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# IS THE GLUCOCORTICOID RECEPTOR IDENTICAL IN VARIOUS TARGET ORGANS?

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SUMMARY

Although glucocorticoid receptors have been extensively studied in a variety of tissues, it is uncertain whether the receptors in all sites are identical or merely similar. To examine this point, the binding properties of cytoplasmic receptors from rat kidney, thymus and white adipose tissue were compared. Scatchard analysis of [$^3$H]-dexamethasone binding at 0°C showed the apparent $K_{diss}$ for all three receptors to be ~5 nM. In a series of competitive binding assays, the relative affinity of a variety of steroids for each receptor was found to be equivalent. The sequence of potency was dexamethasone > corticosterone > progesterone > cortexolone > testosterone > oestradiol. Non-steroidal anti-inflammatory drugs also compete for dexamethasone binding sites; competitive binding studies with a variety of non-steroidal agents showed roughly equivalent binding to glucocorticoid receptors in the three tissues. The data suggest that the steroid binding site is functionally identical in glucocorticoid receptors from various target organs.

## INTRODUCTION

Glucocorticoid receptors have been demonstrated in a multitude of organs in several species [for reviews see 1–4]. Although an extensive literature exists on specific receptors in each target organ, there are few studies in which glucocorticoid receptors from different organs have been directly compared [5, 6, 7]. In general, the data obtained by different groups, each usually examining a given target organ, suggest that receptors in the various target organs are similar. There are, however, sufficient reports of differences between organs that the issue of whether receptors in the various organs are identical, or merely similar, remains open [1]. Since the question has important ramifications, a direct comparison of the binding properties of the receptors from several target organs in the same species employing the same techniques was undertaken. The aim of the study was to evaluate the steroid binding site of the glucocorticoid receptors in rat kidney, thymus and white adipose tissue by examining the ability of a wide variety of compounds to compete for [$^3$H]-dexamethasone binding sites in cytosol. These tissues were selected because our laboratory has had prior experience working with them [8–10], because the tissues are of diverse embryological origin, and finally because they represent different kinds of glucocorticoid action—anabolic, catabolic, and "permissive". The data indicate an apparent identity of the binding site in the receptors examined.

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## MATERIALS AND METHODS

[1,2(n), $^3$H]-Dexamethasone 26 Ci/mmol was purchased from Amersham/Searle (Arlington Heights, IL). Corticosterone, progesterone and testosterone were purchased from Calbiochem (La Jolla, CA), 17$\beta$-oestradiol and cortexolone (11-deoxy-cortisol) from Steraloids (Wilton, NH) and aspirin from Sigma (St. Louis, MO). The following drugs were generously donated by the respective companies: indomethacin (IDM) (1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid), desmethyl IDM (1-(*p*-chlorobenzoyl)-2-methyl-5-hydroxy-3-indolylacetic acid), desbenzoyl IDM (2-methyl-(3-carboxymethyl)-5-methyoxy indole), MK 410 (1-p-methylthiobenzyl-2-methyl-5-methoxy-3-indolyl-$\alpha$-propionate sodium salt), and dexamethasone (9-fluoro-11$\beta$, 17, 21-trihydroxy-16$\alpha$-methyl-1, 4-pregnadiene, 3, 20-dione) by Merck Sharp and Dohme (West Point, PA); SC 19220 (1-acetyl-2-(8-chloro-10, 11-dihydrodibenz-(b, f) (1,4) oxazephine-10-carbonyl hydrazine) by G. D. Searle (Chicago, IL); ibuprofen (2-(*p*-isobutylphenyl)-propionic acid) by UpJohn (Kalamazoo, MI); phenylbutazone (4-butyl-1, 2-diphenyl-3, 5-pyrazolidine-dione) and sulfinpyrazone (1,2-diphenyl-4-(2′-phenylsulfinethyl)-3,5-pyrazolidinedione) by CIBA/Geigy (Summit, NJ); naproxen ((+)-6-methoxy-$\alpha$-methyl-2-naphthalene acetic acid) by Syntex (Palo Alto, CA); meclofenamic acid (N-(2,6-dichloro-M-tolyl) anthranilic acid) by Parke, Davis (Ann Arbor, MI); 5,8,11,14 eicosatetraynoic acid by Hoffman LaRoche, (Nutley, NJ); and polyphloretin phosphate by A.B. Leo (Helsingborg, Sweden). All drugs were used without further purification. The drugs were dissolved as described

by the manufacturers either in alcohol or base. Final alcohol concentration was always less than 1% and pH of solutions was always titrated back to 7.4 before use. Solutions of drugs were made fresh daily.

Male Sprague–Dawley rats of body weight 100–200 g were purchased from Simonsen (Gilroy, CA). The rats were adrenalectomized and maintained on 0.9% saline drinking water until used (3–10 days after adrenalectomy). Details of the binding methods have been published previously [8–11]. In brief, rats were killed by decapitation, the viscera perfused with iced saline to wash out blood, and the kidneys, thymus and epididymal fat pads removed. The kidneys were decapsulated, the medulla and papilla discarded and cytosol prepared from cortex. The thymic capsule was peeled off, along with any adherent lymph nodes; epididymal fat pads were washed free of blood and used *in toto*. Tissues were homogenized in 0.25M sucrose, 10mM monothioglycerol, 1.5 mM EDTA, 10mM Tris pH 7.4, and cytosol prepared by centrifugation at 100,000 *g* for 1 h. Aliquots of cytosol were incubated with 13nM [$^3$H]-dexamethasone ± competitors at 0°C for 3 h. Protein bound [$^3$H]-dexamethasone was separated from free steroid by passing cytosol through G-50 fine Sephadex columns as previously described. "Non-specific" binding was assessed in all experiments by incubating parallel samples with 1000-fold unlabeled dexamethasone and subtracting that binding resistant to this treatment from total binding to give specific binding.

## RESULTS

The same technique, buffers, and usually the same animals were employed to prepare cytosol from the three target tissues. Pilot experiments indicated that a 3 h incubation at 0°C with [$^3$H]-dexamethasone allowed evaluation of maximal steady-state binding in the three cytosols being studied (Fig. 1). Considerable loss of binding occurred by 24 h. Table 1 summarizes the results of data for each cytosol as determined by linear regression analysis of Scatchard plots. Illustrative data for adipose tissue and kidney have recently been published [9, 10]. In the three tissue cytosols the plots were linear, indicating a single class of non-interacting sites. The apparent $K_{diss}$ was very similar in the three tissues, and well within the range of error of the method. The $N_{max}$ (extrapolation to the abscissa) was lowest in WAT, slightly higher in kidney and very much higher in thymus. The $N_{max}$ values given in Table 1 represent the concentration of receptors per mg of cytosol protein; since each

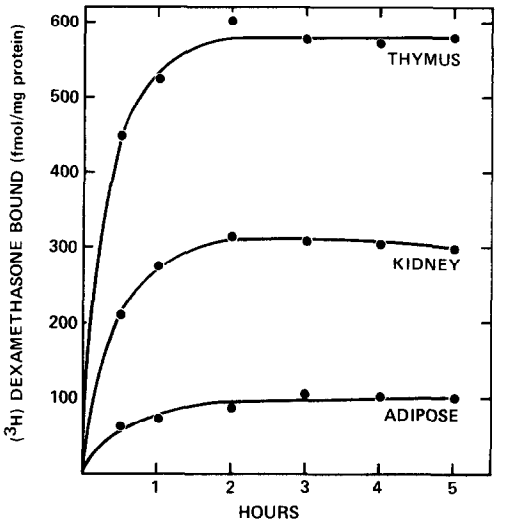


Fig. 1. Time course of specific [$^3$H]-dexamethasone binding in cytosol from kidney, thymus and adipose tissue. Cytosols were incubated with 25 nM [$^3$H]-dexamethasone ± 1000-fold unlabeled dexamethasone for various time intervals. Total binding was determined by Sephadex chromatography and non-specific binding subtracted. Values are the means of duplicate measurements.

tissue is heterogeneous, probably with different proportions of receptor and non-receptor containing cells, the values cannot be interpreted as indicating the actual number of receptors per cell.

An additional means of evaluating the three receptors was to determine the relative affinity of each receptor for a variety of steroid hormones. The results of these competitive binding analyses are shown in Table 2. Increasing concentrations of unlabeled steroids (1-fold to 1000-fold) were used to prepare dose-dependent competition curves. The relative affinity was determined by comparing the concentration of competitor steroid required to achieve 50% inhibition of [$^3$H]-dexamethasone binding to the concentration of unlabeled dexamethasone required to achieve 50% inhibition of binding. The profiles of the steroid affinities are very similar in each tissue—corticosterone is slightly less potent than dexamethasone, progesterone is quite active but considerably less potent than corticosterone, cortexolone exhibits a low affinity for the receptors and both testosterone and oestradiol possess only minor binding activity. Some published data indicates different relative potencies for some of these steroids for any given receptor but the conditions of the experiments differed [1].

In order to expand the range of compounds used to characterize the receptors by competitive analysis,

Table 1. A comparison of [$^3$H]-dexamethasone binding in kidney, thymus and adipose tissue cytosol by Scatchard analysis

| Property | Kidney | Thymus | Adipose tissue |
|---|---|---|---|
| Apparent $K_{Diss}$ 0°C (nM) | 4.4 ± 1.0 | 4.5 ± 1.3 | 6.0 ± 1.0 |
| $N_{max}$ (fmol/mg protein) | 246 ± 36 | 996 ± 171 | 199 ± 6 |

Values are the means ± S.E. calculated from at least 3 separate Scatchard plots.

Table 2. Relative affinities of a variety of steroids for glucocorticoid receptors

| Steroid | Kidney | Thymus | Adipose |
|---|---|---|---|
| | (%) | (%) | (%) |
| Dexamethasone | 100 | 100 | 100 |
| Corticosterone | 80 | 80 | 70 |
| Progesterone | 50 | 39 | 42 |
| Cortexolone | 7 | 4 | 7 |
| Testosterone | <1 | <1 | <1 |
| Oestradiol | <1 | <1 | <1 |

Of the steroids tested dexamethasone had the highest affinity and is set at 100%. Other values are expressed as a percent of dexamethasone affinity. Values are calculated from dose response curves employing the concentration required to achieve 50% inhibition of binding.

we took advantage of our recent finding that non-steroidal anti-inflammatory drugs (NSAID) also inhibit [$^3$H]-dexamethasone binding to glucocorticoid receptors [11]. Figs. 2–4 depict the binding profiles obtained in kidney, thymus and adipose tissue cytosols employing a series of NSAID as competitors. Although not identical the similarity of the patterns is quite striking.

Table 3 lists the results of competition studies using a variety of NSAID, NSAID analogues and prostaglandin antagonists. Although these data are single-point analyses at arbitrary concentrations, the uniformity of the results in each cytosol is apparent. Only polyphloretin phosphate failed to show consistent results in the 3 cytosols being more potent in adipose tissue than kidney or thymus. However, in mixing experiments using heat killed adipose cytosol, the ability of polyphloretin phosphate to compete for receptors in kidney and thymus cytosols was improved to 33% and 20% respectively. These data suggest that this difference is probably a result of environmental factors in adipose cytosol rather than a true difference in the receptor binding sites.

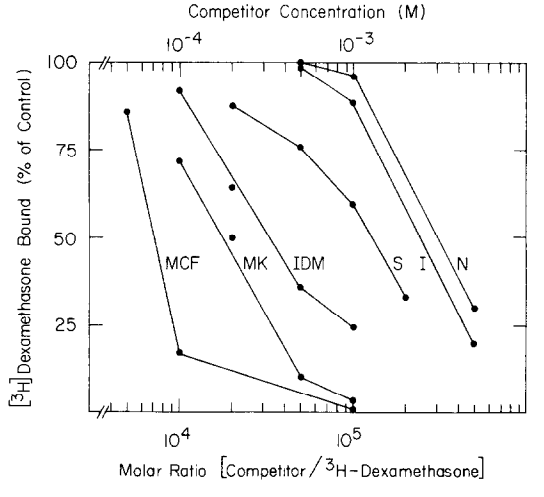


Fig. 2. NSAID Competition for glucocorticoid receptors in kidney cytosol. Cytosol was incubated with 10 nM [$^3$H]-dexamethasone ± competitors for 3 h at 0°C and specific binding determined. Total specific binding in the absence of competitor was taken as 100% and averaged 224 ± 10 fmol/mg protein (mean ± S.E.). Each point is the mean of at least 3 determinations. The symbols are: MCF, meclofenamic acid; MK, MK-410; IDM, indomethacin; S, sulfinpyrazone; I, ibuprofen; N, naproxen.

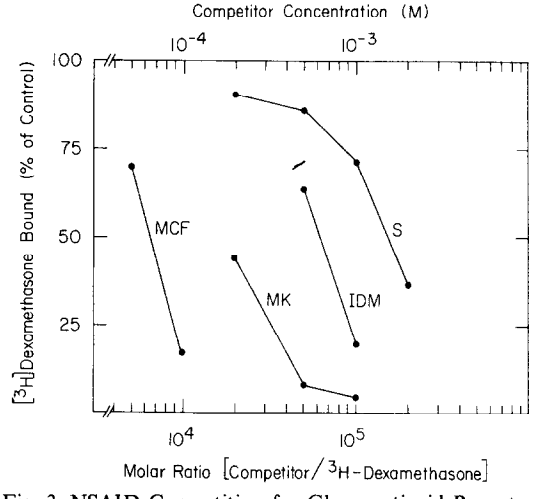


Fig. 3. NSAID Competition for Glucocorticoid Receptors in Thymus Cytosol. Details as in Fig. 2. Total specific binding in the absence of competitor was 714 ± 10 fmol/mg protein.

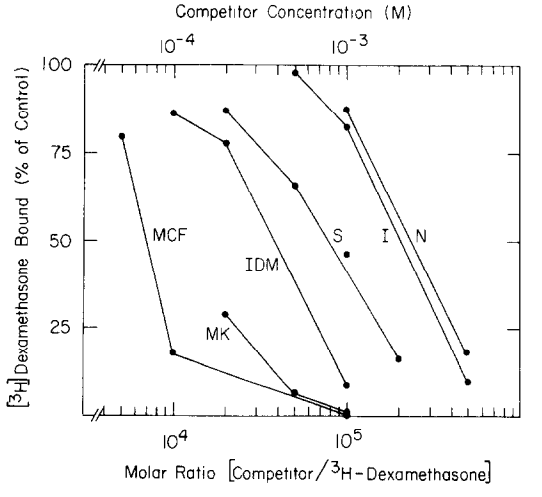


Fig. 4. NSAID Competitor for glucocorticoid receptors in white adipose tissue cytosol. Details as in Fig. 2. Total specific binding in the absence of competitor was 202 ± 16 fmol/mg protein.

Table 3. Ability of a variety of non-steroidal drugs to compete for [$^3$H]-dexamethasone binding sites in cytosol

| Drug | Concentration (% of control binding) | Kidney (% of control binding) | Thymus (% of control binding) | Adipose tissue (% of control binding) |
|---|---|---|---|---|
| MK 410 | 1mM | 4 ± 2 | 7.3 ± 2 | 4.0 ± 2 |
| Phenylbutazone | 1mM | 85 ± 3 | 94 ± 5 | 81 ± 7 |
| Aspirin | 1mM | 101 ± 5 | 104 ± 5 | 99 ± 10 |
| Desmethyl indomethacin | 1mM | 85 ± 13 | 96 ± 5 | 91 ± 4 |
| Desbenzoyl indomethacin | 1mM | 91 ± 6 | 108 ± 5 | 99 ± 6 |
| SC 19220 | 50 μg/ml | 90 ± 6 | 95 ± 6 | 104 ± 6 |
| Eicosatetraynoic acid | 100 μg/ml | 26 ± 2 | 25 ± 1 | 27 ± 7 |
| Polyphloretin phosphate | 100 μg/ml | 56 ± 3 | 42 ± 7 | 23 ± 3 |

[$^3$H]-Dexamethasone concentration was 10nM. Values are means ± S.E. of at least 3 determinations. The only significant difference is the ability of polyphloretin phosphate to compete better in adipose tissue than kidney or thymus ($P < 0.05$ by non-paired $t$-test).

## DISCUSSION

The strong similarity in binding properties exhibited by the glucocorticoid receptors in kidney, thymus and adipose tissue for a wide array of steroidal and non-steroidal compounds is apparent from the data presented. [$^3$H]-Dexamethasone was used as the basic binding probe in order to restrict bound steroid to the classical physiological receptor and to avoid confusion with plasma binders or intracellular "CBG-like" molecules which probably subserve other functions [12, 13]. It should be emphasized that these experiments were performed at 0°C and employed a crude cytosol preparation as the source of receptors. Similar studies performed at other temperatures or with intact tissue would not necessarily be expected to provide the same absolute or relative values. These conditions were selected to minimize non-receptor factors—steroid entry into cells, nuclear transfer rates, and receptor degradation—in an attempt to focus on receptor binding properties. We conclude from the data that the steroid binding site is probably identical in the receptors examined. Multiple physical-chemical properties have not been examined (and the receptors have obviously not been purified) so it is not possible to conclude that the proteins are identical. Moreover, the data do not deal with potential differences in subunit structure or nuclear binding sites and conclusions on these aspects of receptor homology are not warranted from the results presented. Nevertheless, the data do strongly suggest that these three receptors bind steroids and other ligands in an identical fashion. In other studies using the same techniques, liver and bone cytosol receptors exhibited equivalent properties [14–16]. It seems reasonable to speculate, therefore, that glucocorticoid receptors in all target tissues may be basically identical.

The apparent identical nature of the steroid binding site of receptors in various target tissues bears on two important concepts. The first relates to the possibility of designing glucocorticoid agonist or antagonist compounds with differential properties, compounds which could act predominantly on certain target organs and not on others. If the conclusion regarding identity of the binding site is accepted, differential target activity would not be possible on the basis of differential affinity for specific receptors; which is not to say that differential activity cannot be achieved by other means. Local delivery of glucocorticoids as with inhalation therapy in asthma [17] or differential duration of effects as in alternate day therapy [18] may achieve a preponderance of desired effects with less undesired target organ activity. However, the binding data suggest that differential activity based on differential affinity for the receptor cannot be achieved.

The second important point stemming from the data presented relates to the mechanism by which diverse glucocorticoid actions are obtained in different target organs. If the hormone and the receptor are identical, the attainment of divergent actions must reside at some distal step in hormone action and is probably a product of the target cell itself. For example, the ability of dexamethasone to stimulate the synthesis of phosphoenolpyruvate-carboxykinase in kidney and inhibit its synthesis in adipose tissue [10, 19, 20] probably reflects different cellular machinery in kidney and adipose tissue at some point in the action pathway beyond the binding of hormone to receptor.

Of interest, in another vein, are the results which indicate that some NSAID possess the capacity to compete for glucocorticoid receptors (Figs 2–4 and Table 3). These data raise the possibility that NSAID may possess intrinsic glucocorticoid activity [11] in addition to prostaglandin synthetase inhibiting activity [21]. The potential contribution by this mode of action to the anti-inflammatory properties of these drugs is presently being explored.

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