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# STRUCTURE–ACTIVITY RELATIONSHIPS FOR GLUCOCORTICOIDS—I: DETERMINATION OF RECEPTOR BINDING AND BIOLOGICAL ACTIVITY

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SUMMARY

As a prerequisite to study structure–activity relationships for glucocorticoids, we have exploited a sensitive method to evaluate the glucocorticoid activity of a series of steroid analogues *in vitro*.

Glucocorticoid effects at the target cell level were determined in cultured rat hepatoma (HTC) cells where glucocorticoids increase the activity of tyrosine aminotransferase. Distinction can be made between agonists, antagonists, partial agonists and inactive steroids.

These steroids were also tested for binding to the glucocorticoid receptor in the same system. Irrespective of their class assignment all active steroids interacted with the same receptor sites. Accordingly, the apparent affinity of the nonradioactive analogues for the receptor protein could be accurately determined in competition experiments.

This approach could be useful for evaluating rapidly and quantitatively glucocorticoid activity of natural or synthetic steroid molecules in microgram quantities.

## I. INTRODUCTION

The possibility to design a corticosteroid that can accomplish certain therapeutic goals is not based on an extensive, theoretical physicochemical formulation, but derives from a number of empiricisms [1]. This statement was enunciated at a time when little was known on the mechanism of glucocorticoid hormone action. However, recent progress in two directions makes it possible to reevaluate the problem of structure–activity relationships for glucocorticoids.

First, it is now recognised that most, if not all physiological effects of these hormones result from their specific binding to an intracellular receptor protein, (for a review, see [2]). Thus, by studying this interaction *in vitro*, one could define the domains of the molecule which are critical for hormonal activity proper, independently of the presumably different structural characteristics involved in drug resorption, plasma transport, tissue distribution and metabolism. Obviously, such a distinction is difficult to make when determining glucocorticoid activity in whole animals. This new approach has also been resorted to in the case of other steroid hormones [3]. A second advance might result from better exploitation of the theoretical and experimental methods now available for approaching the complete molecular structure of steroid hormones.

In this series of papers, we present some results of a concerted effort, directed along these lines, to study structure–activity relationships for glucocorticoids at the receptor level. One prerequisite was to describe a family of steroid molecules with respect to both their biological activities at the target cell and their structural characteristics.

On the one hand, we have determined receptor binding as well as the activity of a large number of steroid analogues, using cultured rat hepatoma (HTC) cells as an *in vitro* system. In these cells, binding of glucocorticoids to their receptor protein [4] leads to an increased rate of tyrosine aminotransferase synthesis [5], as observed in rat liver. Moreover, induction of liver tyrosine aminotransferase in the rat can be correlated with thymolytic as well as antiinflammatory activity of glucocorticoids [6]. Contrary to what occurs in the case of the androgen testosterone [7], the natural glucocorticoids cortisol and corticosterone released by the adrenal cortex need not be converted to more active compounds in target cells in order to exert their effects [8–11]. HTC cells, which express much less functions than the differentiated hepatocyte, reportedly lack 11$\beta$-hydroxylase and dehydrogenase activities and do not metabolize glucocorticoids to a significant extent [9, 12]. Further justification of this model stems from the similarity between rat and human glucocorticoid receptors [13, 14]. This part of the study is reported here.

On the other hand, we have assessed and exploited a theoretical approach of molecular structures based on energy optimisation by internal strain relaxation. The method gives access to the geometry of molecules for which no crystallographic data are available. It also allows comparison of steroids in a presumably similar situation. The validity of this approach is discussed in the accompanying paper.

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*Abbreviations*: HTC, hepatoma tissue culture; Tyrosine aminotransferase, L-tyrosine: 2-oxoglutarate aminotransferase (EC 2.6.1.5).



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Based on geometry optimisation, together with other techniques such as nuclear magnetic resonance spectroscopy, a coherent series of glucocorticoid agonists, partial agonists and antagonists has been depicted, including some of their structural characteristics, electron densities and conformational plasticity. These data, which will be presented later on†, should make it possible to study the physico-chemical basis of steroid–receptor interactions resulting in either an active or a biologically inactive complex, and also to present a tentative model of the glucocorticoid receptor binding site.

## II. MATERIALS AND METHODS

(a) *Materials.* Phosphate buffer contained 20 mM sodium-potassium phosphate, pH 7.4, 0.2 mM dithiothreitol and 0.1 mM EDTA. Buffered saline consisted in 150 mM NaCl, 2.5 mM KCl, 8 mM $Na_2HPO_4$, 1.5 mM $KH_2PO_4$, pH 7.45. [$^3$H]-dexamethasone (29 Ci/mmol) was from the Radiochemical Centre, Amersham, U.K. Unlabelled steroids, either purchased from Steraloids, Pawling, N.Y., U.S.A., or generously supplied by Roussel-UCLAF, Romainville, France, were dissolved in absolute ethanol to achieve 1–5 mM steroid. Actual concentration was verified by spectrophotometry on the basis of molar extinction coefficients. Final dilutions were made in phosphate buffer.

(b) *Interaction of steroids with glucocorticoid receptors.* HTC cells, originally from Dr G. M. Tomkins laboratory (clone 4), were grown in suspension to a density of about $8 \times 10^5$ cells/ml as described previously [15]. All following procedures were carried out at 0°, unless mentioned otherwise. The cells were washed three times in a total vol. of buffered saline equivalent to half of the culture, frozen in liquid nitrogen and stored at −20° for a maximum of 6 weeks. Cell pellets were homogenized in two vol. of phosphate buffer with 70 strokes of a Dounce homogeniser (pestle B). The homogenate was centrifuged at 30 000 *g* for 20 min and the supernate further cleared at 200 000 *g* for 60 min to yield the cytosol. This material was used immediately as a source of glucocorticoid receptor. Incubations of cytosol with [$^3$H]-dexamethasone were run in duplicate and, after at least 2 h, the concentration of macromolecule-bound radioactivity was determined by a charcoal adsorption method [15]. Duplicate incubations conducted in parallel with a large excess of unlabelled dexamethasone allowed estimation of nonspecific binding. This was subtracted from total bound steroid to yield receptor-bound steroid concentration. Free steroid concentration was calculated by difference between added steroid and total bound steroid. Minute quantities of steroid are sufficient to perform a test since each experimental point requires no more than a 0.4 ml incubation.

† Manuscripts in preparation.

(c) *Induction of tyrosine aminotransferase.* HTC cells in the log phase of growth were resuspended at a density of about $8 \times 10^5$ cells/ml in serum-free medium containing 0.1% (w/v) bovine serum albumin and 0.1% (w/v) $NaHCO_3$. Ethanol solutions of steroids were added to 10 ml aliquots of cell suspensions, final ethanol concentration not exceeding 0.5% (w/v). After 16 h of incubation in tightly capped flasks on a rotary shaker (100 rev./min) at 37°, the cells were harvested and tyrosine aminotransferase activity was determined as described [9]. Even if this test requires more steroid than the receptor-binding assay, 0.05 mg of drug is still enough for three determinations at the highest concentration (10 μM) necessary to achieve with the low affinity steroids.

(d) *Steroid chromatography.* Steroids were chromatographed in chloroform acetone (70:30, v/v) on Merck No. 60 F254 silica gel thin layer plates at room temperature. This system separates cortisol ($R_f$:0.23) from cortisone ($R_f$: 0.41) and from cortisol acetate ($R_f$: 0.60). [$^3$H]-Cortisol acetate was prepared by acetylation of [$^3$H]-cortisol (New England Nuclear Corp.) in presence of equal vol. of pyridin and acetic anhydride for 3 h at 60°. The acetylated product was purified by t.l.c. in the solvent system just mentioned using nonradioactive cortisol acetate as a marker.

Possible metabolism of cortisone and cortisol acetate under the conditions of steroid-receptor binding assay was investigated by incubating cytosol at 0° in presence of 2.5 μM radioactive (100 000 c.p.m.) steroid in a total vol. of 0.4 ml. After 2 h, steroids were extracted once using 10 vol. of methylene chloride. The organic phase was evaporated to dryness under a stream of air at 37° and the steroids redissolved in ethyl acetate. The samples were chromatographed as described, together with nonradioactive standards. The latter were located with an ultraviolet lamp and radioactivity on the plates was detected using a Berthold scanner. The radioactive spots were scraped off the plates and counted by liquid scintillation spectrometry.

Steroid metabolism in the cultures was examined by incubating radioactive standards at a final concentration of 12 μM with 20 ml of HTC cells under the conditions of tyrosine aminotransferase induction at 37°. Radioactivity was at least 100 000 c.p.m. per ml culture. After 16 h, the cells were centrifuged and the supernatant culture medium was saved. The pellet was washed with phosphate-buffered saline and resuspended in 1 ml of the buffer used for tyrosine aminotransferase assay. The suspension was sonicated (Branson B-12 sonifier) at 70 watt for 30 s. Both the culture medium and the homogenate were extracted twice with two vol. of methylene chloride and processed as above. Radioactivity extracted from the cells amounted to 0.1% of that in the medium.

(e) *Other assays.* Protein was determined by the method of Lowry *et al.*[16], using bovine serum albumin as a standard. Radioactivity was counted by liquid scintillation spectrometry in a mixture contain-

ing 700 ml toluene, 250 ml Triton X-100, 50 ml $H_2O$, 0.1 g POPOP and 5.5 g PPO per l. Counts were systematically converted to moles of steroid, using an external standard system of calibration.

## III. RESULTS AND DISCUSSION

(a) *Affinity of steroid analogues for the glucocorticoid receptor.* Based on dose-response curves for tyrosine aminotransferase in HTC cells, steroids have been classified as optimal, suboptimal and anti-glucocorticoids [9]. Optimal inducers, i.e. pure agonists give rise to the maximum tyrosine aminotransferase activity; suboptimal inducers increase enzyme activity to an intermediate value, whatever their concentration; anti-inducers, i.e. antagonists, are unable to stimulate tyrosine aminotransferase activity but can, at sufficient concentration, decrease enzyme induction by optimal or suboptimal inducers. Within each class, the potency of a given steroid can be estimated from the concentration required for half-maximum effect. Steroids from the three classes all bind to the same glucocorticoid receptor sites [15, 17] and it has been proposed [9, 15] that both the potency and class assignment entirely depend on interaction of the steroid with the receptor.

Thus, the first biological parameter to quantify in our study was the potency, namely the affinity of the various steroids for the receptor, regardless of their class of activity. Since glucocorticoid receptor is not readily available in pure form [18], we used receptor-containing HTC cytosol that has proven suitable for this type of analysis [15, 17].

Because HTC cells used for the present work had not been cloned for some time, it was necessary to verify whether glucocorticoid receptor sites actually belonged to a homogenous population, as originally described [17]. Figure 1 shows the saturation kinetics of the receptor with [$^3$H]-dexamethasone. Non-specific binding amounts to only a small proportion of the total. The graph suggests that specific binding is a hyperbolic function of free dexamethasone concentration. In fact, a Scatchard plot of the data is compatible with a single population of binding sites, confirming earlier work [17]. Consistent with this interpretation, the slope of the Hill plot, $n = 1$, indicates that dexamethasone binding sites are not interacting (Fig. 2). The affinity of dexamethasone for the receptor varied somewhat between different batches of cells. In 20 experiments, the apparent equilibrium dissociation constant at 0° was 5.6 ± 0.5 (S.E.M.) nM.

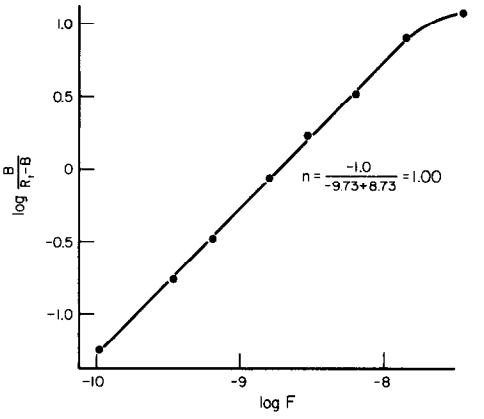


Fig. 2. Hill plot of data presented in Fig. 1. F: free steroid concentration; B: concentration of specifically-bound steroid; $R_T$: total concentration of glucocorticoid receptor sites.

Very few of the steroid analogues tested can be obtained labelled with a radioisotope. We therefore determined the affinity of the unlabelled steroids by competition with [$^3$H]-dexamethasone for receptor binding. Steroids were tested at a constant concentration against four or five different concentrations

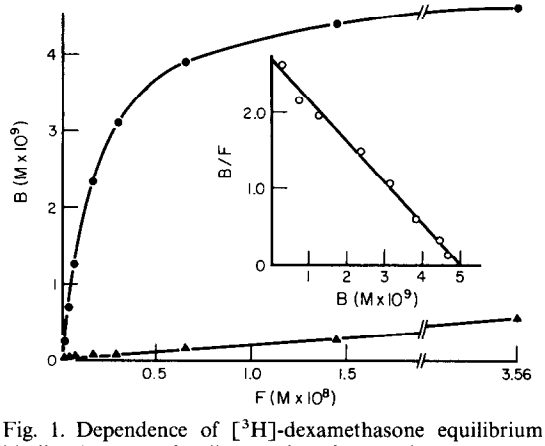


Fig. 1. Dependence of [$^3$H]-dexamethasone equilibrium binding (B) to HTC cell cytosol on free (F) dexamethasone concentration. Nonspecific binding (▲) was subtracted from total binding to yield specific binding to glucocorticoid receptor (●). The inset shows a Scatchard plot of the data.

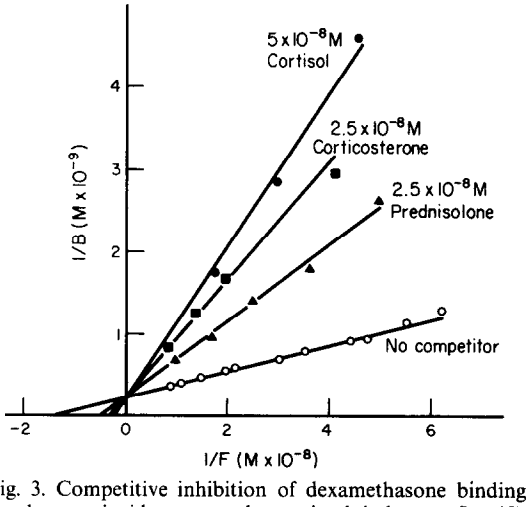


Fig. 3. Competitive inhibition of dexamethasone binding to glucocorticoid receptor by optimal inducers. Specific [$^3$H]-dexamethasone binding to HTC cell cytosol at equilibrium was determined in absence and presence of a constant concentration of non-radioactive competitors as indicated on the figure. Symbols are same as in Fig. 2.

of [$^3$H]-dexamethasone. As a control, a dexamethasone binding curve was also run in the absence of competitor in order to determine the actual affinity of dexamethasone in the particular experiment. This protocol was repeated on four occasions for most steroids, using different batches of cells. Lineweaver-Burk plots of the data were constructed to determine the mechanism of inhibition of dexamethasone binding by each analogue. In all cases, these plots were compatible with competitive inhibition for the same receptor sites. Representative experiments are presented for steroids from the three activity classes, optimal (Fig. 3), suboptimal (Fig. 4) and anti-inducers (Fig. 5). From these graphs the apparent equilibrium dissociation constant $K_i$ at 0° was calculated using the formula:

$$K_i = \frac{K_D \cdot F_i}{K_{app} - K_D}$$

where $K_D$ is the equilibrium dissociation constant of dexamethasone, $K_{app}$ is the apparent affinity of dexamethasone in the presence of competitor and $F_i$ the free concentration of competitor.

Table 1 summarizes the results obtained on 44 steroids. All of them but two contain only—like the natural glucocorticoids—carbon, hydrogen and oxygen atoms because, in its present operational state, the geometry optimisation procedure is not suited for the halogenated steroids. The affinities have been calculated by reference to the mean affinity of dexamethasone ($K_D$ = 5.6 nM), based on the affinity ratios of competitor over dexamethasone in individual experiments. Some of the steroids bear an acetyl group on the $C_{21}$ position. Since this substitution is very susceptible to hydrolysis, we have examined the stability of cortisol acetate under our experimental conditions. The acetyl was not cleaved-off in the presence of cell-free cytosol at 0° in the incubations used for the steroid binding assay. It can be seen in Table 1 that the series comprises the entire spectrum of affinities

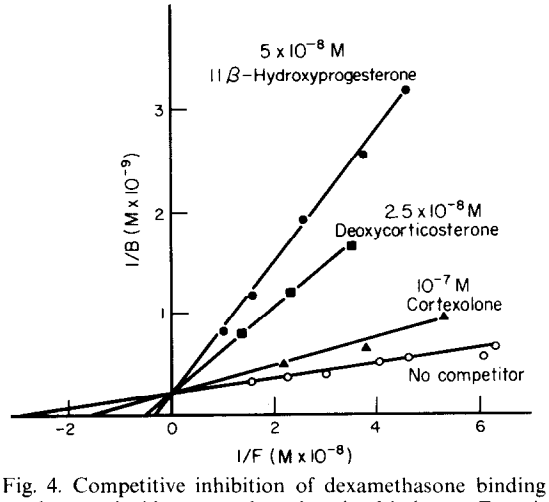


Fig. 4. Competitive inhibition of dexamethasone binding to glucocorticoid receptor by suboptimal inducers. Experiments conducted as described in Fig. 3.

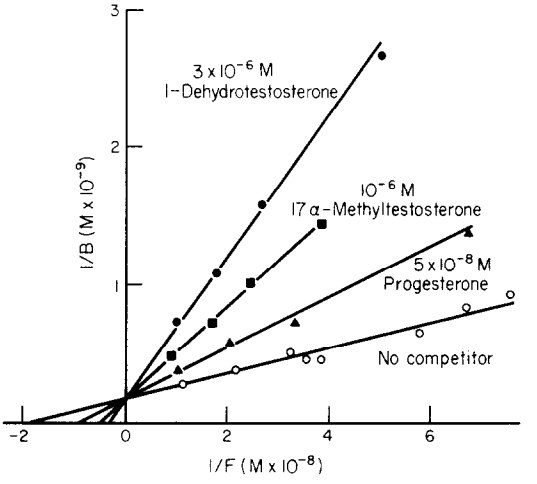


Fig. 5. Competitive inhibition of dexamethasone binding to glucocorticoid receptor by anti-inducers. Experiments conducted as described in Fig. 3.

ranging from steroids with a $K_D$ of about 1 nM down to those with a $K_D$ higher than 10 μM, an interaction too weak to be accurately determined in our assay. A few of the steroids tested had already been examined using the same system [13, 15, 17]. Their relative affinities reported here are in agreement with these earlier studies.

b. *Activity of steroid analogues for tyrosine aminotransferase induction.* As said above, a given steroid belongs to one of four glucocorticoid activity classes, if one includes that of inactive glucocorticoids. The latter are steroids which do not influence tyrosine aminotransferase activity, although they may display non-glucocorticoid hormonal actions in other systems. Thus, inactive glucocorticoids can be identified by their lack of affinity for the HTC receptor. It is noteworthy that the affinity for the receptor determined at 0° in cell-free cytosol is about three-fold higher than at 37° in the intact cell [7]. As mentioned, the *in vitro* system used here allows detection of weak interactions, beyond the range of biological relevance (Table 1). Given the physiological concentrations of free glucocorticoids in plasma, about 10–50 nM, one can consider that steroids with a $K_i$ (0°) above 2000 nM belong to the class of inactive glucocorticoids. Indeed, this $K_i$ would correspond to a dissociation constant of about 6 μM at 37°. By virtue of the law of mass action, the concentration of such steroids required to saturate over 90% of the receptors would be 60 μM, that is three to four orders of magnitude higher than is physiological. This conclusion would still be valid even if the increase in affinity with temperature alluded to above were less pronounced for steroids other than dexamethasone. Actually, none of these steroids (compounds XXXV through XLIV in Table 1) influence tyrosine aminotransferase activity at 10 μM.

On the other hand, assigning active glucocorticoids to one of the three other classes requires quantitative determination of their effect on tyrosine aminotrans-

ferase activity. Table 1 shows the induction of the enzyme above basal levels obtained with 10 $\mu$M analogue. The degree of induction should be commensurate with the extent of receptor saturation, unless the steroid is a suboptimal or an anti-inducer. Thus, a 10 $\mu$M concentration of compounds I–XXVIII is five times or more higher than their postulated $K_i$ at 37° and, therefore, sufficient for occupation of more than 80% of the receptor sites. This concentration would be in even greater excess over $K_i$ for steroids characterized by a smaller influence of temperature on affinity than is the case for dexamethasone. Thus, for these compounds, class assignment can be directly deduced from the extent of induction of tyrosine aminotransferase.

Among steroids XXIX–XXXIV, those which do not influence enzyme activity are anti-inducers since their affinity is such that a sizable proportion of receptor sites is occupied at 10 $\mu$M steroid. The lack of enzyme induction by steroids classified as anti-inducers was not due to their inability to penetrate the cells. Indeed, each of these steroids at 10 $\mu$M effectively inhibited induction of tyrosine aminotransferase by 25 nM dexamethasone (data not shown). For compounds which lead to increased tyrosine aminotransferase activity, Table 1 shows that induction is not proportional to the expected saturation of receptor sites: they should be considered as suboptimal inducers.

In general, classification according to the induction data is consistent with earlier findings [9, 15] concerning some of the steroids studied here. It is clear that high affinity for the receptor is not the exclusive of optimal inducers. Some antagonists have a higher affinity than partial agonists. Borderline situations apply to compounds XIII and XXII. Aldosterone (XIII), which has been considered as a weak optimal inducer [9] has been classified here as a suboptimal glucocorticoid. However, its biological effect can be as high as 91% of that of dexamethasone (Table 1). Compound XXII falls here in the category of antagonists, while it has been found to produce 5% of the effect of cortisol [9].

Some reservations have to be made, however, concerning the five acetylated steroids. Although these steroids are stable in cytosol at 0°, as mentioned, hydrolysis of the C21 acetyl group does take place in cell cultures at 37° under conditions of induction of tyrosine aminotransferase. We found that, after overnight incubation, more than 90% of the cortisol acetate in the medium and about 70% within the cells have been converted to cortisol. Actually, this conversion does not result from cellular metabolism since the same extent of hydrolysis in induction medium is found when the overnight incubation at 37° is conducted in the absence of cells. Therefore, the affinities reported correspond to the acetylated form of these compounds while the activities presumably reflect the interaction of the non-acetylated derivative. From earlier work [15, 17], it is likely that the presence of the acetyl group decreases the affinity for the receptor. Indeed, contrary to compound XXXIII, the affinity of 5$\alpha$-dihydrocortisol is reportedly at least as strong as that of 17$\alpha$-methyltestosterone (XXVI) or of 17$\alpha$-hydroxyprogesterone (XXII). The difference is even more striking between 5$\beta$-dihydrocortisol and compound XXXIX since the affinity of the former appears to be even higher than that of 5$\alpha$-dihydrocortisol [15]. Consequently, in the non-acetylated form, compounds XV, XXIV and XXXIII may be more potent antagonists or partial agonists than suggested by Table 1, while compounds XXXIX and XLI may be antagonists.

Another anomaly was noted concerning tyrosine aminotransferase induction by compound XXVIII, which is considered to be either an inactive steroid [8] or a very weak antagonist of glucocorticoids [9]. It was suspected that reduction of the 11-keto group could take place as has been reported to occur in fetal rabbit lung [10]. As described in *Methods*, the possible metabolism of [$^3$H]-cortisone was examined under conditions of both receptor binding and enzyme induction. Cortisone was not metabolized by cell-free cytosol at 0°. However, after overnight incubation with intact cells, 40% of this steroid had been converted to cortisol and 20% to slowly migrating unidentified metabolites, while the remaining 40% still corresponded to intact cortisone. This conversion did not occur in absence of cells. Thus, unlike other clones of HTC cells [9] the cells used here possess some 11-reductase activity. Therefore, induction of tyrosine aminotransferase by compounds XXVIII, XXXII and XXXIV is very likely due to the presence of sufficient concentrations of the optimal 11-hydroxy derivative of these steroids.

c. *Effects of substitutions in the steroid molecule on receptor binding and tyrosine aminotransferase induction.* Each of all but three of the steroids tested (XV, XIX and XLIV) differs only by one single substitution from another steroid in the series. The consequences of these changes on both the affinity for the receptor and the biological activity are shown in Table 2. Some substitutions, such as 5$\beta$-H, 11-keto, 17$\alpha$-OH and 21-deoxy consistently reduce the affinity for the receptor. Others, like 1-$CH_3$, 11$\alpha$-OH and reduction of the 20-ketone group are incompatible with binding and yield inactive steroids. Receptor binding may also be increased as in the case of 6$\alpha$-$CH_3$ and 17$\alpha$-$CH_3$ substitutions. A change for an 11$\beta$-OH either increases the affinity of steroids derived from the pregnane series or decreases binding of steroids derived from the androstane series. On the other hand, the activity class may or may not be affected. Examples of the latter case are given by substitutions on C17 and desaturation of the C1-C2 bond. These results are in keeping with an earlier but more limited study [15].

Obviously, it would be naive to attempt at drawing correlations at this stage between such chemical changes and biological activity. The consequences of

Table 1. Biological constants of glucocorticoid analogues in cultured hepatoma (HTC) cells

| No. | Steroids: Systematic name | Trivial name | Affinity (nM)* Mean | Affinity (nM)* Range | Activity† Mean | Activity† Range | Class |
|---|---|---|---|---|---|---|---|
| I | 9α-Fluoro-16α-methyl-11β, 21-dihydroxy-1,4-pregnadiene-3,20-dione | Desoxymethasone | 0.82 | 0.34 → 1.3 | 88 | 80 → 96 | O |
| II | 9α-Fluoro-16α-methyl-11β, 17α, 21-trihydroxy-1,4-pregnadiene-3, 20-dione | Dexamethasone | 5.6 | 1.9 → 8.8 | 100 | 88 → 116 | O |
| III | 11β, 21-Dihydroxy-4-pregnene-3, 20-dione | Corticosterone | 7.0 | 6.4 → 7.4 | 85 | 80 → 94 | O |
| IV | 6α-Methyl-11β, 17α, 21-trihydroxy-4-pregnene-3, 20-dione | 6α-Methylcortisol | 8.3 | 6.1 → 104 | 100 | 94 → 104 | O |
| V | 21-Hydroxy-4-pregnene-3, 20-dione | 11-Deoxycorticosterone | 9.1 | 4.1 → 14.1 | 31 | 28 → 33 | S |
| VI | 11β-Hydroxy-4-pregnene-3, 20-dione | 11β-Hydroxyprogesterone | 10.3 | 10.0 → 10.6 | 9 | 6 → 12 | S |
| VII | 11β, 17α, 21-Trihydroxy-4-pregnene-3, 20-dione | Cortisol | 10.5 | 5.0 → 18.1 | 111 | 101 → 119 | O |
| VIII | 11β, 17α, 21-Trihydroxy-1, 4-pregnadiene-3, 20-dione | Prednisolone | 12.4 | 9.5 → 15.3 | 112 | 109 → 114 | O |
| IX | 11β, 17α, 21-Trihydroxy-1, 4, 7-pregnatriene-3, 20-dione | 7-Dehydroprednisolone | 19.1 | 12.2 → 26.1 | 62 | 55 → 64 | S |
| X | 1,4-Pregnadiene-3, 20-dione | 1-Dehydroprogesterone | 20.7 | 12.2 → 29.2 | 0.2 | −1.3 → 1.0 | A |
| XI | 11β, 16α, 17α, 21-Tetrahydroxy-4-pregnene-3, 20-dione | 16α-Hydroxycortisol | 30.5 | 17.2 → 43.8 | 61 | 52 → 71 | S |
| XII | 6α, 16α-Dimethyl-4-pregnene-3, 20-dione | 6α, 16α-Dimethyl-Progesterone | 32.3 | 26.6 → 37.9 | 15 | 14 → 16 | S |
| XIII | 11β, 21-Dihydroxy-18-al-4-pregnene-3, 20-dione | Aldosterone | 40.1 | 23.6 → 49.4 | 71 | 65 → 80 | S |
| XIV | 4-Pregnene-3, 20-dione | Progesterone | 51.6 | 31.2 → 72 | 2.5 | 2.3 → 2.7 | A |
| XV | 17α, 21-Dihydroxy-1, 4, 9(11)-pregnatriene-3, 20-dione (21-acetate) | 1,9 Dehydrocortexolone (acetate) | 53.7 | 31.3 → 76.2 | 8.1 | 7.0 → 10 | S |
| XVI | 11β, 17α, 21-Trihydroxy-4, 6-pregnadiene-3, 20-dione | 6-Dehydrocortisol | 54.8 | 23.6 → 86 | 52 | 48 → 56 | S |
| XVII | 16α-Methyl-17α, 21-dihydroxy-4-pregnene-3, 20-dione | 16α-Methylcortexolone | 61.3 | 43.8 → 78.8 | 14 | 12 → 17 | S |
| XVIII | 16α-Methyl-4-pregnene-3, 20-dione | 16α-Methylprogesterone | 80.1 | 60.3 → 119 | 0 | −4 → 2 | A |
| XIX | 6α-Methyl-4, 16-pregnadiene-3, 20-dione | 6α-Methyl-16-dehydroprogesterone | 88.7 | 69.8 → 108 | 0.6 | 0.2 → 1 | A |
| XX | 17α, 21-Dihydroxy-4-pregnene-3, 20-dione | Cortexolone | 128 | 74.5 → 181 | 14 | 13 → 15 | S |
| XXI | 16α, 17α-Dihydroxy-4-pregnene-3, 20-dione | 16α, 17α-Dihydroxyprogesterone | 180 | 130 → 230 | 1.2 | 0.6 → 1.5 | A |
| XXII | 17α-Hydroxy-4-pregnene-3, 20-dione | 17α-Hydroxyprogesterone | 192 | 138 → 245 | 1.4 | 0.0 → 1.8 | A |
| XXIII | 17α-Methyl-17β-hydroxy-1,4-androstadiene-3-one | 17α-Methyl-1-dehydro-testosterone | 304 | 283 → 326 | 0.6 | 0.2 → 0.9 | A |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| XXIV | 16α, 21-Dihydroxy-4-pregnene-3, 20-dione (21-acetate) | 16α-Hydroxydeoxycorticosterone (acetate) | 318 | 267 → 368 | 0.2 | −0.6 → 1.1 | A |
| XXV | 21-Hydroxy-5β-pregnane-3, 20-dione | 5β-Dihydrodeoxycorticosterone | 419 | 329 → 509 | 0.4 | 0.2 → 0.7 | A |
| XXVI | 17α-Methyl-17β-hydroxy-4-androstene-3-one | 17α-Methyltestosterone | 429 | 360 → 498 | 0.1 | −1 → 1.3 | A |
| XXVII | 16α-Methyl-17α-hydroxy-4-pregnene-3, 20-dione | 16α-Methyl-17α-hydroxyprogesterone | 586 | 469 → 703 | 0 | −1 → 1 | A |
| XXVIII | 17α, 21-Dihydroxy-4-pregnene-3, 11, 20-trione | Cortisone | 631 | 521 → 740 | 61 | 55 → 66 | (S) |
| XXIX | 14α, 17α, 21-Trihydroxy-4-pregnene-3, 20-dione | 14α-Hydroxycortexolone | 692 | 552 → 831 | 0.4 | 0 → 0.6 | A |
| XXX | 17β-Hydroxy-4-androstene-3-one | Testosterone | 695 | 631 → 759 | 0 | 0 → 0 | A |
| XXXI | 17β-Hydroxy-1,4-androstadiene-3-one | 1-Dehydrotestosterone | 869 | 579 → 1286 | 0.1 | −2 → 0.7 | A |
| XXXII | 21-Hydroxy-4-pregnene-3, 11, 20-trione | 11-Dehydrocorticosterone | 919 | — | 31 | 26 → 37 | (S) |
| XXXIII | 11β, 17α, 21-Trihydroxy-5α-pregnane-3, 20-dione (21-acetate) | 5α-Dihydrocortisol (acetate) | 932 | 642 → 1216 | 11 | 7.8 → 14 | S |
| XXXIV | 17α, 21-Dihydroxy-1,4-pregnadiene-3, 11, 20-trione | Prednisone | 1019 | — | 42 | 34 → 51 | (S) |
| XXXV | 17-Keto-4-androstene-3-one | Androstenedione | 2060 | — | 1.6 | 0.7 → 2.6 | i |
| XXXVI | 11β-Hydroxy-17-keto-4-androstene-3-one | 11β-Hydroxyandrostenedione | 2200 | — | 1.3 | 0.9 → 1.7 | i |
| XXXVII | 11α-Hydroxy-4-pregnene-3, 20-dione | 11α-Hydroxyprogesterone | 2231 | 1628 → 2835 | 0.4 | 0 → 0.6 | i |
| XXXVIII | 16β-Methyl-4-pregnene-3, 20-dione | 16β-Methylprogesterone | 2401 | 2313 → 2489 | 1.2 | 0.8 → 2.3 | i |
| XXXIX | 11β, 17α, 21-Trihydroxy-5β-pregnane-3, 20-dione (21 acetate) | 5β-Dihydrocortisol (acetate) | 3035 | 1495 → 4574 | 2.1 | 0.5 → 3.5 | (i) |
| XL | 11β, 17β-Dihydroxy-4-androstene-3-one | 11β-Hydroxytestosterone | 3231 | 1630 → 4051 | −0.2 | −0.8 → −0.4 | (i) |
| XLI | 21-Hydroxy-4, 16-pregnadiene-3, 20-dione (21 acetate) | 16-Dehydrodeoxycorticosterone (acetate) | 7973 | 3893 → 12053 | 1.3 | 0.5 → 1.8 | i |
| XLII | 1-Methyl-11β, 17α, 21-trihydroxy-1,4-pregnadiene-3, 20-dione | 1-Methylprednisolone | 8144 | — | 1.4 | 0.7 → 1.7 | i |
| XLIII | 20α-Hydroxy-4-pregnene-3-one | 20α-Hydroxyprogesterone | >10000 | — | 1.1 | −1.2 → 2.5 | i |
| XLIV | 3α, 11β, 21-Trihydroxy-5β-pregnane-20-one | Tetrahydrocorticosterone | >10000 | — | 2 | 1.5 → 2.1 | i |

* Equilibrium dissociation constant (0°) of steroid–receptor interaction determined in HTC cell cytosol by competition with [$^3$H]-dexamethasone, as described in the text.
† Based on four determinations of the effect of $10^{-5}$ M steroid on tyrosine aminotransferase activity in HTC cells at 37°.

Data are in percent of steady-state induction (61–84 mU/mg protein) over basal level (3.0–3.8 mU/mg protein) obtained with dexamethasone. Activity classes are symbolized as follows: O: optimal inducers; S: suboptimal inducers; A: anti-inducers: i: inactive glucocorticoids. Classifications in parentheses are discussed in the text.

Table 2. Effect of substitutions in the steroid molecule on receptor binding and glucocorticoid activity

| Carbon | Substitutions Before (A) | After (B) | Steroids (A) | (B) | Affinity (A) | (B) | Activity (A) | (B) |
|---|---|---|---|---|---|---|---|---|
| 1 | —H | —$CH_3$ | VIII | XLII | 12.4 | 8144 | O | i |
| 1,2 | —H,H | dehydro | VII | VIII | 10.5 | 12.4 | O | O |
| | | | XIV | X | 51.6 | 20.7 | A | A |
| | | | XXVI | XXIII | 429 | 304 | A | A |
| | | | XXX | XXXI | 695 | 839 | A | A |
| 4,5 | dehydro | 5β-H | V | XXV | 9.1 | 419 | S | A |
| 5 | α-H | β-H | XXXIII | XXXIX | 932 | 3035 | S | (i) |
| 6 | α-H | α-$CH_3$ | VII | IV | 10.5 | 8.3 | O | O |
| | | | XVIII | XII | 80.1 | 32.3 | A | S |
| 6,7 | —H,H | dehydro | VII | XVI | 10.5 | 54.8 | O | S |
| 7,8 | —H,H | dehydro | VIII | IX | 12.4 | 19.1 | O | S |
| 11 | β-H | β-OH | V | III | 9.1 | 7.0 | S | O |
| | | | XIV | VI | 51.6 | 10.3 | A | S |
| | | | XX | VII | 128 | 10.5 | S | O |
| | | | XXX | XL | 695 | 3231 | A | i |
| | | | XXXV | XXXVI | 2060 | 2200 | i | i |
| | $H_2$ | =O | XX | XXVIII | 128 | 631 | S | A* |
| | α-H | α-OH | XIV | XXXVII | 51.6 | 2231 | A | i |
| | β-OH, α-H | =O | III | XXXII | 7.0 | 919 | O | A* |
| | | | VII | XXVIII | 10.5 | 631 | O | A* |
| | | | VIII | XXXIV | 12.4 | 1019 | O | A* |
| | β-OH | α-OH | VI | XXXVII | 10.3 | 2231 | S | i |
| 14 | α-H | α-OH | XX | XXIX | 128 | 692 | S | A |
| 16 | α-H | α-OH | VII | XI | 10.5 | 30.5 | O | S |
| | | | XXII | XXI | 192 | 180 | A | A |
| | α-H | α-$CH_3$ | XIV | XVIII | 51.6 | 80.1 | A | A |
| | | | XX | XVII | 128 | 61.3 | S | S |
| | | | XXII | XXVII | 192 | 586 | A | A |
| | β-H | β-$CH_3$ | XIV | XXXVIII | 51.6 | 2401 | A | i |
| | α-$CH_3$ | β-$CH_3$ | XVIII | XXXVIII | 80.1 | 2401 | A | i |
| 16,17 | 16α-OH | dehydro | XXIV | XLI | 318 | 7973 | A | (i) |
| 17 | α-H | α-OH | I | II | 0.82 | 5.6 | O | O |
| | | | III | VII | 7.0 | 10.5 | O | O |
| | | | V | XX | 9.1 | 128 | S | S |
| | | | XIV | XXII | 51.6 | 192 | A | A |
| | | | XVIII | XXVII | 80.1 | 586 | A | A |
| | α-H | α-$CH_3$ | XXX | XXVI | 695 | 429 | A | A |
| | | | XXXI | XXIII | 869 | 304 | A | A |
| | α-H, β-OH | =O | XXX | XXXV | 695 | 2060 | A | i |
| 18 | $H_2$ | =O | III | XIII | 7.0 | 40.1 | O | S |
| 20 | =O | α-OH, β-H | XIV | XLIII | 51.6 | >10000 | A | i |
| 21 | —OH | —H | III | VI | 7.0 | 10.3 | O | S |
| | | | V | XIV | 9.1 | 51.6 | S | A |
| | | | XVII | XXVII | 61.3 | 586 | S | A |
| | | | XX | XXII | 128 | 192 | S | A |

Data and symbols are from Table 1. *Unmetabolized 11-ketone substitution confers anti-inducer activity [9, 15].

any substitution must be weighed in terms of geometric and electronic modifications in the steroid molecule. A given substitution needs not result in identical modifications in two different steroid molecules. This will be analyzed in greater detail in the second part of this study.

In conclusion, we have exploited an *in vitro* method for evaluating glucocorticoid activity of drugs at the target cell level. This approach emphasizes the distinction to be made between affinity for the receptor and activity of the complex in terms of the biological effects it elicits. The method is sensitive enough to detect differences in affinity over more than four orders of magnitude for the equilibrium dissociation constants. These constants give the apparent affinity, unlike the relative affinities expressed in percentage with reference to a particular concentration of an arbitrary steroid. Thus, any new steroid can be added to the series in the future. Finally, the molecules tested need not be in radioactive form and minute amounts are sufficient for a quantitative and rapid assay.

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