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# THE EFFECT OF PROSTAGLANDINS ON PROTEIN AND NUCLEIC ACID SYNTHESIS IN A CELL-FREE SYSTEM

R. W. Ruddon* and J. M. Johnson

Department of Pharmacology, The University of Michigan Medical School
Ann Arbor, Michigan

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THE prostaglandins are a family of hydroxy unsaturated $C_{20}$ fatty acids which have been classified into an E and F series. Six different compounds have been isolated from various natural sources, including semen, vesicular glands, menstrual fluid, lung, brain, thymus, and iris (1). The prostaglandins have a variety of effects on biological systems. Prostaglandin $E_1$, for example, inhibits the tone of vascular, respiratory, and reproductive smooth muscle, inhibits the mobilization of fatty acids from adipose tissue in response to catecholamines, and depresses the central nervous system (1,2).

On the basis of present evidence it seems that the prostaglandins are not circulating hormones and that they may act intracellulary as co-enzymes or as a factor in cellular control mechanisms (1). If this were true, these agents would be expected to affect biosynthetic mechanisms. The present investigation was designed to examine the effects of two prostaglandins, $PGE_1$ and $PGF_{1\alpha}$, on protein and nucleic acid synthesis in a cell-free system derived from E. coli B cells.

## Materials and Methods

Escherichia coli (strain B) were grown at 37° in a glucose enriched Difco broth (3) and harvested in the early log phase of growth. The cells

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were disrupted, extracted, and S-30, ribosomal, and S-100 fractions prepared by the methods of Nirenberg and Matthaei (4).

Prostaglandins $PGE_1$ and $PGF_{1\alpha}$ were obtained in purified form from the Upjohn Company (Kalamazoo, Michigan). Stock solutions were prepared by dissolving 1 mg prostaglandin in 0.1 ml 95% ethanol and 0.9 ml of 0.02% sodium carbonate solution. Dilutions of the stock solution were made with distilled water, and the final pH of the solutions was 6-7. It should be noted that these solutions of prostaglandins lost their activity in the cell-free system after being stored at 0 - 4°C for 12-14 days.

The reaction mixture used to determine effects of the agents on protein synthesis contained the following in μmoles/ml unless otherwise noted: 50 Tris - HCl, pH 7.8; 15 magnesium acetate; 70 KCl; 4.2 β-mercaptoethanol; 2.5 PEP; 1.0 ATP; 0.3 GTP; 0.05 each of 19 amino acids; 0.1 μCurie phenylalanine - $^{14}C$ (specific activity = 7.0 mCuries/mmole); 20 μg pyruvate kinase; 1.5-2.0 mg S-30 protein, and 50 μg poly U. $PGE_1$ and $PGF_{1\alpha}$ were added in 0.05 ml volumes. Total volume of the reaction mixtures was 0.3 ml. The reaction mixtures were incubated at 37° for 40 min., and the reactions were stopped with 3 ml of cold 10% trichloroacetic acid. Washing and removal of the nucleic acids from the precipitates were performed by a modification of the method of Siekevitz (5). The protein precipitate in TCA was poured on a Millipore filter (0.45 μ pore size) and washed with 5% TCA. The filters were dried and placed in counting vials. Toluene phosphor (10 ml) was added, and the samples counted in Packard Tri-Carb Liquid Scintillation Spectrometer. Results were calculated as counts per minute (CPM) per reaction mixture and all determinations were done in duplicate.

In some of the experiments the washed ribosomes and the S-100 supernatant fractions were preincubated with various concentrations of prostaglandins prior to assay of poly U-directed phenylalanine - $^{14}C$ incorporation as above. The effect of prostaglandins on the binding of $^{3}H$-poly U to

ribosomes was determined by the method of Moore (6). The effect of prostaglandins on the synthesis of phenylalanyl - $^{14}C$ tRNA was determined by adding the agents (0.05 ml) to an 0.25 ml reaction mixture containing 1.0 mg "stripped" sRNA; 0.8 μmoles ATP; 9 μmoles PEP; 2 μg pyruvate kinase; 14 mμmoles of 19 amino acids; 14 μmoles Tris-HCl, pH 7.2; 0.125 μCuries phenylalanine - $^{14}C$ (sp. act. 222 mC/mmole); and 0.3 mg S-100 protein. The reaction mixtures were incubated for 12 min. at 37°. The reactions were stopped with 5% TCA; and the mixtures were poured on Millipore filters, washed with 2.5% TCA, dried, and counted as above. It was demonstrated that most of the labeled amino acid was in the RNA fraction and not incorporated into protein, as heating the mixtures at 80-90° in 5% TCA prior to filtration removed more that 97% of the radioactivity.

The incubation mixture employed for determining the effect of prostaglandins on uridine - $^{14}C$ incorporation into RNA contained the following (in μmoles/ml unless otherwise specified): 50 Tris - HCl, pH 7.8; 15 magnesium acetate; 70 KCl; 4.2 β-mercaptoethanol; 2.5 PEP; 0.5 ATP; and 0.2 each of GTP and CTP; 20 μg pyruvate kinase; 0.1 μC of uridine - $^{14}C$ (sp. act. 30 mC/mmole); and 1.3 mg S-30 protein. The same reaction mixture was used to measure DNA synthesis with the exception that 0.2 μmole/ml each of dATP, dGTP, dCTP were employed, and thymidine - $^{14}C$ (sp. act. 30 mC/mmole) was used in place of uridine. In some experiments 84 μg of "primer" DNA (calf thymus) was added. The reaction mixtures (0.3 ml) were incubated for 40 min at 37°, and the reactions stopped with 3 ml cold 5% TCA. The precipitates were filtered on Millipore filters, washed with cold 2.5% TCA, and counted as above.

## Results

The effects of the prostaglandins $PGE_1$ and $PGF_{1\alpha}$ on protein synthesis in the S-30 fraction from E. coli is shown in Table 1.

TABLE 1

Effect of Prostaglandins $E_1$ and $F_{1\alpha}$ on Poly U-Directed Incorporation of Phenylalanine - $^{14}C$ into Protein in the S-30 Fraction from E. Coli

| Modifications | CPM | % Decrease from Control |
|---|---|---|
| Complete | 13,952 | - |
| -Poly U | 3,064 | - |
| +$PGE_1$ | | |
| $10^{-4}$M | 14,781 | - |
| $10^{-5}$M | 13,214 | 7 |
| $10^{-6}$M | 12,067 | 17 |
| $10^{-7}$M | 12,081 | 17 |
| +$PGF_{1\alpha}$ | | |
| $10^{-4}$M | 14,193 | - |
| $10^{-5}$M | 13,241 | 8 |
| $10^{-6}$M | 12,453 | 14 |
| $10^{-7}$M | 11,965 | 18 |
| Deproteinized at zero time | 2,622 | |

It can be seen that as the concentration of the agents in the medium is decreased the amount of incorporation of phenylalanine - $^{14}C$ into protein is decreased. This suggests 1.) a dual mechanism of action on the protein-synthesizing system, i.e., an inhibitory action which is sensitive to low concentrations of drug and a stimulatory action which becomes apparent at a higher concentration and overrides the inhibitory effect, or 2.) a possibility of molecular association of the prostaglandins at high concentrations.

This same apparent dual action was noted (Table 2) when the S-100 supernatant fraction, containing the protein-synthesizing enzymes, was pre-incubated with the prostaglandins prior to addition of ribosomes for assay of protein-synthesizing activity. However, preincubation of ribosomes with the agents had no significant effect.

One of the enzyme systems in the S-100 fraction whose activity can be conveniently examined are the aminoacyl - tRNA synthetases. The results expressed in Table 3 indicate that neither $PGE_1$ nor $PGF_{1\alpha}$ had an effect on the synthesis of phenylalanyl - $^{14}C$ - tRNA. The prostaglandins also had no

TABLE 2

Effects of Prostaglandins on the Activity of Ribosomes and S-100 Fraction in Poly U-Directed Polyphenylalanine Synthesis*

| Experiment | Modifications | | CPM Phenylalanine-$^{14}$C Incorporated | % Decrease from Control |
|---|---|---|---|---|
| 1 | Complete | | 15,936 | - |
| | -Ribosomes | | 584 | - |
| | -S-100 | | 1,643 | - |
| | Ribosomes preincubated with: | | | |
| | $PGE_1$ | $10^{-4}$M | 15,788 | - |
| | | $10^{-7}$M | 16,325 | - |
| | $PGF_{1\alpha}$ | $10^{-4}$M | 17,000 | - |
| | | $10^{-7}$M | 15,176 | - |
| | Deproteinized at zero time | | 233 | |
| 2 | Complete | | 7,458 | |
| | -S-100 Fraction | | 1,031 | |
| | S-100 Preincubated with: | | | |
| | $PGE_1$ | $10^{-4}$M | 7,654 | - |
| | | $10^{-7}$M | 6,320 | 17** |
| | $PGF_{1\alpha}$ | $10^{-4}$M | 6,200 | 18** |
| | | $10^{-7}$M | 5,853 | 25** |
| | Deproteinized at zero time | | 508 | |

*Ribosomes and the S-100 fraction were preincubated with the prostaglandins in 0.005 M Tris-HCl, pH 7.4, for 30 min at 37° prior to assay of function in poly U-directed protein synthesis.

**Repetition of this experiment (n = 4) demonstrated these changes to be significant ($p < 0.01$).

effect on the binding of $^{3}$H-poly U to ribosomes nor the binding of phenylalanyl - $C^{14}$ - tRNA to a ribosome-poly U complex.

Table 4 shows the effect of these agents on DNA synthesis in the E. coli cell free system. Concentrations of $10^{-4}$M and $10^{-7}$M of both

TABLE 3

Effect of Prostaglandins on the Synthesis of Phenylalanyl - $^{14}C$ - tRNA in the S-100 Fraction of E. Coli

| Modifications | | CPM |
|---|---|---|
| Complete | | 37,261 |
| -S-100 | | 1,047 |
| Complete (Heated to 90° for 10 min in 2.5% TCA) | | 1,263 |
| +$PGE_1$ | $10^{-4}$M | 38,467 |
| | $10^{-7}$M | 37,510 |
| +$PGF_{1\alpha}$ | $10^{-4}$M | 37,310 |
| | $10^{-7}$M | 36,675 |

TABLE 4

Effect of Prostaglandins on DNA Synthesis in an E. Coli Cell-Free System

| Experiment | Modifications | | CPM Thymidine - $^{14}C$ Incorporated | % Decrease from Control |
|---|---|---|---|---|
| 1 | Complete (no exogenous DNA primer) | | 11,232 | - |
| | +$PGE_1$ | $10^{-4}$M | 12,402 | - |
| | | $10^{-6}$M | 12,056 | - |
| | +$PGF_{1\alpha}$ | $10^{-4}$M | 10,948 | - |
| 2 | Complete (with DNA primer)* | | 19,028 | - |
| | +$PGE_1$ | $10^{-4}$M | 13,134 | 31 |
| | | $10^{-7}$M | 13,924 | 27 |
| | +$PGF_{1\alpha}$ | $10^{-4}$M | 12,011 | 37 |
| | | $10^{-7}$M | 9,671 | 50 |

*Calf thymus DNA (25μg), denatured by heating at 100° for 10 min and cooling rapidly, was added to the 0.3 ml reaction mixture, and incorporation of thymidine - $^{14}C$ into DNA was determined in duplicate samples.

prostaglandins inhibited exogenous DNA-directed thymidine - $^{14}C$ incorporation into DNA; however, DNA synthesis directed by endogenous DNA template was not inhibited. In addition, the prostaglandins had no significant effect on the incorporation of uridine - $^{14}C$ into RNA as directed by endogenous template (Table 5).

TABLE 5

Effect of Prostaglandins on Uridine - $^{14}C$ Incorporation into RNA in an E. Coli Cell-Free System*

| Modifications | | CPM |
|---|---|---|
| Complete | | 3015 |
| +$PGE_1$ | $10^{-4}$M | 2846 |
| | $10^{-5}$M | 2952 |
| | $10^{-7}$M | 2925 |
| +$PGF_{1\alpha}$ | $10^{-4}$M | 3281 |
| | $10^{-5}$M | 3038 |
| | $10^{-7}$M | 3286 |

*No exogenous DNA primer was added to the system

## Discussion

The effects of the prostaglandins $PGE_1$ and $PGF_{1\alpha}$ on protein and nucleic acid synthesis in an E. coli cell-free system have been examined in order to ascertain whether these agents might have a function in the intracellular control of these synthetic mechanisms. It was demonstrated that these agents have a slight inhibitory effect on protein synthesis, which was evident only at low concentrations, and that the inhibitory effect was produced in the fraction of the cell-free system containing the protein-synthesizing enzymes (S-100 fraction). The reason for the apparent dual mechanism of action of these compounds is not yet evident. Perhaps low concentrations partially inhibit the function of an enzyme necessary for protein synthesis, and higher concentrations inhibit the enzymatic degradation of messenger RNA, thereby overriding the small inhibitory effect. The prostaglandins had no effect on the binding of poly U to ribosomes, the binding of phenylalanyl - tRNA to a poly U - ribosome complex or the synthesis of phenylalanyl - tRNA, suggesting that these agents act on the polymerization of amino acids into polypeptide. The lack of effect of the prostaglandins on DNA or RNA synthesis directed by endogenous template suggests that the E. coli DNA is in

such a configuration that interaction with these agents does not occur, whereas exogenous DNA may interact with the agents.

In summary, it appears that the prostaglandins $PGE_1$ and $PGF_{1\alpha}$ have only slight effects on the synthesis of macromolecules in a cell-free system. If these agents have a function in intracellular control mechanisms, it may be at some other level of integration of cellular activity, or effects on biosynthetic mechanisms may only be evident in certain target tissues in vivo.

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