivity—** Protein and DNA samples (0.5 to 2 mg) were placed in 20-ml glass vials and dissolved in 2 ml of NCS solubilizer (Amersham/Searle) by shaking for 3 hours in a water bath at 50°C. After the addition of 15 ml of liquid scintillation solution (6 g of 2,5-diphenyloxazole (PPO), 250 mg of dimethyl-1,4-bis-[2-(5-phenyloxazolyl)]benzene (dimethyl-POP) in 1 liter of toluene), the samples were counted in a model 3375 Packard Tri-Carb liquid scintillation spectrometer. With the optimal settings for counting \(^{3}H\) and \(^{14}C\) or \(^{3}H\) and \(^{32}P\) simultaneously, the average background was 32 cpm in the red channel, 34 cpm in the green channel, and 11 cpm in the blue channel. Under optimum counting conditions the mean efficiencies were 92% for \(^{3}H\) in the blue channel, 41% for \(^{14}C\) in the green channel, and 3.5 for \(^{32}P\), 45% for \(^{14}C\), and 26% for \(^{3}H\) in the red channel. Quenching was determined by the automatic...
external standardization method. All measurements were corrected to 100% counting efficiency and were recorded as disintegrations per min.

RESULTS

Criteria of Purity of Nuclear Proteins—All nuclear preparations used in the isolation of nuclear acidic and basic proteins were extensively purified to eliminate unspecific contamination by cytoplasmic particles. In order to reduce the likelihood of such contamination, nuclei obtained after sedimentation in 0.32 M sucrose were sedimented again through 2.1 M sucrose. After this purification step, nuclei were found to be relatively free of cytoplasmic particles when examined under a phase contrast microscope. Spectral ratios of all nuclear preparations measured at 260/330 nm were at least 10:1 or higher. Determination of the protein to DNA mass ratios yielded values between 2.1 and 2.4.

It has been recognized that crude histone fractions obtained by selective acid extraction of nuclei contain substantial amounts of contaminating materials, in particular, inorganic phosphate, non-histone phosphoproteins, and RNA (28, 29). To circumvent the difficulties arising from the analysis of such crude contaminated histone fractions, more reliable methodology has been developed, such as the isolation of specific histone peptide fragments (10, 28), providing a greater analytical specificity. Contaminations can also be effectively minimized by column chromatography or acrylamide gel electrophoresis (29). We have chosen to purify histones and acidic proteins chromatographically on Con-Sephadex or Sephadex G-200. It was previously established (21, 24) that histones purified in this manner are free of contaminating DNA and RNA and do not change their specific activity (disintegrations per min of 3H (respectively 32P) per mg of protein) significantly after additional purification by acrylamide gel electrophoresis. The identity and composition of histones and nuclear acidic protein fractions API and AP2 were routinely evaluated by acrylamide gel electrophoresis after column chromatography. With the electrophoretic procedures used, we have not detected any alterations of the qualitative electrophoretic patterns of ovarian histones and acidic proteins isolated from rats before and after HCG treatment.

Effect of HCG on Histone, Acidic Protein, and DNA Biosynthesis—Fig. 1 compares the effects of HCG on the synthesis of histones, acidic proteins, and DNA isolated from ovarian nuclei 1 hour following injection of [3H]lysine or [3H]thymidine. Since the content of lysine differs in each protein fraction, a comparison of the rate of synthesis of the individual fractions expressed on a protein-weight basis alone is not accurate. For this reason, we have corrected the specific activities for the lysine content of the individual protein fractions.8 The rates of synthesis of nuclear proteins and DNA are stimulated in all instances at various times following injection of HCG to immature rats. Histones, acidic proteins API and AP2, and DNA clearly exhibit a distinct inequality in their increase of precursor incorporation in response to hormonal stimulation. A marked stimulation of about 130% of [3H]lysine incorporation into acidic protein API (pH 11.6-soluble fraction) is observed as early as 10 min following HCG injection. Similarly, the rates of biosynthesis of histones F2a1, F2a2, and F3 are significantly increased 10 min after hormone treatment. Acidic protein AP2 (0.05 x NaOH-soluble fraction) and the relatively lysine-rich histones F1 and F2b exhibit a delayed response and significant increases of their specific activities are not observed until 60 min after gonadotropin injection. The data of Fig. 1 also clearly indicate that gonadotropin-stimulated increases of the biosynthesis of histones F2a1, F2a2, F3, and acidic protein AP1 precede stimulation of [3H]thymidine incorporation into DNA. The earliest noticeable increase in DNA biosynthesis occurs at 30 min and reaches a maximum 18 hours following HCG treatment. Interestingly, the rate of radioactive precursor incorporation into DNA and histones F2a1, F2a2, and F3 remains significantly increased 5 days after HCG administration, in contrast to API, AP2, and histone F1 which no longer exhibit increased [3H]lysine incorporation 5 days after hormone stimulation.

Enzymatic Phosphorylation of Histones and Nuclear Acidic Proteins—Administration of HCG to immature female rats leads
to significant increases of $^{32}$P incorporation into ovarian histones and acidic proteins. The extent of $^{32}$P labeling at various times following HCG treatment is illustrated in Fig. 2 which compares the specific activities of histones F1, F2a1, F2a2, F2b, and F3. Labeling of histone F2a1 with $^{32}$P is already significantly stimulated 10 min after HCG injection. When longer time intervals are allowed to elapse after HCG injection, the extent of phosphorylation is markedly higher in the F2a1, F2a2, and F3 histones than in histones F2b and F1 (with the exception of the marked increase of F1 phosphorylation 60 min after HCG treatment). Comparison of the rate of phosphorylation of histones in relation to their biosynthetic rate (Fig. 3) reveals a significantly increased turnover of protein-bound $^{32}$P in the arginine-rich F2a1 histone at all times following hormone stimulation with an early peak 10 min after HCG injection. Interestingly, the relative phosphorylation of histone F2b is not increased, whereas histones F1, F2a2, and F3 exhibit hormone-stimulated $^{32}$P turnover at various times following HCG injection.

The effect of hormone treatment on the incorporation of $^{32}$P into acidic proteins AP1 and AP2 as well as the corresponding relative phosphorylation activities are shown in Fig. 4. Incorporation of $^{32}$P into AP1 is markedly increased at all times following hormone stimulation, with the exception of Day 5 when no increased phosphorylation is found. However, correlation of the rate of phosphorylation with biosynthesis, expressed as relative phosphorylation activity, suggests that the increased incorporation of $^{32}$P is to a large extent coupled to the biosynthesis of AP1, since only slight (but significant) increases in relative activity are calculated from the data listed in Fig. 3. Each rat received an intraperitoneal injection of sodium $^{32}$P orthophosphate (100 μCi per 100 g) (together with $^{3}$H]lysine; see Fig. 1) 1 hour before death. At the indicated times after HCG treatment, the rats were killed. Histones were isolated and purified as described under “Experimental Procedure.” Values are the arithmetic mean of four experiments, 30 rats per experiment; brackets indicate S.D. $\triangle$, histone F1; $\bigcirc$, histone F2a1; ■, histone F2a2; $\blacktriangle$, histone F2b; $\bullet$, histone F3.

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Relative activity = \( \frac{\text{dpm of } ^{32}\text{P per mg of protein} \times 100}{\text{dpm of } ^{3}\text{H per mg of protein}} \)
Fig. 5. Effect of HCG treatment on the time course of in vivo $^{14}$C-acetate incorporation into ovarian histones of the immature rat. The rats were treated with HCG as listed in legend of Fig. 1. Each rat received an intraperitoneal injection of sodium $[1-^{14}$C]acetate (20 $\mu$Ci per 100 g) (together with $[^3$H]lysine; see Fig. 1) 1 hour before death. See Fig. 2 for additional details and symbols.

Relative phosphorylation activity of API are found 10, 30, and 60 min following hormone treatment. Three and 18 hours after hormone injection, the relative phosphorylation of API is significantly decreased and returns to control levels at the fifth day after HCG injection. $^{32}$P incorporation into AP2 as well as the relative phosphorylation activity are stimulated at 10 min, and decrease 60, 180 min, and 18 hours after HCG administration.

Enzymatic Acetylation of Histones and Nuclear Acidic Proteins—The effect of gonadotropin on the incorporation of $^{14}$C into histones and the corresponding relative rates of acetylation are illustrated in Figs. 5 and 6. In general, incorporation of $^{14}$C-acetate into histones is enhanced following hormone treatment, but the time course of increased $^{14}$C labeling differs for each fraction depending on the time elapsed after HCG injection. The extent of hormone-stimulated $F_1$ and $F_2b$ $^{14}$C labeling is of a rather small order of magnitude, when compared to the extensive increases in $^{3}$C labeling of $F_2a_1$, $F_2a_2$, and $F_3$ (Fig. 5), and appears to be related to the accelerated biosynthesis of $F_1$ and $F_2b$ as evidenced by their relative acetylation rates (Fig. 6). Increased $^{3}$C labeling of histone $F_3$ also seems to be related to its hormonally stimulated increased rate of biosynthesis, with the exception of a small increase in relative acetylation 60 min after HCG injection. Histone $F_2a_1$ is significantly modified by increased acetylation 10 min after HCG injection (Fig. 6). In addition, HCG injection causes enhanced relative acetylation of $F_2a_1$ and $F_2a_2$ 3 hours to 5 days following hormone treatment.

As Fig. 7 illustrates, treatment of immature rats with HCG for 10 to 60 min results in significant increases of the rates of $^{14}$C incorporation into ovarian nuclear acidic protein fractions API and AP2. However, when the rates of $^{14}$C incorporation are related to biosynthesis, API and AP2 exhibit markedly different patterns of relative acetylation. The increased turnover of the $^{14}$C label of AP2 at 10, 30, and 60 min is contrasted by marked}

Fig. 6. Effect of HCG treatment on the time course of in vivo $^{14}$C-acetate incorporation into rat ovarian histones in relation to their rate of biosynthesis. The values of relative activity were calculated from the data listed in Figs. 1 and 5. See Fig. 2 for symbols.

Relative activity = \frac{\text{dpm of }^{14}\text{C per mg of protein} \times 100}{\text{dpm of }^{3}\text{H per mg of protein}}

Fig. 7. Effect of HCG treatment on the time course of in vivo $^{14}$C-acetate incorporation into ovarian nuclear acidic proteins of the immature rat and $^{14}$C-acetate incorporation in relation to the rate of acidic protein biosynthesis. The rats were treated with HCG as listed in legend of Fig. 1. Each rat received an intraperitoneal injection of sodium $[1-^{14}$C]acetate (20 $\mu$Ci per 100 g) (together with $[^3$H]lysine; see Fig. 1) 1 hour before death. At the indicated times after HCG treatment, the rats were killed. API and AP2 were isolated and purified as described under "Experimental Procedure." Values are the arithmetic mean of four experiments, 30 rats per experiment; brackets indicate S.D. The values of relative activity were calculated from the specific activities listed in this figure and from the data listed in Fig. 1 using the formula given in Fig. 6. See Fig. 4 for symbols.
Actinomycin D (600 μg per 100 g) was injected intraperitoneally 4 hours, and cycloheximide (5 mg per 100 g) was injected intraperitoneally 40 min before death. When histones were studied, 200 i.u. of HCG were injected intraperitoneally 18 hours before death; acidic proteins were isolated 10 min following HCG treatment. One hour before killing, all rats received an intraperitoneal injection of either [3H]lysine (40 ¼s per 100 g) together with sodium [32P]orthophosphate (100 PCi per 100 g), or [3H]lysine (40 ¼s per 100 g) together with sodium [32P]orthophosphate (100 PCi per 100 g), respectively. Histones and acidic proteins were isolated and purified as described under "Experimental Procedure." The values listed are arithmetic means of three experiments, 20 rats per experiment.

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<th>Specific activity of nuclear proteins</th>
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<th>Acidic proteins</th>
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Table 1

Comparative effects of actinomycin D and cycloheximide on [3H]lysine, [14C]acetate, and [32P]phosphate incorporation into ovarian nuclear proteins

Actinomycin D (600 μg per 100 g) was injected intraperitoneally 4 hours, and cycloheximide (5 mg per 100 g) was injected intraperitoneally 40 min before death. When histones were studied, 200 i.u. of HCG were injected intraperitoneally 18 hours before death; acidic proteins were isolated 10 min following HCG treatment. One hour before killing, all rats received an intraperitoneal injection of either [3H]lysine (40 ¼s per 100 g) together with sodium [32P]orthophosphate (100 PCi per 100 g), or [3H]lysine (40 ¼s per 100 g) together with sodium [32P]orthophosphate (100 PCi per 100 g), respectively. Histones and acidic proteins were isolated and purified as described under "Experimental Procedure." The values listed are arithmetic means of three experiments, 20 rats per experiment.

Values for S.D., which are not shown, did not exceed 20% of the arithmetic mean in any experiment. The specific activities (disintegrations per min of [3H] per mg of protein) of histones and acidic proteins after [3H]lysine incorporation are corrected for the lysine content of each fraction.

decreases of 14C turnover at 3 and 18 hours, and return to control levels 5 days after HCG treatment. The relative rates of acetylation of AP1 are generally decreased following HCG injection, with the exception of Day 5, when the relative rate of acetylation returns to control levels.

**Effect of Actinomycin D and Cycloheximide on [3H]Lysine, [14C]Acetate, and [32P]Phosphate Incorporation into Ovarian Nuclear Proteins Following HCG Administration**—The effects of actinomycin D and cycloheximide on HCG-stimulated biosynthesis, acetylation, and phosphorylation of ovarian nuclear proteins are listed in Table I. The data indicate that under the experimental conditions administration of actinomycin D and of cycloheximide generally decreases biosynthesis of all histones and nuclear acidic proteins. Administration of HCG together with actinomycin D or cycloheximide has little or no effect on [3H]lysine incorporation, which remains significantly decreased. In actinomycin D- or cycloheximide-treated rats phosphorylation and acetylation of all histones and acidic proteins are greatly reduced. However, it is quite evident that HCG administration causes marked increases in 32P and 14C labeling of histones and acidic proteins in actinomycin D- or cycloheximide-treated rats, and partly overcomes the inhibitory effects of actinomycin D and cycloheximide.

**DISCUSSION**

The above data demonstrate that several metabolic processes involving nuclear basic and acidic proteins are significantly modified by HCG in ovaries of immature rats. The biosynthesis of all nuclear proteins and DNA, as measured by radioactive precursor incorporation, becomes significantly elevated at various times following hormonal stimulation. More importantly, the increased rates of biosynthesis of acidic protein AP1 and histones F2a1, F2a2, and F3 are readily detectable within 10 min after intraperitoneal injection of HCG and clearly precede hormonal stimulation of ovarian DNA biosynthesis (see Fig. 1). These findings are of considerable interest in view of the fact that accumulation of characteristic synthesis of histones in mammalian cells cultures is known to be temporally coupled to the synthesis of DNA during chromosome replication (30–32). There is also indirect evidence that biosynthesis of histones precedes DNA biosynthesis in regenerating liver (31–35).

Increased rates of 32P and 14C labeling of most nuclear proteins are seen at various times following HCG injection. In interpreting these data, it has to be considered that the increased rates of 32P and 14C labeling may partly be due to the concomitantly proceeded accelerated biosynthesis of acidic and basic proteins rather than to a postsynthetic acetylation and phosphorylation of existing nuclear proteins. We have, therefore, applied corrections for the changes in [3H]lysine incorporation, and the corrected data (relative activities of phosphorylation and acetylation) should more accurately reflect HCG-induced chemical modifications of previously formed nuclear protein molecules and metabolic turnover of phosphate and acetyl groups. Although incorporation of 14C and 32P into ovarian acidic and basic nuclear
proteins can be significantly inhibited by actinomycin D or cycloheximide, inhibition can be partly overcome by HCG treatment of the animal. This indirectly indicates that (a) only \(^{3}^{14}C\) and \(^{3}^{14}P\) labeling, which occurs as an integral part of nuclear protein biosynthesis, is inhibited; (b) HCG stimulates acetylation and phosphorylation of previously formed intact protein molecules, and this process is not inhibited by actinomycin D or cycloheximide; and (c) phosphorylation and acetylation are direct effects of the hormone and are not dependent on increased synthesis of protein phosphokinases, acetylases, or other enzymes.

Evidence has accumulated in recent years indicating that the biosynthesis of nuclear proteins or their chemical modification by phosphorylation and acetylation can be increased at times of gene activation and increased RNA synthesis (36–38). Specific examples are provided by the effects of 17β-estradiol and cortisol, both of which specifically increase the selective synthesis of a nuclear acidic protein fraction and the acetylation of histones in uterus (39, 40), or liver (37, 41), respectively. Studies by Langan (10) have demonstrated that glucagon and insulin cause marked increases in the phosphorylation of rat liver histone F1. Based on his studies, considered in relation to previous observations showing that histone phosphokinase activity is stimulated by cyclic 3′,5′-AMP (42, 43), Langan proposed that histone phosphorylation may be part of a mechanism by which hormones, mediated by cyclic 3′,5′-AMP, induce RNA synthesis in target tissues. In view of the reported stimulation of ovarian adenyl cyclase by gonadotropins (44–47) and the presence of cyclic 3′,5′-AMP-dependent protein kinase in bovine ovary (48), a closely related mechanism of hormonal stimulation of RNA synthesis might be expected to operate in the ovary.

In summary the data presented in this paper establish stimulation of biosynthesis of nuclear acidic protein fraction AP1 and of histones F2a1, F2a2, and F3 as being an early event in the acute effect of HCG upon the ovaries of the immature rat. Increased phosphorylation of histone F1 and F2a1, and acidic proteins AP1 and AP2, as well as acetylation of histone F2a1 and acidic protein AP2 are also observed within 10 min following HCG administration. Whether these events are of primary consequence in the action of HCG and lead to the early hormone-stimulated synthesis of ovarian nuclear RNA remains to be determined. Recent reports suggest that gonadotropins may not enter the ovarian target cells but instead bind in some manner to cellular membrane receptor sites (49, 50), thereby activating membrane bound adenyl cyclase and increasing intracellular cyclic 3′,5′-AMP levels. It remains a distinct possibility, therefore, that early hormonal stimulation of biosynthesis and chemical modification of nuclear proteins, ultimately leading to increased RNA synthesis, may be mediated by cyclic 3′,5′-AMP. Further studies are necessary to confirm such a mechanism.

Acknowledgment—We thank Mr. Peter Hiestand and Mrs. R. Jonassen for their technical assistance.

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