Involvement of Glucocorticoid Receptors in Steroid-induced Inhibition of Prostaglandin Secretion*

(Received for publication, December 28, 1978, and in revised form, April 19, 1979)

François Russo-Marie, Micheline Paing, and Dominique Duval
From the Institut National de la Santé et de la Recherche Médicale Unités 90 et 7, Necker Hospital, 75015 Paris, France

In order to study the mechanism of steroid-induced inhibition of prostaglandin (PG) secretion, we have used rat reno-medullary interstitial cells grown in tissue culture as an in vitro model.

These cells have been shown by radioimmunoassay to produce high amounts of prostaglandins, mainly PGE$_2$ and PGF$_{2a}$. This secretion is abolished in the presence of 10 $\mu$M of indomethacin, a well known inhibitor of prostaglandin synthesis.

Using [$^3$H]dexamethasone, we have demonstrated in our cultures the existence of glucocorticoid binding sites which exhibit all the characteristics of physiological glucocorticoid receptors.

Comparison between the biological activity (i.e. the ability to inhibit PG secretion) of the various steroids tested (dexamethasone, corticosterone, aldosterone, progesterone, testosterone, and estradiol) and their affinities for the glucocorticoid binding sites reveals a good correlation between these two parameters. Steroids which bind to the receptors also inhibit prostaglandin secretion whereas testosterone and estradiol, which have a very weak affinity for the glucocorticoid binding sites, do not inhibit PG secretion.

Steroid-induced inhibition of PG secretion is not related to an effect on cell metabolism or cell growth as judged by [$^3$H]thymidine uptake studies and determinations of protein content. In addition, actinomycin D (0.1 $\mu$g/ml) and cycloheximide (0.1 $\mu$g/ml) are both able to abolish the inhibitory effect of dexamethasone on PG secretion.

Our results indicate that the action of corticosteroids on prostaglandin secretion, which is believed to be the basis of their anti-inflammatory property is mediated through receptor occupancy and requires RNA and protein synthesis. This mechanism is thus in good agreement with the classical scheme of steroid hormone action.

It is now widely accepted that the mechanism of action of anti-inflammatory agents is mediated in part through an inhibition of prostaglandin E$_2$ and F$_{2a}$ (PGE$_2$ and PGF$_{2a}$) secretion (1). Indomethacin and aspirin-like drugs have been shown (2) to inhibit the cyclooxygenase enzyme which catalyzes the transformation of arachidonic acid into hydroperoxides, the precursors of PGE$_2$ and PGF$_{2a}$. In contrast, the exact mechanism of action of anti-inflammatory steroids is still unknown. Several authors (3-6) have now reported that steroids inhibit the release of arachidonic acid from the membrane phospholipidic stores mainly through a regulation of phospholipase A$_2$ activity (7).

Lewis and Piper (8) have shown that steroids act by inhibiting the release of prostaglandins from cells. More recently, Chandrabose et al. (9) have suggested that steroids interact directly with the cyclooxygenase enzyme, inhibiting phospholipase activity only secondarily.

Nevertheless, other authors (10-12) have postulated that the anti-inflammatory role of steroids was mediated through stabilization of plasma or lysosomal membranes.

In all steroid target tissue hitherto investigated, the mechanism of action of steroid hormones follows a similar pattern which involves the following steps: 1) The steroid enters the target cell and combines with a high affinity cytoplasmic receptor. 2) The steroid-receptor complex is then transferred to the nucleus where it binds to selective sites in the chromatin. 3) The interaction between the hormone-receptor complex and the genome leads to a modulation of RNA and protein synthesis. 4) This synthesis of specifically induced protein is finally responsible for the physiological response of the hormone.

Given this general scheme, we have investigated in cultures of rat reno-medullary interstitial cells, which produce high amounts of PGE$_2$ and PGF$_{2a}$, the inhibitory effect of steroids on prostaglandin production in parallel with the search for glucocorticoid binding sites.

We now present evidence that the anti-inflammatory effects of steroids are mediated through glucocorticoid receptor occupancy and require RNA and protein synthesis.

**EXPERIMENTAL PROCEDURES**

Materials—Spontaneously hypertensive rats of the Okamoto strain were obtained by local breeding (Necker Hospital, Paris). All culture media were obtained from Gibco Bioulc (Glasgow, Scotland). Prostaglandins E$_2$ and F$_{2a}$, (PGE$_2$ and PGF$_{2a}$) were a gift of Dr. J. E. Pike (Upjohn Company, Kalamazoo, Mich.). Tritiated products (prostaglandins, steroids, arachidonic acid, uridine, leucine and thymidine) were purchased from the Radiochemical Centre (Amersham, U.K.). Unlabeled steroids, cycloheximide, and actinomycin D were obtained from Sigma Chemical Corp. (St. Louis, Mo.). All tissue culture flasks were obtained from Falcon. Prostaglandin E$_2$ and F$_{2a}$ antisera were obtained from the Pasteur Institute (Paris), organic solvents and silica gel plates were purchased from Merck (Darmstadt, Germany), silic acid was obtained from Mallinkrodt, Inc. (St. Louis, Mo.).

Cell Culture and Cell Lines—Monolayer tissue cultures of rat reno-medullary interstitial cells were obtained by the technique previously described (13, 14). Renal medullae from two rats were removed, finely minced, and gently homogenized in a modified Eagle’s medium containing 10% fetal calf serum (Medium A: Basal Eagle’s medium containing 1% v/v of BME$^2$ amino acids, 2.5 g of lactalbumin hydrolysate, 0.5 g of yeast extract, 1.1 g of sodium bicarbonate, 50,000 units of penicillin, 500 mg of streptomycin, and 500 mg of fungizone in a final volume of 500 ml to which was added 10% v/v of fetal calf serum) and the homogenate injected subcutaneously into four differ-
Glucocorticoid and Inhibition of Prostaglandin Secretion

Ent places in the abdominal wall of a syngeneic rat. The recipient was killed 4 days later, and the yellow vascularized nodules formed were removed aseptically. These nodules were minced in medium A, dispersed by trypsin treatment (15 min at 37°C in the presence of 0.25% trypsin), and washed by centrifugation before seeding into culture flasks. Cultures were maintained at 37°C in a 5% CO₂ in air atmosphere and achieved confluence in 21 to 25 days. The cells were dissociated by trypsin treatment (10 min at 37°C in the presence of 0.05% trypsin and 0.02% EDTA), washed, and seeded in flasks containing fresh medium A. Under similar culture conditions, these cells have been shown by Muirhead (13) to maintain their morphologic and karyotypic characteristics up to the 40th passage. In our experiments, the cells were seeded before the 25th passage.

Identification of the Prostaglandin Production Using Thin Layer Chromatography—Cells were grown for 1 or 2 days after seeding in 25-cm² flasks. At the time of the experiments, the medium was discarded and replaced by fresh medium containing 5 µCi of [³H]-arachidonic acid (86 Ci/mmol). After a 24-h incubation, the culture medium was removed, acidified to pH 3.5 with 70% citric acid, and extracted twice with 3 volumes of cyclohexane:ethyl acetate (1:1). The organic phase was then evaporated under nitrogen. The residue was dissolved in 50 µl of ethanol, applied to silica gel plates and developed in the following solvent system: chloroform:methanol:acetic acid:water (90:8.5:1:0.5). Nonradioactive PGE₂ and PGF₂α were run as parallel standards. After exposure to iodine vapor, the plates were scraped and each square centimeter counted by liquid scintillation spectrometry.

Extraction and Radioimmunoassay of PGE₂ and PGF₂α—The extraction and radioimmunoassay of PGE₂ and PGF₂α were performed as previously described (15). One-milliliter aliquots of the culture medium were acidified to pH 3.5 with 70% citric acid, and extracted twice with 3 ml of cyclohexane:ethyl acetate (1:1). The organic phases were then evaporated under nitrogen. The residue was dissolved in 50 µl of ethanol, and applied to silica gel column (0.5 g of silicic acid, 100 mesh), suspended in 2 ml of benzene:ethyl acetate (60:40), and applied to silica gel column (0.5 g of silicic acid, 100 mesh), suspended in 2 ml of benzene:ethyl acetate (60:40), in 25-cm² flasks. At the time of the experiments, the medium was discarded and replaced by fresh medium containing 5 µCi of [³H]-arachidonic acid (86 Ci/mmol). After a 24-h incubation, the culture medium was removed, acidified to pH 3.5 with 70% citric acid, and extracted twice with 3 volumes of cyclohexane:ethyl acetate (1:1). The organic phase was then evaporated under nitrogen. The residue was dissolved in 50 µl of ethanol, applied to silica gel plates and developed in the following solvent system: chloroform:methanol:acetic acid:water (90:8.5:1:0.5). Nonradioactive PGE₂ and PGF₂α were run as parallel standards. After exposure to iodine vapor, the plates were scraped and each square centimeter counted by liquid scintillation spectrometry.

FIG. 1. Time course of prostaglandin secretion and protein content in rat renal-medullary cells grown in tissue culture. One million cells were inoculated into a 75-cm² flask, containing 20 ml of fresh culture medium + 10% FCS. At various intervals after seeding, 1 ml of culture medium was removed and assayed for PGE₂ (●) and PGF₂α (○) content as described under “Experimental Procedures.” In parallel, 300,000 cells were inoculated into 25-cm² flasks containing 4 ml of fresh culture medium + 10% FCS. At various intervals, three flasks were sampled and assayed for protein content (■). Each value is the mean ± S.E. of six determinations in two separate experiments.

Measurement of Inhibition of Prostaglandin Secretion by Indomethacin and Steroids—Cells were grown for 24 h after seeding in 4 ml of fresh culture medium, in 25-cm² flasks. The medium was then removed for assay of basal PGE₂ content and replaced by 4 ml of fresh medium with or without various concentrations of the test substances. After an additional 24-h incubation period, the medium was removed and assayed for PGE₂ content. The cells were then solubilized with 1 ml of 0.1 N NaOH for 20 min at room temperature and the protein content determined by the method of Lowry et al. (16).

[³H]Dexamethasone Binding Studies Cells were grown for 2 or 3 days after seeding in 25-cm² flasks. At the time of the experiments, the medium was discarded and replaced by fresh medium containing various concentrations of [³H]dexamethasone (25 Ci/mmol) in the range: 5 to 80 nM. Nonspecific binding was measured by parallel incubations in the presence of a 1,000-fold excess of nonradioactive dexamethasone. After 20-min incubation at 37°C, the medium was removed and the flasks placed on ice. The cells were then washed several times with ice cold saline until no radioactivity remained in the supernatant. After cell solubilization by 1 ml of NaOH, 0.1 N, 500-μl aliquots were used for determination of cellular bound radioactivity by liquid scintillation spectrometry and protein content.

In competition experiments, a single concentration of [³H]dexamethasone (50 nM) was used whereas the competing steroids were added at various concentrations (3-, 10-, 30-, 100-, and 1,000-fold excess). The residual binding in the presence of competitor was expressed as a percentage of the specific binding of [³H]dexamethasone alone.

Measurements of Precursor Uptakes—The cells were preincubated for 24, 46, and 70 h in the presence of 0.1 μM dexamethasone and then received 1 μCi of [³H]thymidine (25 to 30 Ci/mmol). After an additional 2-h incubation period at 37°C the medium was removed, the flasks were placed on ice, and the cells were washed using ice cold saline. Measurements of thymidine uptake and cellular protein content were done as previously described (15). One-milliliter aliquots of the culture medium were acidified to pH 3.5 with 70% citric acid, and extracted twice with 3 volumes of cyclohexane:ethyl acetate (1:1). The organic phases were then evaporated under nitrogen. The residue was dissolved in 50 µl of ethanol, applied to silica gel plates and developed in the following solvent system: chloroform:methanol:acetic acid:water (90:8.5:1:0.5). Nonradioactive PGE₂ and PGF₂α were run as parallel standards. After exposure to iodine vapor, the plates were scraped and each square centimeter counted by liquid scintillation spectrometry.

FIG. 2. Inhibition of PGE₂ secretion by indomethacin. 300,000 cells were grown for 24 h after seeding in a 25-cm² flask containing 4 ml of fresh culture medium + 10% FCS under 5% CO₂ in air atmosphere at 37°C. The medium was then removed, assayed for PGE₂ content, and replaced by 4 ml of fresh medium + 10% FCS containing various concentrations of indomethacin. After an additional 24-h incubation period, the culture medium was again assayed for PGE₂ content. The secretion of PGE₂ over a 24-h period in the absence of indomethacin was taken as 100% and the secretion of PGE₂ in the presence of various concentrations of indomethacin (0.1 to 10,000 nM) expressed as a percentage thereof. Comparisons were made on the basis of cell protein content. Each value is the mean ± S.E. of six determinations in two experiments.
RESULTS

Pattern of PGE\textsubscript{2} and PGF\textsubscript{2\alpha}, Synthesis: Effect of Indomethacin—Incubation with [\textsuperscript{3}H]arachidonic acid followed by thin layer chromatography showed that the only radioactive prostaglandins released into the culture medium corresponded to PGE\textsubscript{2} and PGF\textsubscript{2\alpha}. Radioimmunoassays of PGE\textsubscript{2} and PGF\textsubscript{2\alpha} were then performed in detailed studies.

As shown in Fig. 1, PGE\textsubscript{2} and protein synthesis proceed almost linearly during the exponential phase of cell growth and both decrease after cell confluence, whereas no similar decrease was observed for PGF\textsubscript{2\alpha}. Routinely, experiments were carried out 1 to 3 days after seeding, i.e. in the linear part of the curve (Fig. 1).

The effect of indomethacin on PGE\textsubscript{2} secretion is shown in Fig. 2, increasing concentrations of the inhibitor decrease PGE\textsubscript{2} secretion, which is almost entirely blocked at 10 \textmu M. A similar pattern of inhibition was also demonstrated for PGF\textsubscript{2\alpha} secretion (not shown). Therefore, in subsequent experiments, levels of PGE\textsubscript{2} were determined as a representative index of total production of prostaglandins.

Inhibition of Prostaglandin E\textsubscript{2}, Secretion by Various Steroids—Steroids studied were glucocorticoids (dexamethasone, corticosterone), mineralocorticoid (aldosterone), and sex steroids (testosterone, progesterone and estradiol). Comparison of the inhibitory effects of these different steroids shows a pattern of specificity (Fig. 3 right panel); only steroids with glucocorticoid properties are able to inhibit prostaglandin secretion. The most potent is the synthetic glucocorticoid dexamethasone, (a widely used anti-inflammatory drug), followed by corticosterone, the endogenous glucocorticoid hormone in rodents. The mineralocorticoid aldosterone, which at high doses has been shown to be a fully active glucocorticoid (17), also inhibits prostaglandin secretion. In contrast, PG secretion appears uninfluenced even by high doses of sex steroids. The inhibitory effect of dexamethasone on prostaglandin secretion does not appear to be related to an inhibition of cell growth. Indeed, protein content and [\textsuperscript{3}H]thymidine uptake, measured after a 24-h incubation with or without 0.1 \textmu M dexamethasone, showed no significant difference between the two groups (Table I). Incubations performed with tritiated steroids show that in our experimental conditions, virtually, no metabolism of the different steroids occurs (<2\%, as estimated by thin layer chromatography).

Binding Studies—Using a single high concentration of [\textsuperscript{3}H]dexamethasone (50 nM), it was determined that the binding of this steroid attained plateau levels after 20 min of incubation at 37°C (Fig. 4).

The binding of increasing concentrations of [\textsuperscript{3}H]dexamethasone (in the range 5 to 80 nM) is shown in Fig. 5. Solid symbols represent the levels of [\textsuperscript{3}H]dexamethasone bound in the absence of competing unlabeled steroid and open symbols denote the binding of the tracer in the presence of a 1000 fold excess unlabeled dexamethasone. [\textsuperscript{3}H]dexamethasone is bound with high affinity to a class of saturable binding sites, since the binding is displaceable by an excess of the unlabeled steroid itself.

A Scatchard (18) plot of the binding data (inset, Fig. 5) is

![Fig. 3. Inhibition of PGE\textsubscript{2} secretion by steroids. Right panel, 300,000 cells were grown for 24 h after seeding in a 25-cm\textsuperscript{2} flask containing 4 ml of fresh culture medium + 10% FCS under 5\% CO\textsubscript{2} in air atmosphere at 37°C. The medium was then removed, assayed for PGE\textsubscript{2} content and replaced by 4 ml of fresh medium containing various concentrations of the steroids tested: dexamethasone (O—O), corticosterone (O—O), aldosterone (A—A), testosterone (O—O), progesterone (A—A), and estradiol (■—■). After an additional 24-h incubation period, the culture medium was again assayed for PGE\textsubscript{2} content. The secretion of PGE\textsubscript{2} over a 24-h period in the absence of steroid was taken as 100% and the secretion of PGE\textsubscript{2} in the presence of various steroids (in the range 0.001 to 10,000 nM) expressed as a percentage thereof. Comparisons of PG secretion were made on the basis of cell protein content. Each value is the mean ± S.E. of nine determinations in three experiments. Competition experiments, left panel. The affinities of the various steroids studied for the [\textsuperscript{3}H]dexamethasone binding sites were determined by competition experiments. The cells were incubated for 20 min at 37°C either in the presence of [\textsuperscript{3}H]dexamethasone alone (50 nM) or with [\textsuperscript{3}H]dexamethasone plus various concentrations of unlabeled steroids. The specific binding of [\textsuperscript{3}H]dexamethasone alone is plotted as 100% and the binding of [\textsuperscript{3}H]dexamethasone in the presence of competitors expressed as a percentage thereof. The unlabeled steroids studied were dexamethasone (O—O), corticosterone (O—O), aldosterone (A—A), testosterone (O—O), progesterone (A—A), and estradiol (■—■) in the range of 50 to 50,000 nM.]

Table I

<table>
<thead>
<tr>
<th>Steroid (nM)</th>
<th>Protein content (mg of protein/flask)</th>
<th>[\textsuperscript{3}H]thymidine uptake (cpm/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.46 ± 0.021</td>
<td>72,028 ± 2,684</td>
</tr>
<tr>
<td>Dexamethasone-treated</td>
<td>0.515 ± 0.021</td>
<td>67,731 ± 11,905</td>
</tr>
</tbody>
</table>
consistent with the existence of a single population of high affinity binding sites \( (K_D, 37^\circ C = 12.50 \text{ nM} \pm 0.17 \text{ in four experiments}) \). In agreement with this interpretation, the slope of the Hill plot (not shown), \( n = 1.19 \), suggests that these sites are not interacting.

After 20-min incubation at 37°C in the presence of 50 nM \([^3H]dexamethasone\), the cells were washed as described above, scraped off with a rubber policeman, and put into 1 ml of chloroform:methanol (2:1). After sonication (Branson Sonifier D12), the homogenate was centrifuged, and the supernatant evaporated to dryness under nitrogen. The residue was dissolved in 50 μl of ethanol and applied to silica gel plates. Nonincubated \([^3H]dexamethasone\) was run in parallel as a standard. After development in a chloroform:methanol:water (90:9:1) system, the plates were scraped and counted by liquid scintillation spectrometry. Under these experimental conditions, virtually all (>95%) the radioactivity remaining bound to the cells after extensive washing represents unmetabolized dexamethasone.

**Specificity of the Dexamethasone Binding Sites**—The affinity of the different steroids tested for the dexamethasone

---

---

![Graph](image1)

**Fig. 4.** Time course of \[^3H]dexamethasone binding.** Cells were incubated at 37°C with a single high concentration of \[^3H]dexamethasone (50 nM) for various periods of time (5 min up to 3 h). At the end of each incubation period, the separation of bound steroid from that remaining free was performed by extensive washing as described under “Experimental Procedures.” Parallel experiments were run in the presence of a large excess of unlabeled dexamethasone in order to determine nonspecific binding.

![Graph](image2)

**Fig. 5.** Determination of \[^3H]dexamethasone binding sites.** Binding studies were performed in a whole assay: the cells were incubated for 20 min at 37°C in the presence of increasing concentrations of \[^3H]dexamethasone (in the range 3 to 80 nM). At the end of the incubation period, the separation of bound steroid from that remaining free was performed by several washes using ice cold saline as described under “Experimental Procedures.” Parallel experiments were performed in the presence of a large excess of unlabeled dexamethasone (50 μM) in order to determine the non-specific binding (○——○). At each concentration, the specific binding (■——■) represents the difference between the total binding (●——●) (i.e., in the absence of unlabeled competitor) and this nonspecific binding. The inset represents a Scatchard plot of the binding data. Each value, expressed as counts per min bound/mg of protein, is the mean of four experiments.

![Graph](image3)

**Fig. 6.** Comparison between receptor occupancy (■——■) and biological activity (○——○) of dexamethasone. For each concentration of dexamethasone used, from the Scatchard plot shown in Fig. 5, we have determined the extent of receptor occupancy (expressed as a percentage of maximum occupancy) and from the data presented in Fig. 3, the biological activity (expressed as a percentage of maximum inhibition of PGE\(_2\) secretion).
binding sites was determined by competition experiments. As shown in Fig. 3 (left panel), the best competitor for the [3H]dexamethasone binding sites is unlabeled dexamethasone itself, followed by corticosterone. Aldosterone and progesterone have similar affinity for the receptor whereas testosterone and estradiol exhibit only weak affinity for dexamethasone binding sites. Such a hierarchy of affinity is consistent with binding to a physiological glucocorticoid receptor.

Correlation between Glucocorticoid Receptor Occupancy and Biological Activity—For each of the steroids studied we have compared the biological activity (i.e., the ability to inhibit prostaglandin secretion) with the affinity for glucocorticoid receptor.

The concentration of a given steroid which gives half maximal inhibition of prostaglandin secretion was taken as an estimate of its biological activity: whereas the affinity of the

**TABLE II**

**Correlation between receptor occupancy and biological activity of various steroids**

The concentration of each steroid which leads to a 50% inhibition of PGE₂ secretion (calculated from the data presented in Fig. 3) was used as an index of its biological activity (Kᵢ). The method of Cheng and Prussof (19) was used to calculate Kᵢ values from the IC₅₀ (i.e., the concentration of a given steroid required to displace 50% of [3H]dexamethasone from its binding sites) Kᵢ = IC₅₀/[1 + ([3H]dexamethasone)/Kᵢ]. Kᵢ denotes the dissociation constant determined for [3H]dexamethasone-receptor interaction by Scatchard plot analysis.

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Affinity for [3H]dexamethasone binding sites (Kᵢ)</th>
<th>Inhibition of PGE₂ secretion (Ki)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosterone</td>
<td>47 nM</td>
<td>78 nM</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>100 nM</td>
<td>100 nM</td>
</tr>
<tr>
<td>Progesterone</td>
<td>100 nM</td>
<td>—</td>
</tr>
<tr>
<td>Testosterone</td>
<td>7 μM</td>
<td>5 μM</td>
</tr>
<tr>
<td>Estradiol</td>
<td>10 μM</td>
<td>—</td>
</tr>
</tbody>
</table>

Steroid for glucocorticoid receptor was calculated from the IC₅₀ which corresponds to 50% displacement from [3H]dexamethasone binding sites by the equation of Cheng and Prussof (19). As shown in Fig. 6, complete occupancy of receptors and inhibitory effect are near maximal at 50 nM dexamethasone. At a low dexamethasone concentration (1 nM), however, the inhibitory effect is still measurable, whereas only a negligible proportion of the receptors appears occupied. For corticosterone and aldosterone, a close correlation exists between receptor occupancy and biological activity (Table II). In contrast, progesterone, testosterone, and estradiol exhibit only weak affinity for dexamethasone binding sites. Such a hierarchy of affinity is consistent with binding to a physiological glucocorticoid receptor.

**TABLE III**

**Effect of inhibitors of macromolecular synthesis on the steroid-induced inhibition of PGE₂ secretion**

300,000 cells were grown for 24 h after seeding in 25-cm² flasks containing 4 ml of fresh culture medium + 10% FCS, under a 5% CO₂ in air atmosphere at 37°C. The medium was then removed and replaced by 2 ml of fresh culture medium + 10% FCS. After a 3-h preincubation period at 37°C, the medium was removed, assayed for basal PGE₂ secretion, and renewed by 2 ml of fresh culture medium containing 10 nM dexamethasone with or without actinomycin D (0.1 μg/ml) or cycloheximide (0.1 μg/ml). After an additional 3-h incubation period, all the samples were assayed again for PGE₂ content.

PGE₂ secretion was expressed on the basis of cell protein content. Each value represents the mean ± S.E. of six determinations.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition of PGE₂ secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0%</td>
</tr>
<tr>
<td>(a) Dexamethasone (10 nM)</td>
<td>53.88 ± 3.67</td>
</tr>
<tr>
<td>(b) Actinomycin D (0.1 μg/ml)</td>
<td>22.90 ± 2.23</td>
</tr>
<tr>
<td>(c) Dexamethasone (10 nM) + actinomycin D (0.1 μg/ml)</td>
<td>33.72 ± 5.30</td>
</tr>
<tr>
<td>(d) Cycloheximide (0.1 μg/ml)</td>
<td>20.10 ± 5.26</td>
</tr>
<tr>
<td>(e) Dexamethasone (10 nM) + cycloheximide (0.1 μg/ml)</td>
<td>23.58 ± 5.21</td>
</tr>
</tbody>
</table>

Differences between (a) and (c) and (a) and (e) are statistically significant (unpaired Student’s t test: p < 0.02 and p < 0.001, respectively).
Glucocorticoid and Inhibition of Prostaglandin Secretion

The two other sex steroids studied (testosterone and estradiol) neither occupy the glucocorticoid receptor nor have any effect on prostaglandin secretion. 

Effects of Cycloheximide and Actinomycin D on Prostaglandin Secretion—In 3-h incubations, both actinomycin D and cycloheximide block macromolecular synthesis as indicated, respectively, by a decrease in [3H]uridine and [3H]-leucine uptake (Fig. 7). In addition, these inhibitors also block prostaglandin secretion in a dose-dependent way (Fig. 7). In a 3-h incubation experiment the addition of submaximal doses of cycloheximide (0.1 μg/ml) or actinomycin D (0.1 μg/ml) abolishes the inhibitory effect of 10 nm dexamethasone on prostaglandin E₂ secretion (Table III).

Discussion

Using [3H]dexamethasone as a tracer, we have demonstrated in cultures derived from rat reno-medullary interstitial cells the presence of steroid binding sites which possess most of the characteristics of hormonal receptors: 1) limited capacity—the saturation being achieved at a concentration close to 50 nm; 2) high affinity—the dissociation constant of the steroid-receptor complex was 12.5 nm, a value similar to that measured for glucocorticoid receptors association in rat liver, kidney, or heart (20-22). 3) specificity—as determined by competition experiments, these binding sites showed a pattern of specificity appropriate to glucocorticoid receptors, with high affinity for dexamethasone and corticosterone, a moderate affinity for aldosterone and progesterone, and virtually no affinity for sex steroids.

Several arguments support the assumption that the observed steroid-induced inhibition of prostaglandin secretion is mediated through glucocorticoid-receptor occupancy:

1) There is a fairly good correlation between the affinity of a given steroid for the receptor and its inhibitory effect on PG secretion. Several factors could explain the observed differences between the receptor affinity and biological activity of aldosterone, progesterone, and corticosterone. First, progesterone and corticosterone are known to interact with steroid-binding proteins in FCS whereas aldosterone does not. It should be pointed out, however, that in our experiments, binding studies were performed in a medium containing FCS. Second, in these experiments, we have compared the initial step in the mechanism of action of steroid (i.e., the binding to the receptor) with a late effect of the steroid (i.e., the inhibition of PG secretion), measured after 24-h incubations. Between these two steps, several intermediate events could be differently modulated by various steroids. As demonstrated by Rousseau et al. (23), in hepatoma tissue culture cells, there are several classes of steroids: (a) optimal inducers, which bind to the receptor and are fully agonist, (b) suboptimal inducers, which bind to the receptor but elicit only a partial response, (c) inactive steroids, which do not bind to the receptor, and (d) anti-inducers, such as progesterone, which bind to the receptor without any subsequent stimulation of tyrosine amino transferase.

2) Both actinomycin D, an inhibitor of RNA synthesis, and cycloheximide, which blocks protein synthesis, abolish the inhibitory effect of dexamethasone. This result is in good agreement with the recent report of Danon and Assouline (24). Similarly, Pong et al. (25) showed that the stimulation of PGE₂ synthesis induced by addition of serum in transformed 3T3 cells could be blocked by inhibitors of protein and RNA synthesis.

3) The finding that the effect of glucocorticoid on prostaglandin secretion acts via macromolecular synthesis could explain perfectly the lag time described by several authors between steroid administration and the onset of inhibition. The discovery by Vane (2) that aspirin-like drugs block prostaglandin synthetase initiated a great deal of research, and there is now considerable evidence that inhibition of synthesis of prostaglandins is indeed the main mechanism of action of these drugs. An obvious question was whether the anti-inflammatory steroids also blocked prostaglandin biosynthesis. Vane (2) demonstrated that hydrocortisone was inactive on prostaglandin biosynthesis in cell-free homogenates, whereas Flower et al. (26) demonstrated that several other anti-inflammatory steroids were also without effect on the cyclooxygenase. Increasing evidence has recently been produced that steroids interfere with prostaglandin biosynthesis in whole cell systems or whole organ systems (3, 8, 27, 28). However, the exact site of action of steroids is still unknown. Nijkamp et al. (29) further investigated this problem and found that the anti-inflammatory steroids prevent the biosynthesis of TXA₂ in guinea pig lungs by preventing the liberation of substrate from intracellular stores. Gryglewski (3) and Blackwell (7) proposed that the mechanism involved was a phospholipase-catalyzed degradation of phospholipid. Evidence for this effect has been given by Hong and Levine (4) showing that the inhibition of arachidonic acid release from membrane was the biochemical pathway for the action of anti-inflammatory steroids. More recently Blackwell et al. (30), further investigating the degradation of phospholipids, demonstrated that phospholipase A₂ activity of guinea pig isolated perfused lungs was blocked by anti-inflammatory steroids. The inhibitory role of steroids would then involve two enzymatic steps which might have a key role in the inflammatory processes: the cyclooxygenase step and the lipoxygenase step. Hammarstrom et al. (31) showed that glucocorticoids inhibited both the cyclooxygenase and the lipoxygenase pathways in inflammatory proliferative skin disease. However, some authors have presented different hypotheses to explain the anti-inflammatory effects of corticosteroids. Lewis and Piper (8) using another target tissue (subcutaneous fat), showed that part of the inhibitory action of steroids on prostaglandin secretion was not due to an inhibition of biosynthesis but to an inhibition of prostaglandin release. Chandrasekhar et al. (9) suggested that the main site of action of glucocorticoids was on the cyclooxygenase enzyme, the inhibition of phospholipase activity being only a secondary event. Our results would appear to rule out the possibility that steroid inhibition of prostaglandin secretion could be mediated by a direct effect of steroid on membrane stabilization. Indeed, the stabilization of lysosomal membranes (10) by steroids has been described at concentrations which are several orders of magnitude higher than those used in our study. Moreover, the hypothesis of a direct interaction between steroids and membrane is hard to reconcile with either the strict specificity or the requirement for the macromolecular synthesis demonstrated for inhibition of prostaglandin secretion. Our experiments do not provide any additional information about the nature of the protein(s) involved in the inhibitory effect of steroids. Pong et al. (25) postulated that it could be either the phospholipase A₂ itself or an unknown modulator of phospholipase activity, or a molecule carrying the substrate arachidonic acid from the membrane to the site of prostaglandin synthesis, whereas Lewis and Piper (8) suggested that steroids act at the level of a membrane carrier releasing prostaglandin into the extracellular medium. The results of Yorio and Bentley (32) and of Goodman et al. (33) showing an effect of aldosterone on phospholipase activity and phospholipid turnover in toad urinary bladder would favor the first hypothesis.
Nevertheless, further experiments are required to distinguish between these possibilities.

Acknowledgments—We gratefully acknowledge Dr. C. Carnaud, Dr. H. Sors, and Dr. M. Dunn for their helpful discussions.

REFERENCES


5. Foman, Y., and Zor, U. (1976) Prostaglandins 12, 405-413


Involvement of glucocorticoid receptors in steroid-induced inhibition of prostaglandin secretion.
F Russo-Marie, M Paing and D Duval