Coordinate Induction of Several mRNA Species in Rat Kidney during Glucocorticoid Treatment*

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The relative rate of synthesis of several renal proteins is increased after a single administration of dexamethasone to the intact rat. Induced proteins were detected among the polypeptides synthesized in vitro by isolated kidney tubules from glucocorticoid-treated animals. In order to investigate this multiple induction process at the mRNA level, total poly(A)-containing RNA was isolated from the kidneys of dexamethasone-treated or control animals and translated in a wheat germ cell-free system. The template activity of a large number of mRNAs was estimated from the synthesis of translation products separated by high resolution two-dimensional gel electrophoresis. Nearly 400 spots were routinely resolved on the fluorograms of these gels. Paired comparison between identical spots revealed that nine products were consistently more actively synthesized at the direction of poly(A)-containing RNA from treated rats than from controls. Quantitative data on the functional level of a restricted subset of mRNAs including six inducible species were obtained by one-dimensional gel electrophoresis of translation products and densitometer scanning of the fluorograms. The translational activity of the six hormone-responsive mRNAs was increased approximately 3-fold above control levels 8 h after dexamethasone administration. An increase in template activity was detectable as early as 2 h after dexamethasone. The buildup of the majority of the inducible mRNAs was not dependent on concomitant protein synthesis since it was not suppressed by an injection of cycloheximide just prior to dexamethasone. However, cycloheximide inhibited the glucocorticoid-dependent increase of one mRNA, which was previously shown to code for the gluconeogenic enzyme phosphoenolpyruvate carboxykinase. The pattern of glucocorticoid-regulated mRNAs was kidney-specific, as shown by comparative translation experiments performed with rat liver poly(A)-containing RNA.

The glucocorticoids influence the function of target cells primarily by inducing or repressing the synthesis of specific proteins. Recent studies concerned with the glucocorticoid-mediated regulation of tryptophan oxygenase and tyrosine aminotransferase in liver (1, 2), P-enolpyruvate carboxykinase (GTP, EC 4.1.1.32) in kidney (3), and growth hormone and preproopiocortin in the pituitary (4-6) have led to the conclusion that changes in the rate of synthesis of these proteins reflect changes in the level of their mRNAs. Additional evidence indicates that a consequence of glucocorticoid hormone action on responsive tissues is an alteration of the transcriptional properties of chromatin (7). A current working hypothesis is that the glucocorticoids modulate the level of specific cellular mRNAs, and thereby of the corresponding proteins, by controlling the rate of transcription of some sensitive genes, apparently as a result of the interaction between glucocorticoid-receptor complexes and chromatin. Adjacent hormonal effects on the processing, degradation, and translation of mRNAs have not been ruled out.

The overall physiological response of target tissues to glucocorticoids may in many cases involve coordinate changes in the synthesis rate of several proteins. In such circumstances, the hormone is said to have a pleiotropic action and the family of affected gene products constitutes the domain of response of the target cell (8). Recently, Ivarie and O'Farrell (9) have provided an estimate of the size of the glucocorticoid domain of response of two hepatoma tissue culture cell lines. After labeling cultures with radioactive amino acid in the presence or absence of dexamethasone, they analyzed the full complement of cell proteins by high resolution two-dimensional gel electrophoresis. Out of 1000 detectable polypeptides, seven or eight had their rate of synthesis increased after dexamethasone addition. On this basis, and as a first approximation, Ivarie and O'Farrell (9) proposed that 0.5 to 1% of the hepatoma genes are under glucocorticoid control.

In this study, we have attempted to delineate the role of the glucocorticoids in the regulation of gene expression in the kidney. The latter, like the liver, is a target site for one of the major metabolic effects of this class of steroids, the stimulation of gluconeogenesis (10). Earlier investigations have established that the cytosolic form of P-enolpyruvate carboxykinase, a rate-limiting enzyme of gluconeogenesis, accumulates in the renal cortex following glucocorticoid administration to the intact rat (11). The increase in P-enolpyruvate carboxykinase level is due to a stimulation of enzyme synthesis (12, 13), which in turn reflects a comparable increase in the level of the specific mRNA coding for the enzyme (3). The objective of the present work was to determine whether the hormonal effect in the kidney is confined solely to the mRNA encoding P-enolpyruvate carboxykinase or, on the contrary, involves a larger family of gene products. Essentially, our strategy has been to examine the coding capacity of the full complement of renal mRNAs isolated from rats treated with dexamethasone for various periods of time, using a mRNA-dependent cell-free protein synthesis system. The functional level of a large number of mRNAs was estimated from the rate of synthesis of polypeptide products resolved by electrophoresis in SDS-polyacrylamide or high resolution two-dimensional gels. This approach has allowed us to identify a subset of several distinct renal mRNA species whose template activity increases during glucocorticoid treatment.

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†The abbreviation used is: SDS, sodium dodecyl sulfate.
EXPERIMENTAL PROCEDURES

Materials and Animals—Collagenase (type IV), bovine serum albumin, and other reagents for electrophoresis were purchased from Sigma. The reagents for RNA isolation by the guanidinium thiocyanate method were obtained from the suppliers identified in the original paper of Chirgwin et al. (14). Oligo(dT)-cellulose as well as wheat germ and biochemicals used in the translation system were from the same commercial sources. Cells were harvested in midlog phase (13, 16). L-[3,4,5-3H]Leucine (specific activity, 110-140 Ci/mmol) was supplied by the Radiochemical Centre (Amersham, England). The amphotolys (pH range, 3.5-10 and 5-7) for isoelectric focusing were bought from LKB (Umea, Sweden) while ultrafiltration and Nonidet P-40 came from Bethesda Research Laboratories (Rockville, MD).

Male rats of the Wistar strain, weighing about 200 g at the time of the experiments, were bought from Iffa-Credo (St Germain-sur l'Arbresle, France). They had free access to food and water until the beginning of the experiments, at which time food was withdrawn from the cages. Dexamethasone (Oradexon, from Orgon, Os, Holland) was injected intraperitoneally at a dose of 2 mg/kg. Cycloheximide (from Sigma) was dissolved in phosphate-buffered saline and injected intraperitoneally at a dose of 2 mg/kg. Control animals received injections of buffer only. The animals were killed by a blow to the head and decapitation.

Protein Synthesis by Isolated Kidney Tubules—Isolated tubules from rat kidney were obtained by a modification of the collagenase technique described by Seglen (17). Surgery was performed under anesthesia induced by 50 mg of pentobarbital/kg i.p. intra-peritoneally. A polyethylene catheter was inserted into the distal part of the abdominal aorta and was connected to a heparinized syringe through a peristaltic pump. The catheter was then perfused in situ by injecting buffer from a syringe attached to the aortic catheter. The perfusion was carried out in two steps, using 15 ml of calcium-free buffer and subsequently 15 ml of collagenase buffer. The composition of the two buffers was as described by Seglen for the preparation of isolated hepatocytes by liver perfusion (18), except that 10 mM glucose and 1% (w/v) bovine serum albumin were added to both solutions. After the perfusion, the kidneys were excised and the cortex was dissected from the medulla. Cortical tissue (approximately 400 mg) was minced and incubated in 5 ml of oxygen-saturated collagenase buffer containing glucose and albumin for 60 min in a shaking water bath at 37°C in order to dissociate the tubules. The tubule suspension obtained at the end of the collagenase incubation was diluted by adding 20 ml of the washing buffer described by Seglen (18), supplemented with 1% (w/v) albumin. The suspension was filtered through a nylon mesh (270-μm openings) and was then centrifuged on a 4% (w/v) bovine serum albumin for 2 min. The tubule pellet was washed twice in 25 ml of ice-cold washing buffer and centrifuged as above. The final pellet was resuspended in 3 ml of oxygenated incubation buffer containing: 100 mM 4-(2-hydroxyethyl)piperazineethanesulfonic acid (pH 7.5), 67 mM NaCl, 5.4 mM KCl, 4.8 mM CaCl2, 1.1 mM KH2PO4, 0.7 mM Na2SO4, 0.6 mM MgCl2, 10 mM glucose, 2 mM Na-citrate, 1% (w/v) albumin, and 0.25 mM/ml of penicillin. Protein synthesis was performed in this buffer in the presence of 100 μCi/ml of each tritiated leucine, lysine, valine, and glutamic acid. An amount of tubules containing between 0.4 and 0.6 mg of protein was incubated in a final volume of 0.6 ml at 37°C for 45 min with occasional shaking. After incubation, the tubules were washed twice as described above, using a washing buffer devoid of albumin. The tubule pellet was then lysed in a solution containing 62.5 mM Tris/HCl (pH 6.8), 2% (w/v) SDS, 3% (v/v) 2-mercaptoethanol, and 10% (w/v) glycerol. Samples of lysate were used for SDS-polyacrylamide gel electrophoresis (see below).

Renal RNA Extraction and Isolation of Poly(A)-containing RNA—In order to minimize the hazard of RNA degradation due to the high ribonuclease content of rat kidney (19), we adopted the guanidinium thiocyanate mixture extraction protocol described by Chirgwin et al. (14) Chirgwin et al. (14) described previously (9) of individual animals were rapidly excised after killing, rinsed in ice-cold saline and directly immersed in the guanidinium thiocyanate mixture. Homogenization was performed with a Polytron (Kinematica, Switzerland) using 18 ml of guanidinium thiocyanate mixture for two kidneys. Repeated cycles of acidification, ethanol precipitation, and extraction in guanidinium hydrochloride were carried out exactly as described by Chirgwin et al. (14). The RNA pellet from the last precipitation from guanidinium hydrochloride was washed in ethanol, dissolved in sterile water, and repurified at -20°C in the presence of 0.2 M potassium acetate, pH 5, by addition of 2 volumes of ethanol. The precipitate was washed in 95% (v/v) ethanol, dried, dissolved in 6 ml of 10 mM Tris/HCl, pH 7.5, containing 0.5 mM NaCl. The RNA solution was then electrophoresed on agarose gels for isolation of the poly(A)-containing fraction as described in another publication (20). The poly(A)-containing RNA was ethanol-precipitated, dissolved in water at a concentration of 0.5 μg/ml, and stored at -80°C. The yields of total RNA and poly(A)-containing RNA from two kidneys averaged 4.5 mg and 0.75 μg, respectively. No significant differences in yield were noted between groups of animals undergoing different treatments.

Translation of RNA—Cell-free protein synthesis directed by exogenous poly(A)-containing RNA was carried out by a nonpenicillase buffered wheat germ extract. Incubation conditions were as described previously (20), except that the total volume of reaction was scaled-down to 80 μl and that the concentration of radioactive leucine was increased to 400 μCi/ml. At the end of the incubation the reaction mixture was diluted at 1:2 by addition of ice-cold phosphate-buffered saline and centrifuged at 110,000 × g for 35 min. Samples of 0.5 μl of high speed supernatant, containing the released polypeptide chains, were used for the estimation of amino acid incorporation into protein by trichloroacetic acid precipitation. The precipitates were collected on glass fiber filters and dissolved in NCS tissue solubilizer (Amersham) for radioactivity measurement by liquid scintillation counting. Additional samples of high speed supernatant were frozen at -80°C and used later for electrophoretic analysis of the translation products.

SDS-Polyacrylamide Gel Electrophoresis and Two-dimensional Gel Electrophoresis—Polyacrylamide slab gel electrophoresis in the presence of SDS was performed according to Laemmli (21), using 12.5% (w/v) polyacrylamide separation gels. The slab gel apparatus was model SE 500 from Hoefer (San Fransisco, CA). Samples of high speed supernatant of translation mixtures (approximately 15 μl) were diluted 1:5 with a buffer containing 75 mM Tris/HCl (pH 6.8), 2% (w/v) SDS, 6% (v/v) 2-mercaptoethanol, and 12% (w/v) glycerol. The samples were then heated at 90°C for 2 min in a water bath containing 0.2% (w/v) bromophenol blue as tracking dye and loading onto the gel. The method for high resolution two-dimensional electrophoresis, which consists of isoelectric focusing in the first dimension and SDS-polyacrylamide gel electrophoresis in the second dimension, was exactly as described by O'Farrell (22, 23). Samples of high speed supernatant (approximately 25 μl) obtained from translation incubation mixtures were first lyophilized and the lyophilized material was then dissolved in 20 μl of O'Farrell's lysate buffer. Two μg each of bovine serum albumin, ovalbumin, and β-lactoglobulin were added to the samples as molecular weight markers. The gels were stained as described by Cleveland et al. (24) and destained by diffusion in a solution containing 30% (v/v) methanol and 8% (w/v) acetic acid.

Fluorography—Immediately after destaining, the gels were immersed in enhancing solution (Enhance, from New England Nuclear) and then dried according to the manufacturer's instructions. Autoradiography of the dried gels was carried out at -80°C, using Kodak X-Omat R film which was sensitized by pre-exposure to flash light as described by Laskey and Mills (25). Strips of developed film were scanned at 540 nm in a PMQ 3 spectrophotometer (Zeiss, Oberochen, Federal Republic of Germany) fitted with a film transport. The existence of a linear relationship between radioactivity in a gel band and absorbance of the radiographic image was verified by exposing preflushed film to a standard slab gel containing various amounts of [3H]leucine-labeled P-enolpyruvate carboxykinase in the different tracks. Labeled P-enolpyruvate carboxykinase for this experiment was obtained by inmunoprecipitation of a translation mixture programmed with poly(A)-containing RNA from the liver of a fasted rat, which is known to contain a high level of mRNA coding for the enzyme (26). In order to determine the exact amount of [3H]leucine incorporated in P-enolpyruvate carboxykinase, a portion of the immunoprecipitate was eluted chromatographed on oligo dT, which was subsequently sliced for liquid scintillation counting, as described previously (3).

RESULTS

Effect of Dexamethasone on Protein Synthesis in Isolated Kidney Tubules—Initial experiments were performed to investigate the effect of glucocorticoid administration on the rate and spectrum of protein synthesis in rat kidney. Protein synthesis was studied in vitro in preparations of isolated...
kidney tubules obtained by collagenase digestion or renal cortex. The donor animals were treated with 2 mg of dexamethasone/kg body weight, or saline as control, 8 h before killing. The tubules were incubated in the presence of tritiated amino acids for 45 min, after which they were lysed in a buffer containing SDS and 2-mercaptoethanol in order to extract

FIG. 1. Effect of dexamethasone on renal protein synthesis. Rats were injected with 2 mg of dexamethasone/kg or with saline intraperitoneally and killed 8 h later. Kidney tubules were isolated as described under “Experimental Procedures” and incubated in the presence of radioactive amino acids for 45 min, after which they were lysed in a buffer containing SDS and 2-mercaptoethanol in order to extract radioactivity in trichloracetic acid-insoluble material. Film exposure was for 48 h. Inset, relationship of fluorographic image absorbance to amount of radioactive protein in a gel band. The radioactive protein was \(^{3}H\)leucine-labeled P-enolpyruvate carboxykinase isolated by immunoprecipitation of a wheat germ translation mixture. See “Experimental Procedures” for technical details. Film exposure was for 48 h.

FIG. 2. Cell-free translation products specified by kidney poly(A)-containing RNA from normal and dexamethasone-treated rats. Rats were injected with 2 mg of dexamethasone/kg or with saline and killed 8 h later. Procedures for the extraction of total kidney RNA, the isolation of poly(A)-containing RNA and its translation in the wheat germ system are described under “Experimental Procedures.” Electrophoresis of the released translation products was performed in a 12.5% polyacrylamide gel. Track 1, polypeptides synthesized in the absence of exogenous RNA (25,000 cpm of \(^{3}H\)leucine incorporated into trichloracetic acid); tracks 2, 4, and 5, translation products specified by three different control RNA preparations; tracks 3, 5, and 7, translation products specified by three different RNA preparations from dexamethasone-treated animals. Samples 2 to 7 contained approximately 270,000 cpm of \(^{3}H\)leucine incorporated into trichloracetic acid-insoluble material. Film exposure was for 48 h (track 1) and 24 h (tracks 2 to 7). 94K, 68K, etc., see Fig. 1.

FIG. 3. Time course of induction of kidney mRNAs after dexamethasone administration. Renal poly(A)-containing RNA was isolated prior to, or at various times after, dexamethasone administration. Translation in the wheat germ system, electrophoresis of the released polypeptide products, and fluorography were performed as in the experiment of Fig. 2. Fluorogram strips were scanned at 540 nm in a spectrophotometer. The spectrophotometer tracings illustrate the pattern of translation products specified by poly(A)-containing RNA isolated at 0 h, 2 h, 4 h, and 8 h after dexamethasone injection. All samples contained approximately 200,000 cpm of \(^{3}H\)leucine incorporated into trichloracetic acid-precipitable material. Film exposure was for 48 h. Inset, relationship of fluorographic image absorbance to amount of radioactive protein in a gel band. The radioactive protein was \(^{3}H\)leucine-labeled P-enolpyruvate carboxykinase isolated by immunoprecipitation of a wheat germ translation mixture. See “Experimental Procedures” for technical details. Film exposure was for 48 h.

total cell proteins. These proteins, including newly synthesized ones, were displayed on 12.5% polyacrylamide slab gels containing SDS. Fluorograms of such gels are shown in Fig. 1: tracks 1 and 4 contain the proteins synthesized by tubules from dexamethasone-injected animals and tracks 2 and 3 contain the proteins synthesized by control tubules. Three polypeptides (denoted PEPCK, P 25, and P 23 in Fig. 1) are more actively synthesized in tubules from treated animals than in their counterparts from normal animals. The largest of these polypeptides (PEPCK) was identified as P-enolpyruvate carboxykinase, both on the ground of its exact comigration with purified hepatic P-enolpyruvate carboxykinase (not shown) and because of its inducibility by dexamethasone. The stimulatory effect of glucocorticoids on the synthesis of cytosolic P-enolpyruvate carboxykinase in rat kidney cortex has been established by earlier experiments involving immu-
Glucocorticoid Domain of Kidney

**TABLE 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PEPCK*</th>
<th>P 64</th>
<th>P 45</th>
<th>P 25</th>
<th>P 24</th>
<th>P 23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.3 ± 1.5</td>
<td>5.0 ± 1.2</td>
<td>3.0 ± 0.8</td>
<td>11.8 ± 1.1</td>
<td>3.7 ± 0.3</td>
<td>38.1 ± 4.3</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>20.2 ± 1.6</td>
<td>10.6 ± 2.4</td>
<td>10.7 ± 2.5</td>
<td>32.3 ± 1.0</td>
<td>8.3 ± 1.8</td>
<td>68.6 ± 8.1</td>
</tr>
</tbody>
</table>

*PEPCK, phosphoenolpyruvate carboxykinase.

**Effect of dexamethasone administration on functional level of six inducible mRNAs of rat kidney**

Total kidney RNA was obtained 8 h after dexamethasone injection. Translation of the poly(A)-containing fraction and electrophoresis of the products was as in Fig. 2. Densitometer scanning of the fluorograms was performed as in Fig. 3. Messenger RNA-directed incorporation of [3H]leucine into specific polypeptides was quantified by measuring the height of the corresponding peaks on the tracings. The base-line was constructed under each peak of interest by drawing a straight line between the adjacent troughs on each side of the peak. Values expressed in arbitrary units are presented as means ± S.E. for four separate assays involving four different RNA samples.

The products was as in Fig. 1. The increased synthesis of P 13) in the studies after in vivo pulse-labeling with radioactive leucine (12, 13). On the other hand, the induction of the two smaller proteins (P 25 and P 23) has not been reported prior to this study. The increased synthesis of P 25 and P 23, as well as that of P-enolpyruvate carboxykinase, was obvious in all experiments. In addition, a stimulation of the synthesis of two other proteins with apparent molecular weights of 52,000 and 45,000 (designated by arrows in Fig. 1) was observed in about half of the experiments.

**Effect of Dexamethasone on Template Activity of Renal Poly(A)-containing RNA—**As a rule, steroid hormone-mediated effects in the synthesis of specific proteins are due to changes in the cellular concentration of the corresponding mRNAs. A logical extension of the above experiments was therefore to extract renal mRNA from dexamethasone-treated or control animals and to examine its template activity in a heterologous cell-free protein synthesis system. Total kidney RNA was prepared 8 h after dexamethasone, or control treatment, by the guanidinium thiocyanate method of Chirgwin et al. (14) and the poly(A)-containing fraction of the RNA was isolated by oligo(dT)-cellulose chromatography. This fraction, which contains the majority of cellular mRNAs, was incubated in a wheat germ protein synthesis system in amounts which elicit a concentration-dependent incorporation of amino acid into protein. The polypeptides synthesized during incubation and released from the ribosomes were resolved by SDS-polyacrylamide gel electrophoresis (Fig. 1); products obtained from the translation of three distinct control RNA batches are displayed in *tracks* 2, 4, and 6, whereas the products specified by RNA samples from dexamethasone-treated rats are shown in *tracks* 3, 5, and 7; products synthesized in the absence of exogenous RNA were electrophoresed in *track 1*. The overall pattern of polypeptides encoded by renal RNA is identical in all cases. On the other hand, six distinct products are consistently more intensively labeled when the translation system’s programmed with RNA from dexamethasone-injected animals. The largest of these polypeptides (denoted PEPCK in Fig. 2) co-migrates with marker P-enolpyruvate carboxykinase (not shown). Earlier experiments have established that this product is specifically precipitated by antibodies against P-enolpyruvate carboxykinase. Furthermore, identical peptide maps were obtained after limited proteolysis from this immunoprecipitable product and from purified P-enolpyruvate carboxykinase from rat liver. On this basis, this product has been formally identified as P-enolpyruvate carboxykinase (16). The other polypeptides whose mRNAs display enhanced template activity after dexamethasone administration are termed P 64, P 45, P 25, P 24, and P 23 according to their apparent molecular mass in kilodaltons. Translation products P 45, P 25, and P 23 have RF values similar to those of the induced proteins shown in the experiments of Fig. 1. The failure to detect induced proteins corresponding to P64 and P24 in the isolated tubules can probably be ascribed to the relatively low specific radioactivity of protein synthesized in this system.

**Time Course and Extent of Induction—**In order to establish the time course of increase in the level of the various glucocorticoid responsive mRNAs, experiments were performed with RNA isolated at different times of treatment. Rats were killed prior to or at 2, 4, and 8 h after an injection of 2 mg of dexamethasone/kg. The isolation of poly(A)-containing RNA, its translation by the wheat germ cell-free system, and the electrophoresis of the polypeptide products were performed as before. The fluorograms were scanned in

**FIG. 4. Effect of cycloheximide on glucocorticoid-mediated induction of kidney mRNAs.** Cycloheximide was injected intraperitoneally at a dose of 2 mg/kg 30 min before dexamethasone. Renal RNA was isolated 8 h after dexamethasone administration. Control animals received injections of saline. Translation of the poly(A)-containing RNA, electrophoresis of the translation products, and densitometer tracing of the fluorogram were as described in the legend of Fig. 3. Lower trace, translation products specified by renal poly(A)-containing RNA from untreated rats; upper trace, translation products specified by renal poly(A)-containing RNA after cycloheximide plus dexamethasone treatment. The samples contained approximately 160,000 cpm of [3H]leucine incorporated into trichloroacetic acid-precipitable material. Letters above the traces correspond to the same products as in Fig. 3.
a spectrophotometer fitted with a film transport. As shown in the inset of Fig. 3, the conditions for fluorography were selected so as to ensure a roughly linear relationship between the density of the radiographic image and the amount of radioactive protein in a gel band. Under these conditions, the height of the peaks of the densitometer tracing provides a semiquantitative estimate of the amount of radioactive amino acid incorporated into the various translation products and thereby of the functional level of the various mRNAs. As may be seen in Fig. 3, the level of four of the six inducible mRNAs is increased within 2 h of dexamethasone administration. At 4 h of treatment, the increased template activity is obvious for all six mRNAs. A further slight rise is observed between 4 and 8 h. Although we failed to observe an increase in the level of the mRNA coding for P-enolpyruvate carboxykinase mRNA normally observed in rat kidney during glucocorticoid treatment (16). It was therefore of interest to investigate a possible effect of cycloheximide on the hormonal regulation of the other renal mRNAs. In the next experiments, a dose of 2 mg of cycloheximide/kg, sufficient to inhibit renal protein synthesis maximally for 6 to 8 h (27), was injected to rats 30 min prior to dexamethasone. The kidneys were removed from these animals and from controls 8 h after the time of hormone administration. Renal poly(A)-containing RNA was isolated and translated in the usual manner. The translation products were analyzed by densitometry of fluorograms. Typical recordings are shown in Fig. 4: the bottom trace depicts the products of control RNA and the upper trace depicts the products of RNA from animals treated with cycloheximide plus dexamethasone. Clearly, cycloheximide does not prevent the increase in translatable mRNAs coding for P 64, P 45, P 25, P 24, and P 23. We therefore conclude that the glucocorticoid-mediated induction of these messages does not require contin-

![Figure 5](image-url)

**Fig. 5.** Two dimensional gel electrophoresis of wheat germ translation products specified by kidney poly(A)-containing RNA from normal (A) and dexamethasone-treated rats (B). The treatment of the animals and the methods for RNA isolation and translation were as described in Fig. 2. High resolution two-dimensional electrophoresis was performed according to O'Farrell (22), using a 12.5% polyacrylamide gel for the second dimension. The samples contained approximately 350,000 cpm of [3H]leucine incorporated into trichloracetic acid-precipitable material. Film exposure was for 6 days. C, electrophoresis of rat liver P-enolpyruvate carboxykinase purified as in Ref. 28 and gel stained with Coomassie brilliant blue; D, electrophoresis of an immunoprecipitate of wheat germ translation mixture formed after addition of antibodies against P-enolpyruvate carboxykinase; film exposure was for 3 days. The pH gradients in electrophoresing gels were measured by slicing duplicate gels and eluting the slices in water. 68K, 45K, and 18K denote the migration of serum albumin, ovalbumin, and β-lactoglobulin, respectively.
ued protein synthesis. On the other hand, in agreement with our earlier results, P-enolpyruvate carboxykinase mRNA does not accumulate in response to dexamethasone pretreatment with cycloheximide.

High Resolution Two-dimensional Gel Electrophoresis of Polypeptides Encoded in Kidney Poly(A)-containing RNA—Mammalian tissues typically contain more than 10,000 different mRNA species. Only a very small percentage of the polypeptides encoded in the total mRNA population are resolved by a single-dimension electrophoretic procedure such as the SDS-polyacrylamide gel electrophoresis used in the above experiments. In order to obtain a more complete picture both of the overall coding capacity of kidney poly(A)-containing RNA and of the small family of glucocorticoid-regulated mRNAs, we also used the high resolution two-dimensional gel electrophoresis of O’Farrell (22) for the analysis of the polypeptides synthesized by the mRNA-dependent cell-free system. Representative fluorograms of gels containing the translation products of control RNA and of RNA from dexamethasone treated animals are shown in Fig. 5 (A and B). Four additional paired comparisons were performed, using a total of 10 different RNA samples. The overall pattern of spots was highly reproducible in all experiments. The total number of identifiable spots was 376 ± 20 (mean ± S.D.). The squares in Fig. 5 delineate products whose synthesis is always more active at the direction of RNA from glucocorticoid-treated animals. As may be seen, there are nine such products, numbered 1 through 9 in order of decreasing molecular weights. Spot 1 was identified as P-enolpyruvate carboxykinase on the basis of the experiments illustrated in the right part of Fig. 5, which depict the electrophoretic migration of P-enolpyruvate carboxykinase purified from rat liver (C) and that of an immunoprecipitate formed upon addition of antibodies against P-enolpyruvate carboxykinase to a wheat germ translation mixture programmed with poly(A)-containing RNA from the liver of fasted rats (D). As judged from its apparent molecular weight, spot 2 is probably the counterpart of P 64 identified in one-dimensional gels. Similarly, spots 3 (or 4), 7, and 8 correspond to P 45, P 25, and P 24. The induced polypeptides in spots 5 and 6, with apparent molecular weights of 33,500 and 31,000, did not show up after conventional SDS-polyacrylamide gel electrophoresis. In addition to these nine spots, three induced polypeptides, singled out by the arrows in Fig. 5, were detected in two out of five paired comparisons. Their apparent molecular weights were 54,500, 54,000, and 23,500. The latter product may correspond to P 23 in single-dimensional gels.

Effect of Dexamethasone on Liver mRNA Population.—In order to evaluate the tissue specificity of the response to dexamethasone, translation studies were performed using poly(A)- containing RNA from rat liver. The treatment of the animals and the experimental procedures for RNA isolation and cell-free protein synthesis were the same as in the experiments described above. As shown by the fluorograms in Fig. 6, an impressive consequence of glucocorticoid treatment in the liver is a marked decline in the functional level of several mRNAs, as evidenced by the decreased cell-free synthesis of the polypeptides delineated by circles. Under the same circumstances, however, there is no consistent deinduction of any renal mRNA (see Fig. 5). As expected from the known inductive effect of dexamethasone on the synthesis of several hepatic proteins, there is also an increase in the template activity of a subset of hepatic mRNAs (delineated by squares in Fig. 6). Notice that there is no overlap in the families of induced gene products in liver and kidney. In particular, there is no increase in the level of mRNA coding for P-enolpyruvate carboxykinase in liver (arrow in Fig. 6). This observation is consistent with the earlier data of Gunn et al. (12) who showed that an injection of triamcinolone to the intact animal does not cause a stimulation of hepatic P-enolpyruvate carboxykinase synthesis, probably because of a counteraction by insulin. The relative rate of synthesis of tyrosine aminotransferase, on the other hand, is clearly increased in the liver of rats treated with a glucocorticoid, an effect resulting from a comparable increase in the level of functional mRNA coding for the enzyme (2, 29–31). We have tentatively identified spot 1 in Fig. 6 as tyrosine aminotransferase, by comparison with the two-dimensional gel data of Ivarie and O’Farrell (9). It appears, moreover, that spot 5 (Fig. 6) in our gels is identical with glucocorticoid-induced protein P 25 detected by Ivarie and O’Farrell in hepatoma cells (9).

**DISCUSSION**

The relative rate of synthesis of several renal proteins is enhanced after dexamethasone administration to the intact animal. These induced proteins were detected in kidney tubules isolated from treated animals and incubated in vitro in the presence of radioactive amino acid. The rather limited protein synthetic capacity of the tubule preparation, however, prevents the detection of polypeptides with low rates of synthesis and makes it difficult to show relatively small changes in the rate of synthesis of a given protein. Furthermore, experiments in the intact cell do not distinguish between hormonal effects at the translational level, i.e. on the protein synthesis machinery, or at the transcriptional/post-transcriptional level, i.e. on the abundance of mRNAs. To circumvent
such difficulties, we have used a strategy involving the extraction of total kidney mRNA and its translation by an efficient heterologous cell-free protein synthesis system, coupled with sensitive detection procedures for the analysis of translation products. Our data show that the functional level of several distinct mRNAs of the rat kidney increases during glucocorticoid treatment. These mRNAs code for polypeptides corresponding to the induced proteins observed in the isolated tubules and for additional gene products that could not be detected in the tubule preparation. The above findings are consistent with the current concept according to which the response of target tissues to the glucocorticoids consists primarily of changes in the abundance of a well defined subset of cellular mRNAs.

A limitation of the present work, inherent to experiments in the whole animal, is the inability to discriminate between direct effects of the glucocorticoids on the kidney and indirect effects due to factors of extrarenal origin which might be produced during glucocorticoid treatment. It was argued earlier that the buildup of the mRNA coding for P-enolpyruvate carboxykinase in the kidney is a direct consequence of glucocorticoid action (9). The basis for this assumption is the well known capacity of the glucocorticoids to induce the synthesis of P-enolpyruvate carboxykinase in vitro in hepatoma cell culture. Our current hypothesis is that the coordinate induction of the entire group of renal mRNAs is a primary response to the injected steroid, although a formal proof of this hypothesis will have to await the availability of a hormone-responsive kidney tissue culture system.

Our approach to monitor changes in the mRNA population of the rat kidney after dexamethasone treatment relied on the electrophoretic analysis of the full complement of polypeptides encoded in poly(A)-containing RNA and translated by a wheat germ cell-free protein synthesis system. Maximal resolution of the translation products was achieved using the high resolution two-dimensional electrophoresis procedure designed by O'Farrell (22). This technique has the potential to separate a thousand proteins in a single experiment and thereby affords a means to survey a substantial fraction of the expressed genome of mammalian cells. On the other hand, in order to get more easily quantifiable data on the level of some mRNAs, the translation products were displayed on conventional SDS-polyacrylamide slab gels. The fluorograms of the gels were scanned to obtain densitometric recordings. Within certain limits, the density of the radiographic image faithfully reflected the amount of radioactivity contained in the corresponding polypeptide band in the gel. The latter procedure was used to follow the time course and evaluate the extent of induction of a restricted subgroup of six mRNAs.

All the assayed mRNAs accumulate with comparable kinetics. Of particular interest is the increase in template activity consistently seen after 2 h of treatment. This observation is in good agreement with our earlier data showing a 70% increase in the level of renal P-enolpyruvate carboxykinase mRNA during this time interval (16). Recently, Diesterhaft et al. (31) observed a 3-fold increase in hepatic tyrosine aminotransferase mRNA 1 h after hydrocortisone injection. Such data, as well as studies of the hormonally induced expression of mouse mammary tumor virus RNA in infected cells (32, 34), demonstrate that glucocorticoid-mediated changes in mRNA levels in mammalian tissues can occur without the several-hour lag period sometimes associated with steroid hormone action (35, 36).

The response of all inducible mRNAs is roughly comparable in terms of induction ratio. This ratio, i.e. the template activity in RNA samples from treated animals compared to the template activity in control preparations, fell within the narrow range of 2 to 3 for the six assayed mRNAs 8 h after dexamethasone administration. According to the present estimates, the level of P-enolpyruvate carboxykinase mRNA is increased 2-fold above the control level, whereas earlier experiments using precise immunoochemical techniques to quantify mRNA-directed synthesis in the wheat germ system showed a 4-fold increase in this particular mRNA (16). A possible source of underestimation in the present experiments may well be an overlap of both induced and non-induced products in one-dimensional gels. Such an overlap would tend to minimize the observed differences in rate of synthesis of individual polypeptides. In fact, the visual inspection of several pairs of two-dimensional gels containing the translation products of RNA from treated or control animals suggests that the induction ratio may be comprised between 4 and 6 for the majority of the mRNAs.

In a variety of model systems, steroid hormone-dependent increases in the abundance of specific mRNAs are suppressed in the presence of protein synthesis inhibitors. Well known examples include the estrogen induction of egg white protein mRNAs in the chick oviduct (36, 37) and the glucocorticoid induction of α-globulin and tryptophan oxygenase in rat hepatocytes (38, 39). We have also reported previously (16), and confirmed here, that the accumulation of P-enolpyruvate carboxykinase mRNA caused by the glucocorticoids is markedly inhibited by cycloheximide. A striking observation in the present work is that the induction of the other kidney mRNAs is fully preserved when a dose of cycloheximide causing a virtually total inhibition of renal protein synthesis is administered just prior to dexamethasone. The absence of a requirement for continued protein synthesis may indicate that the accumulation of these mRNAs is a direct consequence of the activation of the corresponding genes by the glucocorticoid-receptor complex. On the contrary, the increase in the level of P-enolpyruvate carboxykinase mRNA would require an intermediary step involving the synthesis of a regulatory protein. Alternative hypotheses accounting for the inhibitory effect of cycloheximide on P-enolpyruvate carboxykinase mRNA accumulation have been discussed previously (16).

Nearly 400 polypeptide products were routinely separated by high resolution two-dimensional electrophoresis of translation mixtures programmed with renal poly(A)-containing RNA. The spot pattern on fluorograms was highly reproducible for all the RNA samples tested. Paired comparisons between identical spots revealed that nine translation products were always more actively synthesized at the direction of RNA from dexamethasone-treated rats than from controls. Three additional products were found to be induced in two out of five experiments. The rate of cell-free synthesis of all the other polypeptides was the same irrespective of whether the input RNA came from treated or control animals. The most straightforward conclusion from these data is that at least nine mRNAs accumulate in the kidney in response to dexamethasone administration. It should not be overlooked, however, that multiple products may arise from single mRNAs or, in other words, that the number of spots in two-dimensional gels may overestimate the number of mRNAs translated. Two possible mechanisms may contribute to generate several spots per mRNA: the release of nascent polypeptide chains at various stages of completion by "early quitting" ribosomes or the post-translational modification of proteins. We do not believe that the latter mechanism was responsible for the genesis of several of the induced products seen in our gels because the pattern of spots does not contain arrays suggestive of posttranslational protein modification. On the other hand, we cannot totally discount the possibility that some of the smaller molecular weight peptides are abortive
translation products from the same mRNAs that encode some of the larger proteins. Thus, spots 1, 2, 3, or 4. Conversely, this study may underestimate the number of glucocorticoid-regulated kidney mRNAs for two reasons. First, the total mRNA population of adult mammalian organs contains 10,000 to 20,000 different polyadenylated mRNA species which are distributed in several abundance classes (40, 41). The 400 gene products monitored in our experiments cover only a small fraction of this population, probably corresponding to the most abundant mRNAs. It is of course possible that additional steroid responsive mRNAs went undetected because they are present at concentrations below the sensitivity limit of a translational assay. Second, this work is concerned only with mRNAs retained on oligo(dT)-cellulose. The minority of messengers devoid of poly(A) tail, which do not hybridize to oligo(dT), were by essence excluded from consideration.

Neither the intrarenal site of synthesis nor the identity of the proteins encoded by the induced mRNAs are known, except for P-enolpyruvate carboxykinase (spot 1). This enzyme is expressed exclusively in the proximal tubule (42). There is also recent evidence suggesting that glucocorticoid receptors are localized predominantly in the proximal tubule (43). This would imply that all induced mRNAs are transcripts of proximal tubular cells (provided of course that induction does result from a direct glucocorticoid effect on the kidney). In support of this inference is the fact that the proximal tubules make up approximately 60% of the total mass of the kidney, the remaining 40% consisting of all the other nephron segments. Therefore, the mRNA population of the proximal cells is prevalent in total tissue RNA extracted from the kidney and consequently encodes the majority of the polypeptides that are translated in a cell-free system. The same argument suggests that proteins synthesized in the isolated tubule preparation are essentially proximal tubule proteins. What are then the nature and function of the induced proteins? A major metabolic effect of the glucocorticoids is to stimulate renal gluconeogenesis (10). The augmentation of P-enolpyruvate carboxykinase activity resulting from the induction of enzyme synthesis is regarded as a key mechanism maintaining the increased flux of substrates along the gluconeogenic pathway. Whether the other glucocorticoid-induced gene products also play a role in gluconeogenesis or related processes is a matter of speculation.

In order to assess the specificity of the renal response, we performed comparative studies of the effect of dexamethasone administration on the coding capacity of liver poly(A)-containing RNA. The glucocorticoids are known to stimulate the synthesis of various hepatic enzymes and other proteins (9, 44). We therefore expected, and found, that the functional level of several hepatic mRNAs is increased during dexamethasone treatment. Interestingly, however, the families of glucocorticoid induced mRNAs in liver and kidney do not overlap, as judged from the migration of their translation products in two-dimensional gels. Furthermore, there is a striking decrease in the translational activity of several mRNAs of liver, whereas no deinduction occurs in the kidney. Unfortunately, the interpretation of these tissue differences is complicated by the multiple hormonal interactions taking place in the intact animal. It is conceivable, for instance, that some of the mRNA changes in liver are due to the action of insulin, which is known to be released in the rat after glucocorticoid administration (12), rather than to dexamethasone itself. Nevertheless, the present experiments clearly establish that the administration of a glucocorticoid to the intact animal results in distinctly different alterations of gene expression in kidney and liver.

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