Gonadotropin Regulation of Nucleotide Biosynthesis in Corpus Luteum†

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ABSTRACT: The incorporation of [2-^{14}C]glycine into 5′-phosphoribosylglycinamide (P-Rib-GlyNH$_2$) and its formyl derivative (P-Rib-fGlyNH$_2$) was measured in high-speed supernatant prepared from homogenates of corpora lutea from the pig, cow, and human ovary. Azaserine, an inhibitor of phosphoribosylformylglycinamidine synthetase (EC 6.3.5.3), was used to stop *de novo* synthesis of purines at the stage of P-Rib-fGlyNH$_2$. Luteinizing hormone (LH) or human chorionic gonadotropin (HCG) added at a level of 0.1–1 μg to 2 ml of incubation medium markedly stimulated the early steps of purine biosynthesis in the presence of added glucose 6-phosphate (Glc-6-P). Adrenocorticotropin, follicle-stimulating hormone, and cAMP had no stimulatory effect. Substrates such as 6-phosphogluconate, Rib-P, and PP-Rib-P stimulated the reaction but there was no additional effect of luteinizing hormone. The stimulatory effect of LH and HCG on *de novo* purine biosynthesis results from activation of corpus luteum Glc-6-P-dehydrogenase (EC 1.1.1.49) leading to an increased generation of PP-Rib-P *via* the pentose phosphate pathway. PP-Rib-P is presumed to be rate limiting to 5′-phosphoribosylpyrophosphate amidotransferase (EC 2.4.2.14), the first enzyme of *de novo* purine biosynthesis. The α and β subunits of LH and HCG inhibited the stimulatory effect of LH or HCG to P-Rib-GlyNH$_2$ formation. Gonadotropin stimulated the formation of $^{14}CO_2$ from [1-^{14}C]Glc-6-P in corpus luteum supernatant. The salvage pathways for the synthesis of UMP from orotate and PP-Rib-P and for the synthesis of AMP, ADP, and ATP from adenine and PP-Rib-P were also studied. Under the experimental conditions used, no effects of gonadotropins were found on the enzymes PP-Rib-P-amidotransferase, Rib-P-pyrophosphokinase, adenine phosphoribosyltransferase, orotidylic pyrophosphorylase, or orotidylic decarboxylase.

An important regulatory mechanism in hormone responsive tissues derives from trophic hormone stimulation of unique species of Glc-6-P[1]-dehydrogenase (EC 1.1.1.49). In addition to generating NADPH, this provides 5′-phosphoribosyl pyrophosphate (PP-Rib-P) *via* the pentose phosphate pathway for several different enzyme systems involved in the synthesis of ribonucleotides (McKerns, 1968, 1969a–c). Regulation of *de novo* biosynthesis of pyrimidine nucleotides is achieved by the concentration of PP-Rib-P available to orotidine 5′-phosphate pyrophosphorylase (EC 2.4.2.10). The same is true with 5′-phosphoribosylpyrophosphate amidotransferase (EC 2.4.2.14) in *de novo* purine biosynthesis. ACTH stimulated the biosynthesis of pyrimidine and purine nucleotides in supernatant from adrenal cortex by inducing an increased generation of PP-Rib-P (McKerns, 1969b, 1972, 1973b). We have shown previously that ACTH activated purified adrenal Glc-6-P-dehydrogenase (McKerns, 1964; Criss and McKerns, 1968, 1969). HCG stimulated purified corpus luteum Glc-6-P-dehydrogenase (McKerns, 1969a). In addition, LH and HCG stimulated Glc-6-P-dehydrogenase, pyrimidine nucleotide, and RNA synthesis in rat ovaries (McKerns, 1969a). To extend these studies, we have examined the role of LH, HCG, their subunits, and other hormones in regulating the biosynthesis of nucleotides in supernatant from corpus luteum of the pig, cow, and human. A preliminary report of this work has been presented (McKerns, 1973a).

Materials and Methods

Ovaries were obtained from young cows and pigs killed at the University Meat Laboratories under the supervision of the Animal Science Department. Corpora lutea from days 8 to 16 of the estrus cycle of cows and from days 10 to 11 of the estrus cycle of pigs were immediately dissected out and kept over crushed ice. Human corpora lutea were obtained immediately after surgery on days 3–5 after ovulation. Tissues were finely minced with scissors and homogenized in an all-glass homogenizer using approximately 200 mg of tissue/ml of buffer. The buffer used in all experiments was 75 mM Tris-HCl (pH 7.4) with 50 mM KCl, 15 mM NaCl, 0.1 mM $CaCl_2$, 10 mM $MgCl_2$, and 4.5 mM K_2HPO_4.

The homogenate was centrifuged at 105,000*g* for 1 hr and the clear supernatant was dispensed into tubes in 1.5-ml aliquots and stored at −30°. Protein content was determined by the method of Lowry *et al.* (1951). Different preparations varied somewhat in control values and in response to added substrates and gonadotropins. This may be because of the age of the corpora lutea and endogenous levels of substrates, LH and other hormones.

Assay Procedures

P-Rib-GlyNH$_2$ and P-Rib-fGlyNH$_2$. Assay 1. The early steps of purine biosynthesis *de novo* were assessed by measuring the formation of radioactive 5′-phosphoribosylglycinamide and 5′-phosphoribosyl-*N*-formylglycinamide as "total P-Rib-GlyNH$_2$." This has been previously described (McKerns, 1973b). The method was modified in that the eluate from the column was not evaporated to dryness under nitrogen. Rather, the 3 ml of eluate was run directly into a scintillation vial to which 17 ml of scintillation fluid was then added. Scintillation fluid was prepared with 2 l. of toluene, 1 l. of Triton X-100, and 11 g of Permablend I (91% 2,5-diphenyloxazole, 9%

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[1] Abbreviations used are: Glc-6-P, glucose 6-phosphate; P-Rib-GlyNH$_2$, 5′-phosphoribosylglycinamide; P-Rib-fGlyNH$_2$, formylated P-Rib-GlyNH$_2$; ACTH, adrenocorticotropin; LH, luteinizing hormone; HCG, human chorionic gonadotropin; FSH, follicle-stimulating hormone; TSH, thyroid-stimulating hormone.

1,4-bis[2-(4-methyl-5-phenyloxazole)]). Counting was carried out in a Nuclear-Chicago automatic scintillation counter.

P-Rib-fGlyNH$_2$. Assay 2. When P-Rib-fGlyNH$_2$ alone was to be measured, the method of Henderson (1962) was used. Aliquots (0.4 ml) of the CCl_3COOH supernatant were diluted with 1.5 ml of Tris-HCl buffer (pH 7.4) and applied to a Pasteur pipet column packed with AG 50W-X8 (formate). The column was washed through with 10 ml of 0.5 M formic acid and the P-Rib-fGlyNH$_2$ was eluted with 6 ml of 4 M formic acid. A 3-ml aliquot was added to a vial containing 17 ml of the scintillation fluid and radioactivity determined by scintillation counting.

$^{14}CO_2$ from [1-^{14}C]Glc-6-P. Assay 3. This method was used as an estimate of the rate of stimulation of Glc-6-P-dehydrogenase and has been described previously (McKerns, 1969b). Methylene Blue was added to the system so that lower levels of NADP could be used. The rapid reoxidation of NADPH gave a linear reaction rate over a longer time than would be possible without Methylene Blue.

Orotidylic Pyrophosphorylase and Orotidylic Decarboxylase. Assay 4. Orotic acid and PP-Rib-P are converted by orotidylic pyrophosphorylase to orotidine 5′-phosphate and this compound in turn is irreversibly decarboxylated by orotidylic decarboxylase to uridine 5′-phosphate. Each incubation mixture of a final volume of 200 μl contained buffer, [6-^{14}C]orotic acid, high-speed supernatant from corpus luteum, PP-Rib-P or Rib-5-P, and gonadotropin where indicated. Incubation at 37° was for 10 min. The reaction was terminated by immersing the tube for 1 min in a boiling-water bath after which the tubes were centrifuged. Ten microliters of superntant with carrier UMP was spotted on 2.5-cm channels ruled on cellulose thin layers with fluorescent indicator (Eastman Kodak Co.). The method of Randerath and Randerath (1964) with butanol–acetic acid–water (2:1:1) was used to develop the sheets. Eastman chromatogram developing apparatus 6071 was used for all thin-layer chromatography. The fluorescent areas corresponding to UMP were cut out and counted in a scintillation spectrophotometer. Some strips were also run through a radiochromatogram scanner (Packard Model 7201) and only one peak of radioactivity corresponding to UMP fluorescence was obtained. Since uridine nucleotides were not separated from each other, they were counted as UMP.

Ribose Phosphate Pyrophosphokinase and Adenine Phosphoribosyltransferase. Assay 5. The assay is based on the method of Henderson and Khoo (1965) in which PP-Rib-P and [^{14}C]adenine are converted into AMP by adenine phosphoribosyltransferase. Rib-P as substrate is converted to PP-Rib-P by Rib-P-pyrophosphokinase. Each incubation mixture of 100 μl contained 50 μl of high-speed supernatant fraction from corpus luteum, [8-^{14}C]adenine, 0.2 μmol of Rib-5-P, 0.5 μmol of UMP to inhibit pyrimidine biosynthesis, gonadotropin where indicated, and buffer to bring the mixture up to volume. Incubation was at 37° with shaking for various time intervals. The reaction was stopped by immersing the tubes for 1 min in a boiling-water bath. The mixture was then centrifuged. Polyethylenimine cellulose thin layers on Mylar sheets (Baker Chemical Co.) were used to separate purine ribonucleotides by the method of Crabtree and Henderson (1971) which is a modification of the method of Randerath and Randerath (1964). These sheets were first developed for 5 hr with 4 M sodium formate buffer (pH 3.4), dried, and then developed overnight with methanol–water (1:1). After these sheets were dried, 10 μl of supernatant plus 20 nmol each of AMP, ADP, and ATP were applied as a 2.0-cm streak 2.0 cm from the bottom of the sheet. The sheets were ruled vertically into seven channels of 2.4-cm widths. A wick of Whatman No. 3MM paper was stapled to the top of the sheet, and it was developed overnight with methanol–water (1:1) to wash salts, purine bases, and ribonucleotides onto the paper wick which was then discarded. All the unused [^{14}C]adenine washes into the wick. To separate the ribonucleotides, the sheets were then developed with increasing concentration of pH 3.4 buffers as follows: 0.5 M formate buffer to a line 2.5 cm above the origin, then 2.0 M formate buffer to a line 7.0 cm above the origin, and finally 4.0 M formate buffer to the top of the sheet. After the sheets were dried, ribonucleotides were visualized under uv light. R_F values were 0.68, 0.43, and 0.15 for AMP, ADP, and ATP, respectively. The areas containing the ribonucleotides were cut out and immersed in scintillation fluid and counted by scintillation counting. Recovery of radioactive standard was 75%. Radioactivity corresponding to the areas for AMP, ADP, and ATP was also located in some experiments using the radiochromatogram scanner.

Azaorotate (*s*-triazine-2-carboxylic acid, 4,6-dihydroxy-sodium salt, hydrate) and azaserine (*O*-diazoacetyl-L-serine) were obtained from Cancer Chemotherapy, National Service Center, National Institutes of Health, Bethesda, Md. Crystalline β^{1-24}-corticotropin (Synacthen) was a gift of Ciba Pharmaceutical Co., Summit, N. J. LH S_{17} and FSH S_9 were obtained from the National Institutes of Health. Dr. Darrell N. Ward supplied *O*-LH (HSN-3-122B) and the α and β chains of LH. HCG and its α and β subunits were obtained from Dr. Robert E. Canfield. [2-^{14}C]Glycine (4.92 or 22.5 Ci per mol), [8-^{14}C]adenine (52 Ci/mol), [6-^{14}C]orotate (13.6 Ci/mol), and [1-^{14}C]Glc-6-P (55 Ci/mol) were purchased from New England Nuclear, Boston, Mass. PP-Rib-P and L-glutamine were obtained from Calbiochem, Los Angeles, Calif.; AMP and ADP from Pabst Laboratories, Milwaukee, Wis.; cAMP from Nutritional Biochemicals, Cleveland, Ohio; NADP, Glc-6-P, 6-phosphogluconate, Rib-5-P, and ATP from Sigma Chemical Co., St. Louis, Mo.; and AG 50W-X8 (hydrogen and formate forms, 200–400 mesh), from Bio-Rad Laboratories. Richmond, Calif. Permablend I was purchased from Packard Instrument Co., Downers Grove, Calif., and Triton X-100 from Rohm and Haas Co., Philadelphia, Pa.; cellulose thin layers with fluorescent indicator from Eastman Kodak Co., Rochester, N. Y., and polyethylenimine cellulose thin layers from Baker Chemical Co., Phillipsburg, N. J.

Results

As shown in Figure 1, the biosynthesis of P-Rib-GlyNH$_2$ was stimulated in high-speed supernatant from bovine corpus luteum by the addition of Glc-6-P and NADP. There was an additional stimulation due to luteinizing hormone (NIH S_{17}) in the range 10–0.1 μg. An optimum response to LH was achieved by adding 1 μg of LH to 2 ml of incubation medium. Assuming a molecular weight of 30,000 and that the LH preparation is pure, 1 μg/2 ml is equivalent to 1.7×10^{-9} M. No stimulation was observed when LH was added at the level of 100 μg. The reason for this is not known, but a similar effect was observed with a solution of crystalline Glc-6-P-dehydrogenase prepared from adrenal cortex in the presence of high concentrations of ACTH. A maximum response was obtained with ACTH in the range 1–10 mol/mol of purified enzyme (Criss and McKerns, 1968).

The effect of protein concentration and time on the formation of P-Rib-GlyNH$_2$ was determined by assay procedure 1. Each reaction mixture contained supernatant, 0.06 μmol of

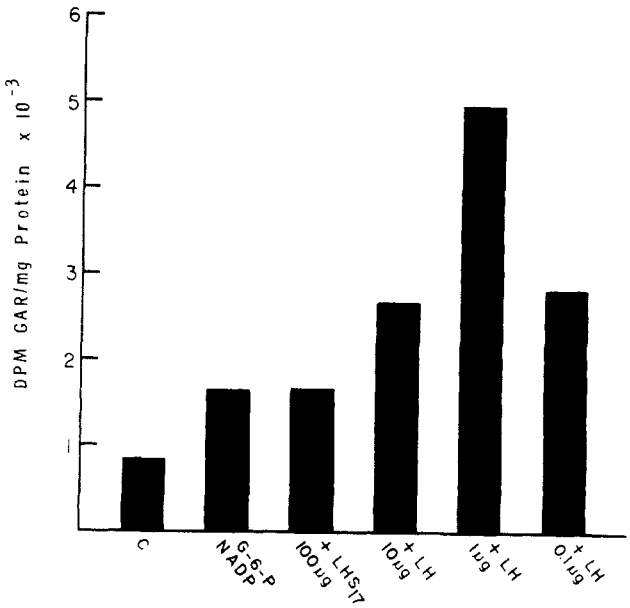

FIGURE 1: Response of supernatant prepared from cow corpus luteum to LH (NIH-S_{17}). Incubation was for 10 min at 37° and P-Rib-GlyNH$_2$ formed was determined by assay 1. The control value was determined by incubation in the absence of added Glc-6-P or NADP. All other values were obtained in the presence of Glc-6-P (8 μmol) and NADP (0.06 μmol). LH was added at the levels indicated. The reaction was started by the addition of 4 μl of a solution containing 0.08 μCi of [2-^{14}C]glycine (4.92 Ci/mol). Total volume was 2 ml. Each value is the mean of three separate determinations. 10^3 dpm indicated the formation of 91 pmol of P-Rib-GlyNH$_2$. The standard error of the mean from left to right was 71, 139, 144, 161, 172, and 61 dpm.

NADP, 8 μmol of Glc-6-P and 0.08 μCi of [2-^{14}C]glycine (4.92 Ci/mol) in a total volume of 2 ml. The effect of protein concentration was measured at 0.1, 0.2, 0.4, and 0.8 mg with a 10-min incubation. The additional LH response in this range was obtained with LH at a level of 1 μg. The time response to substrates (Glc-6-P and NADP) and to substrates plus LH was carried out with a protein concentration of 0.324 mg/2 ml of incubation mixture and time intervals of 3, 6, 9, and 12 min. In spite of the long chain of enzymatic reactions from Glc-6-P to P-Rib-GlyNH$_2$, the response to time and protein concentration was linear under the assay procedure described. In most determinations, a 10-min incubation was used with a quantity of protein intermediate in the range 0.1–0.8 mg.

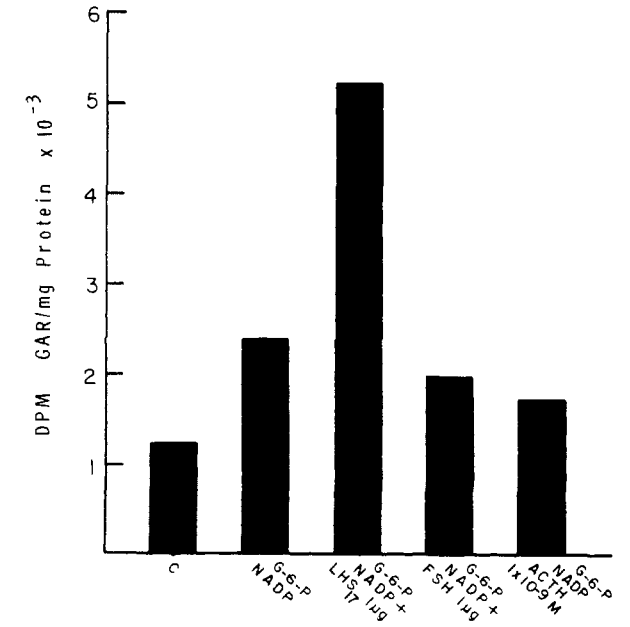

FIGURE 2: Effect of various trophic hormones on the formation of P-Rib-GlyNH$_2$ in supernatant prepared from cow corpus luteum. Assay 1 with an incubation time of 10 min was used. Each reaction mixture contained supernatant, and where indicated 0.06 μmol of NADP, 8 μmol of Glc-6-P, LH, FSH, or ACTH, and 0.08 μCi of [2-^{14}C]glycine (4.92 Ci/mol) in a total volume of 2 ml. Each value is the mean of 3. From left to right the standard error of the mean was 91, 214, 476, 122, and 25 dpm.

TABLE I: Response of Supernatant from Cow Corpus Luteum to LH and the β Subunit of LH.[a]

Additives	Dpm of [^{14}C]P-Rib-GlyNH$_2$/mg of Protein
None (control)	180
Glc-6-P + NADP	570[b]
Glc-6-P + NADP + LH S_{17}	1310[b]
Glc-6-P + NADP + *O*-LH	1120[b]
Glc-6-P + NADP + LH-β	280[b]

[a] Assay 1 was used. Incubation time was 10 min. Supernatant (0.41 mg of protein) with Glc-6-P (8 μmol), NADP (0.06 μmol), 0.08 μCi of [2-^{14}C]glycine (22.5 Ci/mol), and LH (1 μg) or LH-β (1 μg) added as indicated. Total volume was 2 ml. Each value is the mean of 3. 49.2 dpm indicated the formation of 1 pmol of P-Rib-GlyNH$_2$. [b] $P < 0.05$.

The specificity of the response to LH is apparent in Figure 2. Both FSH and ACTH had a slight inhibitory effect on the stimulus to the formation of P-Rib-GlyNH$_2$ in the presence of added Glc-6-P and NADP.

By using assay 2 for the measurement of P-Rib-fGlyNH$_2$, Glc-6-P and NADP increased the production of P-Rib-fGlyNH$_2$ almost fourfold in 10 min compared with the control. LH increased P-Rib-fGlyNH$_2$ synthesis an additional threefold.

Table I shows the response to P-Rib-GlyNH$_2$ formation with two preparations of LH and the effect of the β subunit of LH. In other experiments it has been shown that both the α and β subunits of LH block the stimulatory effect of LH on P-Rib-GlyNH$_2$ biosynthesis.

In Table II, the effect of HCG and its α and β subunits on the formation of P-Rib-GlyNH$_2$ in a high-speed supernatant prepared from human corpus luteum is shown. The addition of Glc-6-P and NADP to the incubation mixture increased the synthesis of P-Rib-GlyNH$_2$ and HCG stimulated an additional synthesis. Both the α subunit and the β subunit of HCG had little intrinsic activity on the formation of P-Rib-GlyNH$_2$ as shown in expt 1. However, both subunits blocked the stimulatory effect of the intact molecule of HCG.

The effect of various substrates on P-Rib-GlyNH$_2$ biosynthesis and the interaction with LH was explored. In Table III, it can be seen that the metabolism of both Glc-6-P and 6-phosphogluconate provide PP-Rib-P for the rate-limiting PP-Rib-P-amidotransferase reaction. There is also a marked response to the synthesis of P-Rib-GlyNH$_2$ when PP-Rib-P itself is added. However, the only stimulatory effect of LH compared with the appropriate control value was with Glc-6-phosphate as substrate. There was some LH stimulus in

TABLE II: P-Rib-GlyNH$_2$ Synthesis by Supernatant from Human Corpus Luteum in Response to HCG, HCG-α, and HCG-β.[a]

Additives	Dpm of [^{14}C]P-Rib-GlyNH$_2$/mg of Protein
Expt 1	
Control (10-min incubation)	1280
Glc-6-P + NADP	1920
Glc-6-P + NADP + HCG	2760
Glc-6-P + NADP + HCG-α	1770
Glc-6-P + NADP + HCG-β	2200
Glc-6-P + NADP + HCG-α + HCG-β	1880
Expt 2	
Control (15-min incubation)	1540
Glc-6-P + NADP	2330
Glc-6-P + NADP + HCG	4900
Glc-6-P + NADP + HCG + HCG-α	2310
Glc-6-P + NADP + HCG + HCG-β	2150

[a] Assay method 1 was used. Each incubation mixture contained in a total volume of 750 μl supernatant from human corpus luteum (0.57 mg of protein in expt 1 and 0.40 mg in expt 2), [2-^{14}C]glycine (22.5 Ci/mol), 4 μmol of Glc-6-P, 0.0075 μmol of NADP, and 0.5 μg of HCG or subunits where indicated. Incubation time was 10 min at 37° with shaking in expt 1 and 15 min in expt 2. Each value is the mean of three separate determinations.

the control series in the absence of added substrates, presumably because of endogenous Glc-6-P.

Since cAMP has been so ubiquitously implicated in cell regulation, the effect of this compound was tested on the supernatant. The results are given in Table IV. cAMP within the range of concentrations shown had a slight inhibitory action. This is probably due to inhibition of PP-Rib-P-amidotransferase since it has been shown that AMP and other "end products" of purine pathways inhibit the pigeon liver enzyme (Wyngaarden and Ashton, 1959; Caskey *et al.*, 1964). It was also demonstrated previously that cAMP has no stimulatory effect on purified Glc-6-P-dehydrogenase prepared from adrenal cortex (Criss and McKerns, 1968).

Figure 3 shows the stimulatory effect of LH on the rate of formation of $^{14}CO_2$ from [1-^{14}C]Glc-6-P. Some of the $^{14}CO_2$

TABLE III: LH Effect on P-Rib-GlyNH$_2$ Biosynthesis in the Presence of Various Substrates.[a]

Additives	Dpm of [^{14}C]P-Rib-GlyNH$_2$/mg of Protein	
	− LH	LH S_{17}
Control	370	660
Glc-6-P	320	940[b]
Glc-6-P + NADP	610[c]	1800[c]
6-P-G	810[c]	940
6-P-G + NADP	730[c]	960
PP-Rib-P	1810[c]	1630

[a] P-Rib-GlyNH$_2$ was measured by the procedure of assay 1 with 0.08 μCi of [2-^{14}C]glycine (22.4 Ci/mol). Each reaction mixture of 2 ml contained supernatant of cow corpus luteum (0.25 mg of protein). Incubation was for 10 min in the presence of Glc-6-P (8 μmol), 6-phosphogluconate (6-P-G) (8 μmol), PP-Rib-P (10^{-6} M), and LH S_{17} (1 μg) where indicated. Each value is the mean of 3. [b] $P < 0.01$. [c] $P < 0.001$.

TABLE IV: Effect of LH and cAMP on P-Rib-GlyNH$_2$ Formation.[a]

Additives	Dpm of [^{14}C]P-Rib-GlyNH$_2$/mg of Protein
Glc-6-P + NADP	1601
Glc-6-P + NADP + LH	3010[b]
Glc-6-P + NADP + cAMP (10^{-5} M)	1360[c]
Glc-6-P + NADP + cAMP (10^{-7} M)	1260[c]
Glc-6-P + NADP + cAMP (10^{-9} M)	1400

[a] Assay 1 was used. Each 2 ml of incubation medium contained supernatant of cow corpus luteum (0.324 mg of protein). LH S_{17} was added at 1 μg where indicated. Glc-6-P (8 μmol), NADP (0.06 μmol), and 0.08 μCi of [2-^{14}C]glycine (22.5 Ci/mol) were added to all vessels. Each value is the mean of 3. [b] $P < 0.01$. [c] $P < 0.05$.

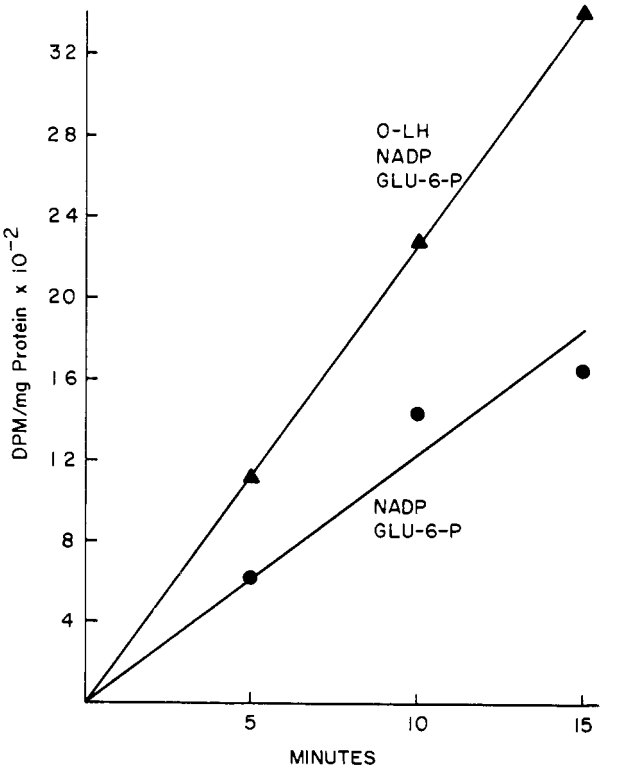

FIGURE 3: Stimulation of Glc-6-P-dehydrogenase by O-LH in high-speed supernatant prepared from human corpus luteum. Assay 3 was used. Each reaction mixture contained 0.25 ml of supernatant (0.75 mg of protein), 2.0 μmol of [1-^{14}C]Glc-6-P (0.09 Ci/mol), 0.005 μmol of NADP, 1.6 μmol of sodium pyruvate, and 0.01 μmol of Methylene Blue in a total volume of 0.5 ml. LH was added at a level of 1 μg. 1 dpm is equivalent to 5 pmol of Glc-6-P metabolized.

TABLE V: Effect of LH on Rib-5-P-Pyrophosphokinase, Orotidylic Pyrophosphorylase, Orotidylic Decarboxylase, and the Formation of UMP in Corpus Luteum Supernatant.[a]

Additives	pmol of UMP/mg of Protein
Experiment A	
Control	184
PP-Rib-P	395
PP-Rib-P + LH	434
Experiment B	
Control	219
Rib-5-P	328
Rib-5-P + LH	286

[a] Assay 4 was used as described in Methods. Each reaction mixture of 200-μl total volume contained 2 μmol of PP-Rib-P or Rib-P, 0.1 μCi of [6-^{14}C]orotic acid (13.6 Ci/mol) in expt A, and 0.05 μCi in expt B, and *O*-LH (0.25 μg in A and 0.125 μg in B). Experiment A contained 100 μl of supernatant (1.14 mg of protein) from pig corpus luteum. Experiment B contained 50 μl (0.623 mg of protein) of supernatant from human corpus luteum. Experiment B also contained 0.2 μmol of ATP and an ATP-regenerating system of phosphoenolpyruvate (0.2 μmol) and lactate dehydrogenase (0.002 μl) containing pyruvate kinase. Incubation was for 10 min at 37°. Each value is the mean of three separate determinations. Under the conditions of the assay 31 dpm is equivalent to 1 pmol of UMP.

TABLE VI: Luteinizing Hormone Effect on Ribose Pyrophosphokinase and Adenine Phosphoribosyltransferase in Corpus Luteum Supernatant.[a]

	pmoles/mg of Protein		
	AMP	ADP	ATP
0 time	17	2	0
1 min	607	61	8
2 min	740	94	12
3 min	599	223	34
1 min + *O*-LH	560	52	5
2 min + *O*-LH	934	137	19
3 min + *O*-LH	690	181	42

[a] Assay 5 was used. Each incubation mixture contained 50 μl of supernatant from pig corpus luteum (0.65 mg of protein), 0.125 μCi of [8-^{14}C]adenine (52 Ci/mol), 0.2 μmol of Rib-5-P, 0.5 μmol of UMP, and 0.125 μg of *O*-LH where indicated in a total volume of 100 μl. Each value is the mean of two separate determinations. A pmole is equivalent to 115 dpm.

is probably derived *via* glycolysis. The additional $^{14}CO_2$ generation induced by LH presumably represents increased metabolism from the pentose phosphate pathway. This experiment provides additional evidence for a specific activating effect of gonadotropin on ovarian Glc-6-P-dehydrogenase.

There is competition for the available PP-Rib-P by a number of systems in ribonucleotide synthesis between the *de novo* pathways and the salvage pathways. Experiments were carried out on major salvage pathways for pyrimidine and purine synthesis to determine if there was any gonadotropin effect. It was also of interest to find out if there was any direct action of gonadotropins on Rib-5-P-pyrophosphokinase. Under the experimental conditions outlined in Table V, it is apparent that there is no LH effect on the utilization of added PP-Rib-P in the salvage system for the formation of UMP. In addition, there is no significant effect of luteinizing hormone on the pyrophosphokinase reaction. As shown in Table VI, there was no significant effect of luteinizing hormone on the formation of adenine ribonucleotides with ribose phosphate as the substrate. The rate of AMP formation was extremely rapid, with a slower rate of formation of ADP. ATP was presumably being used up in the ribose pyrophosphokinase reaction for the formation of PP-Rib-P. In other experiments, it was found that with the addition of ATP (0.5 μmol) and an ATP-regenerating system consisting of phosphoenolpyruvate (0.2 μmol) and Sigma lactic dehydrogenase type I containing pyruvate kinase (2 μl), most of the counts were in ATP. Our main concern in these experiments was to determine that there was no inhibitory effect on salvage systems which would allow for the *de novo* systems to utilize the excess PP-Rib-P.

Discussion

PP-Rib-P is an essential substrate for *de novo* purine and pyrimidine biosynthesis, for the purine and pyrimidine salvage pathways, and for pyridine synthesis in man (Fox and Kelley, 1971). The high-speed supernatant fractions from corpora lutea were found to contain, among other enzymes, those of the pentose phosphate pathway for the metabolism of Glc-6-P to Rib-P, phosphoribopyrophosphokinase, and the enzymes of *de novo* purine and pyrimidine nucleotide biosynthesis as well as the enzymes of the salvage pathways. The evidence presented here suggests that LH and HCG activate corpus luteum Glc-6-P-dehydrogenase to increase the supply of Rib-P required in nucleotide biosynthesis. Direct stimulatory effects of ACTH on purified adrenal Glc-6-P-dehydrogenase (Criss and McKerns, 1968) and of HCG on corpus luteum Glc-6-P-dehydrogenase (McKerns, 1969a) have been demonstrated previously. These trophic hormones have no effect on nontarget tissues.

Whereas 6-phosphogluconate, Rib-5-P, and PP-Rib-P stimulated P-Rib-GlyNH_2 formation in the *de novo* pathways of purine biosynthesis, no additional stimulatory effect of LH or HCG was noted. Stimulatory effects were also found with the addition of Rib-5-P and PP-Rib-P as substrates in the salvage pathways for pyrimidine and purine ribonucleotides synthesis. Again, the further addition of gonadotropins was without effect. The various experiments indicate no direct effect of gonadotropin on PP-Rib-P-amidotransferase, the presumed rate-limiting enzyme of *de novo* purine nucleotide biosynthesis. Gonadotropins were without effect on the conversion of Rib-5-P to PP-Rib-P by the enzyme ribose phosphate pyrophosphokinase. Adenine phosphoribosyltransferase, orotidylic pyrophosphorylase, and orotidylic decarboxylase in the salvage pathways were neither stimulated nor inhibited by the addition of gonadotropins in high-speed supernatant from corpora lutea. However, it has not been ruled out that gondotropins may have an allosteric effect on enzyme activity at lower substrate concentrations.

The only site of action of gonadotropins that has been demonstrated in supernatant from corpora lutea has been on Glc-6-P-dehydrogenase. The net synthesis of PP-Rib-P seems

to be regulated by gonadotropin activation of ovarian Glc-6-P-dehydrogenase to stimulate the metabolism of Glc-6-P by oxidative pathways.

The subunit nature of ovine LH was shown by Li and Starman (1964), De La Llosa *et al.* (1967), and Papkoff and Amantha Samy (1967). Studies on the subunits of bovine and human LH were carried out by Reichert *et al.* (1970), and on human LH by Rathnam and Saxena (1971). Ward *et al.* (1966) have presented evidence for dissimilar α and β subunits. Liu *et al.* (1972a,b) have determined the primary structure of LH-α and LH-β from ovine luteinizing hormone. The α subunit of LH has properties in common with other glycoprotein hormone α subunits: TSH (Liao and Pierce, 1970; Pierce *et al.*, 1971), HCG (Bell *et al.*, 1969; Swaminathan and Bahl, 1970), and FSH (Papkoff and Ekblad, 1970). The β subunit seems to confer the hormone specificity to the molecule.

The experiments reported here demonstrate that the α and β subunits of LH and HCG block the stimulatory action of the intact molecules on P-Rib-GlyNH_2 formation. It is not proven whether this interaction is at the level of Glc-6-P-dehydrogenase or whether the subunits inhibit an enzyme such as PP-Rib-P-amidotransferase in the *de novo* pathway for purine biosynthesis. These effects are being studied further in a number of test systems including those described in this paper. Smaller peptide subfractions of the α and β subunits are also being tested for possible inhibitory actions. If these interactions are substantiated, it would suggest that effective contraceptive compounds could be designed based on the structure or partial structure of the subunits.

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