No correlation between binding of glucocorticosteroids to specific cytoplasmic proteins *in vivo* and enzyme induction in the rat liver

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Time- and dose-dependence of the formation of the different cytoplasmic hormone–protein complexes were studied in the rat liver after administration *in vivo* of [³H]cortisol or [³H]dexamethasone and compared with the stimulation of RNA polymerase B and induction of tyrosine aminotransferase and tryptophan oxygenase. No correlation could be found between formation *in vivo* of any of the five cytoplasmic hormone–protein complexes found and stimulation of RNA polymerase B activity or enzyme induction. After administration of [³H]cortisol, different metabolites of cortisol could be demonstrated in the isolated hormone–protein complexes. No time- or dose-dependence of the metabolite patterns could be observed after application of hormone doses that were in the range of the biologically active doses. After administration of [³H]dexamethasone, the same hormone–protein complexes were observed, which contained, however, the injected steroid instead of metabolites. These results seem to indicate that the cytoplasmic binding components present in the rat liver are enzymes involved in the metabolism of the glucocorticosteroids and that dexamethasone binds to these enzymes as a substrate analogue.

The induction by glucocorticosteroids of tryptophan oxygenase (L-tryptophan:oxygen 2,3-oxidoreductase; EC 1.13.11.11) and tyrosine aminotransferase (L-tyrosine:2-oxoglutarate aminotransferase; EC 2.6.1.5) in the rat liver depends on a specific increase of the concentrations of the corresponding mRNA species (Schütz *et al.*, 1973; Roewekamp *et al.*, 1976; Nickol *et al.*, 1976) and can be inhibited by actinomycin D (Greengard *et al.*, 1963; Garren *et al.*, 1964; Schütz *et al.*, 1975; Voigt *et al.*, 1978) and α -amanitin (Sekeris *et al.*, 1970; Voigt *et al.*, 1978). It is generally regarded, as reported for other steroid hormones (Raspe, 1971; Jensen & DeSombre, 1972), that the first step of the induction process after penetration of the hormones into the cell is the formation of complexes with specific proteins, called 'receptors' (Rousseau, 1975; Cake & Litwack, 1975; King & Mainwaring, 1974). Cortisol or corticosterone, depending on the animal species is thought to be the active inducer for tryptophan oxygenase and tyrosine aminotransferase (Baxter & Tomkins, 1971), as well as for proangiotensin (Voigt & Köster, 1980). Evidence for this assumption stems from observations that after injection of radioactive cortisol or corticosterone, the

most prominent radioactive compound demonstrated in purified nuclei as well as in at least one of the isolated cytoplasmic hormone–protein complexes was the administered hormone, whereas the main part of the unbound radioactivity present in the cytosol corresponded to different metabolites (Cake & Litwack, 1975; Beato *et al.*, 1969; Carlstedt-Duke *et al.*, 1975, 1977). On the other hand, acid metabolites of cortisol were isolated from rat liver, which differentially induced tryptophan oxygenase and tyrosine aminotransferase (Voigt & Sekeris, 1980). Re-investigation of the metabolites bound to the different cytoplasmic proteins *in vivo* revealed that cortisol could be demonstrated in all the isolated hormone–protein complexes after application of an extremely low dose of radioactive hormone, whereas after application of biologically active doses only metabolites of cortisol could be found in the same complexes (Voigt *et al.*, 1981).

Beato *et al.* (1972a) have tried to correlate the dose–response relationship of tryptophan oxygenase and tyrosine aminotransferase to cortisol with the degree of saturation of cytoplasmic dexamethasone binding sites. We, however, have not been able to confirm the dose–response curves of tryptophan oxygenase and tyrosine aminotransferase published by these authors (Voigt & Sekeris, 1978). All these findings prompted us to study in detail whether or not there is a correlation between saturation *in vivo*

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of any of the cytoplasmic glucocorticosteroid-binding proteins, the hormones bound *in vivo* to these proteins and the data available about the process of enzyme induction.

Materials and methods

Chemicals

Dexamethasone was a gift from Schering AG (Berlin/Bergkamen, Germany). α -Amanitin was kindly provided by Professor Th. Wieland, Heidelberg, Germany. [^3H]Dexamethasone (sp. radioactivity 24 Ci/mmol); [^3H]cortisol (sp. radioactivity 87 Ci/mmol) and [^3H]UTP (sp. radioactivity 15 Ci/mmol) were obtained from Amersham-Buchler (Braunschweig, Germany). The non-labelled nucleoside triphosphates were purchased from Pharma Waldhof G.m.b.H. (Mannheim, Germany), pyridoxal 5-phosphate from Boehringer (Mannheim, Germany) and bovine serum albumin from Sigma (St. Louis, MO, U.S.A.). Actinomycin D, haematin hydrochloride and Scintigel were obtained from Roth (Karlsruhe, Germany), DEAE-cellulose (DE-52) was from Whatman (Maidstone, Kent, U.K.), Sephadex G-25 from Pharmacia (Uppsala, Sweden) and filter papers from Schleicher & Schüll (Dassel, Germany). $11\beta,17\alpha$ -Dihydroxy-3-oxo-androst-4-ene-17 β -carboxylic acid and $11\beta,17\alpha,20$ -trihydroxy-3-oxopregn-4-en-21-oic acid were prepared as described previously (Voigt & Sekeris, 1980). Cortisol, tetrahydrocortisol, cortisone, thin-layer plates (silica gel 60 F₂₅₄), diphenyloxazole and all other chemicals were purchased from Merck (Darmstadt, Germany).

Animals and injections

Male Wistar rats (8–12 weeks old; 150–200 g), kept under standard conditions, were used throughout. The animals were bilaterally adrenalectomized 6–8 days before the experiment through the paravertebral dorsal approach. The animals then received a 0.14 M-NaCl solution instead of normal drinking water. Unlabelled cortisol was dissolved in dimethylformamide and mixed with [^3H]cortisol obtained as a solution in benzene/ethanol (1:1, v/v). After removing most of the benzene and ethanol by a gentle stream of N₂ the organic solution was mixed with a 0.14 M-NaCl solution. Portions of this mixture corresponding to 0.5, 5, 15, 20 or 50 mg of cortisol and 25 μCi of radioactive hormone per kg body wt. were injected intraperitoneally between 09:00 and 10:00 h; 15, 20 and 50 mg of steroid per kg body wt. were found to be active with respect to induction of tyrosine aminotransferase and tryptophan oxygenase (Voigt & Sekeris, 1978). The dose of dimethylformamide never exceeded 0.5 ml per kg body wt. The animals were killed by cervical dislocation 5, 15, 30 or 60 min after injection.

Preparation of liver fractions and separation of hormone-protein complexes

The preparation of the liver fractions was carried out as previously described (Voigt *et al.*, 1981). The isolation and fractionation of DEAE-cellulose (Whatman DE-52) of cytoplasmic hormone-protein complexes was performed essentially by the method of Schmid *et al.* (1976). To determine the radioactivity of the different fractions 1 ml portions were mixed with 4 ml of Scintigel and measured in a liquid-scintillation counter (Mark II; Nuclear Chicago).

Assays

Tyrosine aminotransferase activity was determined by the method of Diamondstone (1966) and tryptophan oxygenase activity essentially as described by Schütz & Feigelson (1972). The preparation of rat liver homogenates for the determination of enzyme activities was carried out as described (Voigt *et al.*, 1978). The activities of tyrosine aminotransferase and tryptophan oxygenase are expressed in μmol of *p*-hydroxybenzaldehyde and μmol of *N*-formylkynurenine plus kynurenine respectively formed/min per mg of protein. Protein concentrations were determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard. DNA concentrations were measured as described by Burton (1956).

Activities of DNA-dependent RNA polymerases were determined by measuring the incorporation of [^3H]UTP into material insoluble in 5% trichloroacetic acid (Chamberlin & Berg, 1962) as described previously (Voigt *et al.*, 1978).

Protein binding of labelled steroids was measured by the charcoal-dextran assay (Schmid *et al.*, 1976).

Extraction and characterization of steroids

Aqueous solutions containing labelled steroids were adjusted to 1 M-acetic acid and extracted with ethyl acetate. The extracts were analysed by t.l.c. on silica gel 60F₂₅₄ as previously described (Voigt & Sekeris, 1980; Voigt *et al.*, 1981).

Results

Specificity of glucocorticosteroid-binding components present in rat liver cytosol

When binding of [^3H]cortisol or [^3H]dexamethasone to rat liver cytosol was analysed by Scatchard plots (Scatchard, 1949), only one class of specific binding sites could be detected (Schmid & Grote, 1977). However, several binding components were found after anion-exchange chromatography of cytosol labelled with [^3H]cortisol, [^3H]corticosterone or [^3H]dexamethasone *in vivo* or *in vitro* (Beato *et al.*, 1972b; Beato & Feigelson, 1972; Sekeris &

Schmid, 1972; Litwack *et al.*, 1973; Cake & Litwack, 1975; Schmid *et al.*, 1976; Carlstedt-Duke *et al.*, 1977; Schmid & Grote, 1977; Grote *et al.*, 1978). Very similar radioactivity profiles were obtained after DEAE-cellulose chromatography of high-molecular-weight components from rat liver cytosols labelled *in vivo* by injection of [3 H]dexamethasone (Fig. 1a), a low (Fig. 1b) or a biologically active dose of [3 H]cortisol (Fig. 1c). Five peaks of radioactivity, termed DE-1, DE-1b, DE-2, T and DE-3, could be detected after administration of [3 H]cortisol eluting at the same salt concentrations as the hormone-protein complexes found after incubation of rat liver cytosol with [3 H]cortisol *in vitro* (Schmid *et al.*, 1976; Schmid & Grote, 1977; Voigt *et al.*, 1981). In the experiments performed with [3 H]dexamethasone, binding component T, which represents transcortin (Beato *et al.*, 1970), could not

be detected, as reported previously (Schmid *et al.*, 1976). On the basis of these results it is impossible to identify the 'classical' cytoplasmic glucocorticosteroid receptor among the different cytoplasmic binding components.

Time course of hormone uptake by cytosol and nuclei, formation of cytoplasmic hormone-protein complexes and stimulation of DNA-dependent RNA-polymerase activities

Time courses of hormone uptake by rat liver nuclei and cytosol, as well as formation of cytoplasmic hormone-protein complexes, were studied after administration *in vivo* of biologically active doses of [3 H]cortisol (Fig. 2) and [3 H]dexamethasone (Fig. 3). The experiments were performed with inducing doses of hormone, in order to make them biologically meaningful. No difference could be observed with respect to the accumulation of hormones in cytosol and nuclei (Figs. 2a and 3a), maximal levels of radioactivity being observed 15 min after injection. Essentially the same time

Fig. 1. DEAE-cellulose chromatography of rat liver cytosol labelled *in vivo* by administration of [3 H]dexamethasone (a) and a low dose (b) or a biologically active dose of [3 H]cortisol (c)

Adrenalectomized male rats of 200g body weight were subjected to intraperitoneal injection of 2 μ g of [3 H]dexamethasone (sp. radioactivity 7000 Ci/mol) (a), 0.4 μ g of [3 H]cortisol (sp. radioactivity 87000 Ci/mol) (b) or 3 mg of [3 H]cortisol (sp. radioactivity 12 Ci/mol) (c) 15 min before preparation of the cytosol., [Cl $^-$]; ●, radioactivity (c.p.m./ml). T = transcortin.

Fig. 2. Kinetics of appearance of radioactivity in rat liver cytosol and nuclei (a) and in the different cytoplasmic hormone-protein complexes (b) after intraperitoneal injection of 2 mg of [3 H]cortisol (sp. radioactivity 40 Ci/mol) per 100g body weight

◆---◆, Total cytosol; ◆---◆, cytosol treated with charcoal; ●---●, nuclei; ■---■, DE-1; ■---■, DE-1b; ●---●, DE-2; ●---●, DE-3.

Fig. 3. Kinetics of appearance of dexamethasone in rat liver cytosol and nuclei (a) and in the different hormone-protein complexes (b) after intraperitoneal injection of $1 \mu\text{g}$ of $[^3\text{H}]$ dexamethasone (sp. radioactivity 7000 Ci/mol) per 100 g body weight

◆—◆, Total cytosol; ◇—◇, cytosol treated with charcoal; ●—●, nuclei; ■—■, DE-1; ■—■, DE-1b; ●—●, DE-2; ●—●, DE-3.

course was found for the formation of total cytoplasmic hormone-protein complexes as determined by the charcoal assay. However, differences were observed with respect to the formation of the individual hormone-protein complexes, measured after separation by DEAE-cellulose chromatography (Figs. 2b and 3b). Maximal levels of hormone-protein complexes DE-1, DE-2 and DE-3 were reached between 5 and 15 min after administration of either $[^3\text{H}]$ cortisol (Fig. 2b) or $[^3\text{H}]$ dexamethasone (Fig. 3b), whereas accumulation of complex DE-1b was significantly slower. In the experiments performed with $[^3\text{H}]$ cortisol, the radioactivities present in DE-1 and DE-2 were maximal 5 and 15 min after injection respectively. Thereafter, radioactivities present in these peaks decreased successively, whereas the levels of DE-1b and DE-3 remained almost constant between 30 and 60 min after injection (Fig. 2b). In the experiments performed with $[^3\text{H}]$ dexamethasone (Fig. 3b), levels of DE-2 and DE-3 reached a plateau between 15 and 150 min after injection, whereas the label was successively lost from DE-1 after reaching the maximal level.

Activity of RNA polymerase B increased after a lag phase of 15 min after injection of dexamethasone, maximal stimulation being observed after 60 min (Fig. 4). Therefore, 15 min after injection glucocorticosteroids must be present at their target sites within the nuclei.

Dose-response relationship of the formation of cytoplasmic hormone-protein complexes, stimulation of RNA polymerase activities and enzyme induction

Beato *et al.* (1972a) have reported that there is a correlation between the dose-response relation of the formation of cytoplasmic hormone-protein complexes and induction by cortisol of tyrosine aminotransferase and tryptophan oxygenase. However, we are not able to confirm these results on the basis of our experiments. As shown in Fig. 5(b), all the binding components could be saturated with hormone by administration of $[^3\text{H}]$ cortisol *in vivo*. No differences could be observed with respect to the dose-dependence of the formation of hormone-protein complexes DE-1, DE-1b, DE-2 and DE-3

Fig. 4. Time course of RNA polymerase (A + C) and B activities after intraperitoneal injection of dexamethasone

Adrenalectomized male rats of 200 g body weight received intraperitoneally $2 \mu\text{g}$ of dexamethasone at zero time. Animals were killed at the indicated time periods, liver nuclei were prepared as described in the text and tested for RNA polymerase (A + C) and B activities. The values are expressed in c.p.m. of $[^3\text{H}]\text{UTP}$ incorporated per μg of DNA and represent means of individual determinations in 12 animals $\pm$ S.E.M. $\blacktriangle$ — $\blacktriangle$, Total incorporation of $[^3\text{H}]\text{UTP}$; ∇ — ∇ , incorporation of $[^3\text{H}]\text{UTP}$ in the presence of α -amanitin (i.e. polymerase A + C activities); $\bullet$ — $\bullet$, RNA polymerase B activity.

Fig. 5. Dose-dependence of uptake of $[^3\text{H}]$ cortisol by rat liver nuclei and cytosol (a), of formation of cytoplasmic hormone-protein complexes (b) and induction of tyrosine aminotransferase and tryptophan oxygenase by cortisol (c).

Adrenalectomized male rats were injected at zero time with the amount of $[^3\text{H}]$ cortisol indicated in the Figure and killed by cervical dislocation after 30 min (a and b) or 5 h (c). Specific radioactivities of nuclei and cytosol were measured and the amounts of individual cytoplasmic hormone-protein complexes were calculated after separation by DEAE-cellulose chromatography. Activities of tyrosine aminotransferase and tryptophan oxygenase were measured as described in the text. $\diamond$ — $\diamond$, total cytosol; $\blacklozenge$ — $\blacklozenge$, cytosol treated with charcoal; $\bullet$ — $\bullet$, nuclei; $\blacksquare$ — $\blacksquare$, DE-1; $\blacksquare$ — $\blacksquare$, DE-1b; $\bullet$ — $\bullet$, DE-2; $\bullet$ — $\bullet$, DE-3; $\blacktriangledown$ — $\blacktriangledown$, tyrosine aminotransferase; $\blacktriangle$ — $\blacktriangle$, tryptophan oxygenase.

(Fig. 5b) and accumulation of radioactivity in nuclei and cytosol (Fig. 5a). However, considerably higher doses of hormone were necessary for induction of tryptophan oxygenase and tyrosine aminotransferase (Fig. 5c) than for the formation of the different cytoplasmic hormone-protein complexes. As already reported (Voigt & Sekeris, 1978), induction of tryptophan oxygenase could be observed at hormone doses lower than those necessary to increase tyrosine aminotransferase activity. Similar results were obtained from the experiments

performed with $[^3\text{H}]$ dexamethasone (Fig. 6). However, enzyme induction as well as apparent saturation with hormone of cytoplasmic binding components was observed at a hormone dose 1/5000th of that necessary in the case of $[^3\text{H}]$ cortisol. Cytoplasmic concentrations of all the hormone-protein complexes with the exception of DE-1b reached a plateau at a hormone dose of 1–2 μg per 100 g body wt. At the same hormone dose specific radioactivities of nuclei and cytosol reached their

Fig. 6. Dose-dependence of uptake of $[^3\text{H}]$ dexamethasone by rat liver cytosol and nuclei (a), of formation of cytoplasmic hormone-protein complexes (b) and induction of tyrosine aminotransferase and tryptophan oxygenase by dexamethasone (c).

Adrenalectomized male rats were injected at zero time with the amount of $[^3\text{H}]$ dexamethasone indicated in the Figure and killed after 30 min (a and b) or 5 h (c). Specific radioactivities of nuclei and cytosols were measured and the amounts of individual cytoplasmic hormone-protein complexes were calculated after separation by DEAE-cellulose chromatography. Activities of tyrosine aminotransferase and tryptophan oxygenase were determined as described in the text. $\diamond$ — $\diamond$, Total cytosol; $\blacklozenge$ — $\blacklozenge$, cytosol treated with charcoal; $\bullet$ — $\bullet$, nuclei; $\blacksquare$ — $\blacksquare$, DE-1; $\blacksquare$ — $\blacksquare$, DE-1b; $\bullet$ — $\bullet$, DE-2; $\bullet$ — $\bullet$, DE-3; $\blacktriangledown$ — $\blacktriangledown$, tyrosine aminotransferase activity; $\blacktriangle$ — $\blacktriangle$, tryptophan oxygenase.

maximal level and optimal induction of tyrosine aminotransferase and tryptophan oxygenase could be observed. However, at a dose of 0.2 μg of dexamethasone per 100g body wt. no significant increase of enzyme activities could be measured (Fig. 6c), whereas at the same dose of [^3H]-dexamethasone specific radioactivities of nuclei and cytosol (Fig. 6a) as well as cytoplasmic concentrations of hormone-protein complexes DE-1, DE-2 and DE-3 were 25–50% of the corresponding maximal levels (Fig. 6b).

Maximal stimulation of RNA polymerase B activity was observed at a dose of 2 μg of dexamethasone per 100g body wt. (Fig. 7). The effects on RNA polymerase activities were, however, too small to determine the dose-response relation below 1 μg of dexamethasone per 100g body wt.

Glucocorticosteroid metabolites present in the different cytoplasmic hormone-protein complexes formed in vivo

Radioactive steroids present in the different cytoplasmic hormone-protein complexes were extracted and analysed by t.l.c. In the cortisol experiments no radioactivity could be extracted from hormone-protein complex DE-3, 5–10% of the radioactivity present in DE-2 and about 60% in the

case of DE-1 and DE-1b. By contrast, almost the whole amount of radioactivity could be extracted from all the different hormone-protein complexes formed *in vivo* after administration of [^3H]-dexamethasone (results not shown). Only radioactive material moving like dexamethasone could be found after t.l.c. of extracts from the different complexes isolated after injection of [^3H]-dexamethasone (results not shown). In the case of cortisol, however, different metabolites, replacing cortisol itself, were found in the different cytoplasmic hormone-protein complexes after administration of biologically active doses of the hormone: 3 ξ ,11 β ,17 α ,20 ξ ,21-pentahydroxypregnanes and various amounts of tetrahydroxypregnanes were found in DE-1, glucuronides in DE-2 and glucuronides plus steroidal carboxylic acids in DE-1b (cf. Voigt *et al.*, 1981). No significant time- or dose-dependence could be observed for DE-1, DE-1b or DE-2 (results not shown).

Discussion

On the basis of dose-response experiments, Beato *et al.* (1972a) have concluded that there is a correlation between formation *in vivo* of the total amount of cytoplasmic hormone-protein complexes and induction by cortisol of tyrosine aminotransferase and tryptophan oxygenase. Comparing the dose-dependence of the formation of the individual cytoplasmic hormone-protein complexes after application *in vivo* of [^3H]-cortisol with the dose-response relation of enzyme induction, we did not observe a correlation between induction of tyrosine aminotransferase or tryptophan oxygenase and saturation *in vivo* of any of the cytoplasmic binding components. Essentially the same results were obtained with dexamethasone, although the differences between binding *in vivo* and enzyme induction were less pronounced than in the case of cortisol. By contrast, a good correlation was observed for dose-dependences of enzyme induction and stimulation of RNA polymerase B. Activity of RNA polymerase B started to increase about 15–20 min after injection of dexamethasone and reached its maximal value after 60 min. This finding is in good agreement with previous studies about the effects of α -amanitin and actinomycin D on the induction process, revealing that the first induced primary transcripts are completed about 25 min after administration of hormone (Voigt *et al.*, 1978). Therefore, the postulated cytoplasmic cortisol-'receptor' complex should be detectable in the range of 5–60 min after administration of biologically active doses of [^3H]-cortisol or [^3H]-dexamethasone. If a significant percentage of the cytoplasmic receptor would enter the nucleus, the cytoplasmic concentration of the glucocorticosteroid-receptor

Fig. 7. Dose-dependence of stimulation of RNA polymerase B activity by dexamethasone

Rat liver nuclei were prepared 30 min after intraperitoneal injection of the amounts of dexamethasone indicated in the Figure. RNA polymerase (A + C) and B activities were determined as described in the text. The values are expressed in c.p.m. of [^3H]-UTP incorporated per μg of DNA and represent mean values of individual determinations in 12 animals $\pm$ S.E.M. Δ --- Δ , Total incorporation of [^3H]-UTP; ∇ --- ∇ , incorporation of [^3H]-UTP in the presence of α -amanitin (i.e. polymerase A + C activities); $\bullet$ --- $\bullet$, RNA polymerase B activity.

complex should be maximal at a time interval shorter than 60 min after injection of the hormone, followed by a decrease due to the postulated translocation into the nucleus, as measured in hepatoma and lymphoblastoma cultures (Rousseau *et al.*, 1973; Gehring & Tomkins, 1974; Yamamoto *et al.*, 1974; Kalimi *et al.*, 1975). In the cortisol experiments, such kinetics were observed for DE-1 and DE-2; in the dexamethasone series of experiments, they were observed only for DE-1. Therefore, these experiments do not support the assumption that DE-2, recently isolated in a very pure form (Govindan & Sekeris, 1978), is the glucocorticosteroid receptor (Beato & Feigelson, 1972; Litwack *et al.*, 1973; Govindan & Sekeris, 1978; Govindan, 1980).

Marković & Litwack (1980) and Munk & Foley (1979) have reported an activation of liver, kidney and thymus glucocorticosteroid-receptor complex to occur *in vivo*. Such an activation process occurring between 5 and 60 min after administration of hormone results in an increase of affinity towards nuclei, DNA and phosphocellulose and a weaker binding to DEAE-Sephadex, such that the non-activated complex is eluted like DE-3, whereas the activated complex is eluted like DE-2. In our experiments, however, we could not detect such an activation process.

In recent years Litwack and co-workers, as well as Feigelson and co-workers, have also described several proteins binding glucocorticosteroids or glucocorticosteroid metabolites in rat liver cytosol that could be separated by DEAE-Sephadex chromatography (Morey & Litwack, 1969; Litwack *et al.*, 1971; Beato & Feigelson, 1972; Litwack *et al.*, 1973; Steeger & Litwack, 1981). Binder II described by Litwack *et al.* (1973) and binder G described by Beato & Feigelson (1972) was expected by each group to be the 'classical' cytoplasmic glucocorticosteroid receptor on the basis of its specificity towards different natural and synthetic glucocorticosteroids. On the basis of its elution point from DEAE-Sephadex, this binding component should correspond to our binder DE-2. Furthermore, our hormone-protein complex DE-1b should correspond to the binder Ib described by Litwack *et al.* (1973), which is interpreted to be a second glucocorticosteroid receptor owing to its binding specificity (Steeger & Litwack, 1981). We, however, have found steroidal carboxylic acid(s) in DE-1b and glucuronides in DE-2 (Voigt *et al.*, 1981; the present paper).

With respect to identification of a possible cytoplasmic glucocorticosteroid receptor in the rat liver, there are two very striking problems, owing to the metabolic function of this tissue: (1) experiments performed at doses of hormone that are biologically inactive are insignificant; (2) synthetic

steroids like dexamethasone and triamcinolone may strongly interact with metabolizing enzymes as substrate analogues without being metabolized (Cleland, 1963) and, owing to their structural similarities and differences compared with the natural steroids, may differentially bind to metabolizing enzymes. Point (2) has been completely overlooked in the literature. Identification of the 'glucocorticosteroid receptor' present in rat liver cytosol has been attempted mainly on the basis of its binding specificity towards biologically active synthetic steroids without studying the effects of these synthetic steroids on the metabolite pattern of the corresponding natural steroid hormones. The necessity of using biologically active doses of hormone in order to look for a receptor has often not been considered in the case of the glucocorticosteroid receptor predicted to be present in rat liver cytosol.

It is known that steroid-protein complexes have the tendency to aggregate with each other and with the nuclei (Bulanyi & Oliver, 1976). Therefore the possibility cannot be excluded that the apparent translocation of hormone-protein complexes observed in some systems is an experimental artifact. On the other hand, it is possible that cytoplasmic binding proteins bind to the nuclear envelope, owing to diffusion of steroids out of the nucleus. In this respect it is noteworthy that the amount of cytoplasmic binding sites measured after administration of [³H]cortisol *in vivo* is much higher than the binding capacity of cytosol measured *in vitro*, although this fact cannot be easily interpreted (Voigt & Sekeris, 1982).

Interpretation of all the present available data about the cytoplasmic binding proteins indicates that a valid conclusion on whether or not there is a cytoplasmic glucocorticosteroid receptor in the rat liver seems to be impossible, particularly owing to the complexity of this system. On the basis of our results, however, we are convinced that all the cytoplasmic binding proteins found so far are enzymes involved in the metabolism of glucocorticosteroids in the rat liver.

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