INHIBITION OF CHEMICAL INDUCTION OF PORPHYRIN SYNTHESIS IN CHICK EMBRYO LIVER CELLS BY PARTIALLY PURIFIED HUMAN CHORIONIC GONADOTROPIN

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SUMMARY: Commercial preparations of human chorionic gonadotropin (hCG) inhibited chemical induction of δ -aminolevulinic acid synthetase and porphyrin formation in chick embryo liver cell cultures. The inhibition was not attributable to hCG since highly purified preparations of the hormone were not inhibitory. After fractionation of crude hCG on Sephadex G-100, inhibitory activity was found in two fractions, one of slightly smaller and one of much smaller molecular weight than hCG. Thus partially purified hCG may have other biologic effects than those caused by the hormone itself. Moreover, the occurrence of substances in crude hCG which at low concentrations can interfere with drug and hormone effects on liver cells is of biologic and potential clinical interest.

Human chorionic gonadotropin (hCG)[1] is a placental polypeptide. Its physiologic function is unknown. The partially purified hormone is used clinically as a pharmacologic agent to stimulate testicular androgen production. Partially purified hCG is also frequently used in studies of the hormone's biochemical effects. Thus, based on the observation that partially purified preparations of hCG inhibit the response of lymphocytes to phytohemagglutinin, it has been suggested that a principal function of hCG is to prevent immune rejection of the fetus (1-3). There is evidence, however, that the purified hormone is not antimitogenic (4). We have observed that partially purified hCG has another potent biochemical effect: it inhibits chemical induction of porphyrin formation. The inhibition is not attributable to hCG itself. The findings extend knowledge of the diversity of

[1] Abbreviations: hCG, human chorionic gonadotropin; APL, A.P.L., Ayerst Laboratories brand of hCG; ALAS, δ -aminolevulinic acid synthetase; AIA, allylisopropylacetamide.

actions of partially purified hCG and emphasize the pitfalls in attributing biochemical effects observed with the partially purified hormone to hCG itself.

METHODS: Chick embryo liver cells were cultured according to the method of Granick (5). 5×10^5 cells were added to 19 x 65 mm shell vials, each of which contained a glass cover slip and one ml medium, or 20 times the amount of cells and media were added to 10 cm petri dishes. Cells were maintained in 5% CO_2 in air at 37° for 24 hours. Media changes, additions to cultures and porphyrin measurements were performed as previously described (6). ALAS was measured in cells (7) from 10 cm petri dishes. Each determination was based on duplicate measurements on cells from two petri dishes. The capacity of the cells to incorporate ^{14}C-leucine into protein was measured as described by Incefy and Kappas (8). Protein was measured by the method of Lowry, Rosebrough, Farr and Randall (9).

Two lots of APL (Ayerst), one of Pregnyl (Organon), and three batches of crude hCG from Organon, the Netherlands, were tested. One lot of APL was a gift of Ayerst Laboratories, Inc. The other lots were purchased. Allylisopropylacetamide (AIA), a potent inducer of ALAS, was a gift of Hoffman-La Roche, Inc.

HCG was purified as previously described (10,11). Briefly, the starting material (Organon) was adsorbed to sulfoethyl Sephadex C-50, eluted, then fractionated on either Bio-gel P-150 or Sephadex G-100. The fractions with the most hCG activity (as determined by bioassay) were then applied to DEAE Sephadex A-50 and the purified hCG was eluted therefrom. The biologic activity of the purified hCG preparations tested ranged from 10,600 IU/mg to 14,300 IU/mg. In a separate experiment to determine the molecular weight of the inhibitor, 2 g of Organon commercial hCG, lot 92145, were passed over a column of Sephadex G-100 as detailed in the legend to Figure 2.

RESULTS: In Figure 1, dose related inhibition of AIA induced porphyrin formation by three separate lots of the crude Organon hCG is shown, and compared to the absence of an effect of hCG purified from each batch. Commercial preparations of Organon hCG (Pregnyl) and Ayerst hCG (APL) also caused dose related inhibition of chemically induced porphyrin formation. Purified hCG added to cultures in concentrations up to 1.8 mg/ml medium did not inhibit porphyrin accumulation.

APL was used to examine characteristics of the inhibitory effect. APL inhibited the increase in porphyrin formation by other chemicals as well as by AIA, including aminopyrine, pentobarbital, hexobarbital, secobarbital, meprobamate, and the steroid etiocholanolone (Table 1). The inhibition was not due to interference of APL with the extraction or measurement of porphyrins because porphyrin levels were not depressed in vials to which APL was added at the end of the incubation period.

APL inhibition of drug and steroid induced porphyrin formation was undiminished after the APL was heated to 60° for ten minutes. Preincubating APL

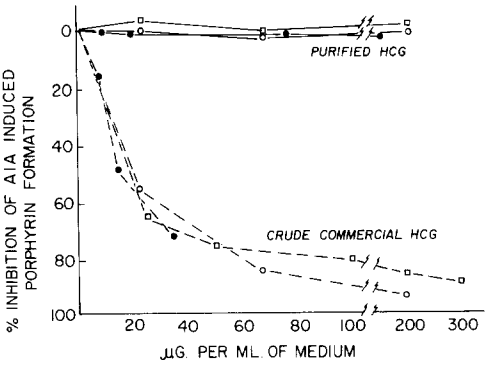

FIGURE 1: Effects of hCG preparations on AIA induction of porphyrin formation. Three separate lots of purified hCG and of the crude commercial preparations from which the hormone was purified were added in the concentrations noted on the abscissa to culture vials containing 5 x 10^5 cells, 1 ml medium and 30 µg AIA. Each point represents the mean porphyrin content in five replicate vials. HCG CR-115 (14,300 IU/mg) was purified from Organon Lot 91783 (3300 IU/mg) symbolized by the closed circles; hCG CR-117 (10,600 IU/mg) was purifed from Organon Lot 91843 (3810 IU/mg) symbolized by open circles; CR-121 (13,450 IU/mg) was purified from Organon Lot 92145 (2700 IU/mg) symbolized by open squares.

with 0.1% trypsin at 37° for 30 minutes resulted in a 50% decrease in inhibition. Thus, the component(s) in APL responsible for the inhibition are heat stable and partially sensitive to trypsin.

APL inhibited drug and steroid mediated increases in porphyrin accumulation, but did not depress the levels of porphyrins in control vials to which no inducing agents were added, suggesting the APL was not toxic to the cells. To further evaluate possible toxicity, we measured ^{14}C-leucine uptake by untreated cells and cells to which AIA, APL, and AIA plus APL had been added 24 hours before (Table 2). APL did not alter the cells' capacity to incorporate ^{14}C-leucine into protein. Like Incefy and Kappas (8), we observed a decrease in ^{14}C-leucine incorporation by AIA treated cells. APL prevented that decrease, as well as the AIA mediated increase in porphyrin accumulation.

There was no inhibition of AIA induced porphyrin formation in vials preincubated with APL for one hour and washed prior to adding AIA. Thus short exposure to APL did not make the cells refractory to AIA. Rather, simultaneous exposure of cells to APL and the inducing agent was required for the inhibition to occur.

Table 1

APL Inhibition of Drug Induced Increases in Porphyrin Formation

Additions to vials	Porphyrin concentration (pmoles coproporphyrin/vial ± S.E.)		% inhibition by APL
None	(10)	5.5 ± 0.8	0
None + APL 100 IU	(10)	5.8 ± 0.9	
Secobarbital 100 μg	(5)	81.5 ± 5.8	33
Secobarbital 100 μg + APL 100 IU	(5)	54.9 ± 2.8	
Meprobamate 100 μg	(5)	118.4 ± 11.3	39
Meprobamate 100 μg + APL 100 IU	(5)	72.4 ± 8.6	
Etiocholanolone 5 μg	(12)	34.3 ± 1.1	44
Etiocholanolone 5 μg + APL 100 IU	(5)	19.1 ± 0.7	
Aminopyrine 383 μg	(4)	51.6 ± 3.8	44
Aminopyrine 383 μg + APL 100 IU	(4)	28.8 ± 3.1	
Hexobarbital 100 μg	(4)	52.0 ± 2.8	75
Hexobarbital 100 μg + APL 100 IU	(4)	12.8 ± 0.8	
Pentobarbital 100 μg	(4)	34.3 ± 4.0	85
Pentobarbital 100 μg + APL 100 IU	(4)	5.2 ± 0.2	

Drugs were added to culture vials containing 5 x 10^5 liver cells and 1 ml medium, in the presence or absence of APL. Porphyrins were measured in the cells and medium 24 hours later. The number of vials in each group is shown in parentheses.

100 IU APL per ml of medium inhibited AIA induction of ALAS by 48%; porphyrin formation was inhibited to a similar degree (40%). When ALAS was by-passed by adding δ-aminolevulinic acid (ALA) to the culture medium, APL in concentrations up to 100 IU/ml did not depress the conversion of ALA to porphyrins. Thus APL did not increase the rate of degradation of porphyrins. It appeared to prevent chemically induced porphyrin synthesis mainly by interfering with the ability of chemicals to induce ALAS.

N-acetyl neuraminic acid, in concentrations of 1 to 100 μg/ml medium, had no effect on AIA or etiocholanolone induced porphyrin formation. Thus, it is unlikely that the inhibitory effect of APL is due to the sialic acid component of hCG.

Table 2

Effect of APL on Protein Synthesis by Chick Embryo Liver Cells

Additions to vials	Porphyrin concentration (pmoles coproporphyrin/vial ± S.E.)		^{14}C-leucine uptake by cells (CPM/cover slip ± S.E.)	
Controls (no additions)	(11)	7.4 ± 0.7	(3)	3849 ± 146
APL 100 IU	(8)	7.3 ± 0.4	(4)	3757 ± 485
AIA 30 μg	(8)	51.6 ± 3.5	(4)	1905 ± 176
AIA 30 μg + APL 100 IU	(8)	25.2 ± 3.0	(4)	3026 ± 252

Vials containing 5 x 10^5 liver cells and 1 ml medium were incubated with no additions (Control), 100 IU APL, 30 μg AIA, or 30 μg AIA plus 100 IU APL. After 24 hours the media were poured off and used for porphyrin measurements. Leucine free medium was added to the cells for five minutes, followed by 1.43 x 10^6 cpm ^{14}C-leucine. The cover slips were then removed, fixed, extracted, and washed, and the ^{14}C-leucine uptake by the cells was measured. The number of vials in each group is shown in parentheses.

Some inhibitory activity persisted with the hCG through the initial steps of the purification procedure. Inhibitory activity was retained after passage of the starting material through SE-Sephadex C-50 and after subsequent passage of the hCG containing fractions through Bio-gel P-150. However, the inhibitory activity was present in other fractions as well as those containing hCG following the gel filtration step. During the final purification of hCG, by ion-exchange chromatography on DEAE-Sephadex, the inhibitory activity and the hormonal activity of hCG were dissociated. The final results shown in Figure 1 indicate that hCG accounts for none of the inhibitory activity of the crude commercial preparation.

In an attempt to characterize the substance(s) responsible for the inhibitory activity, the crude commercial material was passed directly over a Sephadex G-100 column, as detailed in the legend of Figure 2. Minimal inhibitory activity was present in the region of highest hCG concentration (fraction C).

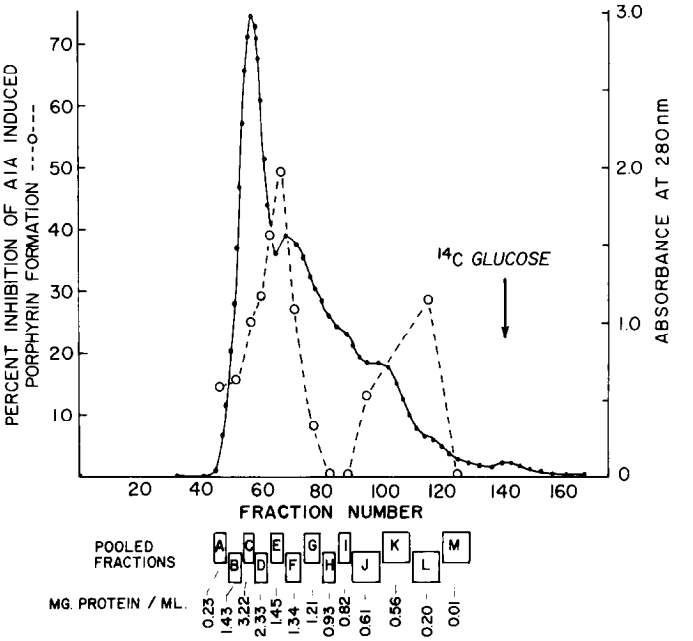

FIGURE 2: Effects of fractions obtained from gel filtration of crude commercial hCG on AIA induction of porphyrin formation. Two grams of Organon Lot 92145 were dissolved in 50 ml of 0.02 M Tris in 0.15 M NaCl, adjusted to pH 7.4, and passed over a 4.0 x 195 cm column of G-100 Sephadex equilibrated with the same buffer. Fractions were pooled as shown at the bottom of the figure (A-M) and aliquots of each were analyzed for protein content with the results listed below. ^{14}C-glucose was added as a marker for the salt peak. Aliquots (5 µl) of each pool were added to 5 replicate culture vials, each containing 5 x 10^5 cells, 1 ml medium, and 30 µg AIA. Porphyrins were measured in the cells and media after 24 hours. In this experiment the porphyrin accumulation in 10 control vials to which no additions were made and in 25 vials to which 30 µg AIA were added were 2.63 ± 0.11 and 26.6 ± 2.6 p moles coproporphyrin/vial ± S.E., respectively. Absorbance at 280 nm is shown by the solid line. The percent of inhibition of AIA induction of porphyrin formation by 5 µl of each pool is illustrated by the dashed line.

Two principal inhibitory peaks, distinct from hCG, were noted. One appeared to have a molecular weight slightly smaller than hCG (highest in fraction E), and the other appeared to have a much smaller molecular weight (fraction L). Inhibitory effects were evident at low concentrations of the fractionated material. Thus, the inhibitory effects of fractions E and L in Figure 2 were obtained at protein concentrations of 7.5 µg and 1 µg per ml of medium, respectively.

DISCUSSION: The data reported here indicate that commercial preparations of hCG isolated from first trimester pregnancy urine contain one or more substances which are potent inhibitors of drug and hormone effects on the activity of the heme

biosynthetic pathway. This inhibitory capacity is not a property of the hCG molecule. The occurrence of inhibitory activity in two regions of the eluate from Sephadex G-100 suggests that the inhibition is attributable either to several different chemical moieties, or to a small molecular weight compound associated with a larger one, but easily dissociated from it. The partial inactivation of the inhibition by trypsin further suggests that a portion of the inhibitor is a protein.

The inhibition of drug induced porphyrin accumulation does not appear to be attributable to a direct action of the inhibitor(s) on the liver cells, but rather to an interaction between the inhibitor(s) and the inducing chemicals. Thus, after adding partially purified hCG to the cultured liver cells, (a) there is a decrease in porphyrin formation in cells exposed to inducing chemicals, but no decrease in porphyrin levels in control cells; (b) there is no inhibition of the cells' capacity to incorporate ^{14}C-leucine into protein; and (c) AIA suppression of leucine incorporation into protein is reversed. Moreover, (d) preincubation of the cells with crude hCG does not alter the cells' responsiveness to AIA after the inhibitor is washed out. The inhibitor(s) could act by binding to chemical inducing agents, preventing access of inducers to a hypothetical repressor of ALAS (5), or accelerating the rate of metabolic inactivation of inducers.

The results of these studies demonstrate the necessity for caution in attributing biologic effects obtained with crude hCG to the pure hormone, since partially purified hCG has potent biochemical actions unrelated to the hormone itself. Moreover, the occurrence of a urinary material which is a potent inhibitor of chemical induction of porphyrin formation is of interest on its own account and merits further investigation. Also, since partially purified hCG has wide clinical use, the possible occurrence in man of interactions between crude hCG and other drugs and chemicals should be considered.

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