The Distribution and Properties of the Glucocorticoid Receptor from Rat Brain and Pituitary*

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The distribution and properties of cytoplasmic binding sites for the synthetic glucocorticoid dexamethasone and the natural glucocorticoid corticosterone in the brain and the pituitary were studied in detail. Cortisol-17β acid, a derivative which does not bind to the glucocorticoid receptor but is a competitor of corticosterone binding to plasma, was used to overcome plasma interference.

In vitro competition assays in the presence of excess cortisol acid reveal that dexamethasone is as effective a competitor for [3H]corticosterone binding as corticosterone itself. Scatchard analysis of equilibrium experiments with bovine steroids, using cytosol from various brain areas and from the pituitary yielded linear plots, suggesting one class of binding sites. The quantitative distribution of the sites follows the pattern: cortex > hippocampus > pituitary > hypothalamus > brain stem white matter. Furthermore, kinetic analysis of corticosterone dissociation showed a first order reaction, thus indicating the presence of one type of receptor in all brain areas examined.

Rat brain cytosolic receptors for corticosterone and dexamethasone elute from DEAE-Sephadex A-50 anion exchange columns at 0.3 M NaCl in the presence of stabilizing sodium molybdate and at 0.15 M NaCl and/or in the buffer wash when heat-activated, thus exhibiting the characteristic activation pattern of rat liver cytosolic glucocorticoid receptor. The ratio of the buffer wash to the 0.15 M NaCl form is low for dexamethasone and very high for corticosterone. Receptor complexes from various brain parts showed the same activation pattern.

In our experiments, brain corticosterone and dexamethasone receptors stabilized by sodium molybdate are indistinguishable by a number of techniques, thus indicating that it is unnecessary to evoke specific binding sites for each glucocorticoid.

Glucocorticoid binding proteins in various regions of the brain and in the pituitary have been described (1-3). Comparison of the accumulation in cell nuclei of the natural glucocorticoid corticosterone and the potent synthetic glucocorticoid dexamethasone, after injection of the steroids in adrenalectomized rats, revealed a much greater labeling of hippocampus cell nuclei by [3H]corticosterone and of pituitary cell nuclei by [3H]dexamethasone (4). Autoradiographic data support these findings (1, 5).

In vitro nuclear uptake experiments in the presence of saturating steroid concentrations, the differences in the distribution of the two glucocorticoids are attenuated (6), thus indicating that in vivo operating factors or the kinetics of distribution of these steroids in the brain is primarily responsible for the preferential localization of [3H]corticosterone in the hippocampus and [3H]dexamethasone in the pituitary. Furthermore, differences in the concentration and the properties of the rat brain hippocampus cytoplasmic binders for [3H]corticosterone and for [3H]dexamethasone were also observed (7). The question was therefore raised whether the labeling preferences of these steroids reflect the presence of two populations of glucocorticoid receptors differing in their steroid specificity and distribution in the pituitary and brain (8, 9).

In view of possible alterations of hormonal specificity due to plasma contamination it was found imperative to re-evaluate the distribution of glucocorticoid receptors under conditions where the possible effects of the presence of CBG could be eliminated. We therefore made use of the cortisol-17β acid, which effectively competes for plasma CBG binding sites but does not bind to glucocorticoid receptors (11). Thus, in the presence of excess cortisol-17β acid, corticosterone will bind exclusively to the corticosterone receptor. The true nature, the anatomic distribution, and the characteristic properties of [3H]corticosterone and [3H]dexamethasone receptors were thus examined in detail.

MATERIALS AND METHODS

RESULTS

The Specificity of Glucocorticoid Receptors from Different Brain Structures—Competition studies were carried out with cytosol from whole brain as well as from hippocampus, hypothalamus, and cortex separately. All brain parts examined exhibited the same competition patterns. Only the results of the experiments with cortex are shown in Fig. 1. Corticosterone was as effective a competitor as dexamethasone itself for [3H]dexamethasone binding sites (Fig. 16). However, dexamethasone binding is more readily displaced by cortisol-17β acid than by corticosterone (11).

1 The abbreviations and trivial names used are: CBG, corticosteroid-binding globulin; PMSF, phenylmethylsulfonyl fluoride; DCC, dextran-coated charcoal; cortisol 17β acid, 11β,17α-dihydroxy-3-one; 4-androstene-17β-carboxylic acid; corticosterone, 4-pregnen-11β,21-diol-3,20-dione; dexamethasone, 1,4-pregna-1,2,4-trien-3-ol-16α-methyl-11β,17α,21-triol-3,20-dione; 17β-estradiol, 1,3,5(10)-estratrien-3,17β-diol; testosterone, 4-androstene-17β-ol-3-one.

2 Portions of this paper (including "Materials and Methods" and Figs. 1-6) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9550 Rockville Pike, Bethesda, MD 20814. Request Document No. 82 M-1889, cite the authors, and include a check or money order for $3.60 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
methasone failed to compete for all \(^{3}H\)corticosterone binding unless an excess of cortisol-17\(\beta\) acid was included in the incubation medium (Fig. 1a). 17\(\beta\)-Estradiol and testosterone were weak competitors for \(^{3}H\)corticosterone binding sites, whereas cortisol-17\(\beta\) acid alone competed for only \(\frac{1}{6}\) of the total \(^{3}H\)corticosterone binding, even at a concentration of 10 \(\mu M\), the highest concentration used in this experiment. These data indicate that the population of binding sites specific only for \(^{3}H\)corticosterone exhibited affinity for cortisol-17\(\beta\) acid.

In a separate experiment, binding of corticosterone to plasma proteins was effectively competed for by appropriate concentrations of cortisol-17\(\beta\) acid (Fig. 2), whereas dexamethasone had no such effect. This is in agreement with the known properties of dexamethasone which has a weak affinity solely for albumin (14). We thus conclude that cortisol-17\(\beta\) acid competes with corticosterone for CBG binding sites. Consequently the cytosolic binding sites specific only for corticosterone detected in all the brain parts examined in this study may be attributed to a CBG-like binder located inside the cell or tightly associated with the cell milieu (21).

**Determination of Dissociation Rate Constants**—In order to determine the dissociation rate constant for corticosterone, a 500-fold excess of radioinert corticosterone was added to brain cytosol, previously saturated with \(^{3}H\)corticosterone in the presence of a 1000-fold excess of cortisol-17\(\beta\) acid to quench plasma-like binding. The dextran-coated charcoal assay was used to measure the remaining bound \(^{3}H\)corticosterone at the indicated time points (Fig. 3) and up to a total of 24 h following the addition of radioinert corticosterone. First order dissociation kinetics were observed with whole brain as well as with hippocampus, hypothalamus, and cortex studied separately, thus indicating one major class of binding sites throughout the brain. The dissociation rate constant was determined from the slope of the plot shown in Fig. 3 and equaled 4.9 \(\times\) 10\(^{-3}\) min\(^{-1}\).

**Determination of Binding Constants and Maximum Number of Sites**—Yeakley et al. (15) have shown that for rather lengthy equilibrium binding experiments, because of receptor inactivation, Scatchard analysis of the binding parameters could not yield accurate estimation of affinity. Furthermore, under conditions of extreme instability, the receptor concentration is also not measured accurately. Binding studies were therefore performed in isotonic buffer \(P\) containing a number of ingredients known to confer maximum stability to the glucocorticoid receptor (16). Sodium molybdate was also present at a concentration of 10 \(\mu M\), since a stable and homogeneous population of binding sites was reproducibly obtained in chromatographic experiments only when molybdate was added in the homogenization buffer. Typical, invariably linear plots were obtained (Fig. 4). When results were corrected for protein concentration, they pointed to the same single class of binding sites for corticosterone and dexamethasone for the whole brain and also for the hippocampus, hypothalamus, and cortex areas studied separately. Table I shows the mean values of affinity constants and of maximum site concentration of several equilibrium experiments with \(^{3}H\)dexamethasone. The affinity constant is in excellent agreement with that reported for the rat liver glucocorticoid receptor (17). The estimated values are: 3.44 \(\pm\) 1.86 \(n\) = 4 for dexamethasone and 10.8 \(\pm\) 1.5 \(n\) = 4 for corticosterone.

**Distribution of Cytosolic Glucocorticoid Binders in Perfused Pituitary and Brain Parts**—The results presented in Table I were further substantiated in a detailed investigation of \(^{3}H\)corticosterone binding in the absence or presence of a 250-fold excess of either radioinert corticosterone, dexamethasone, or cortisol-17\(\beta\) acid. Evidently, a 250-fold excess of acid suppresses 80\% or more of corticosterone binding to plasma (Figs. 1a and 2). Specific \(^{3}H\)corticosterone binding was identified with that abolished by excess radioinert corticosterone. Binding to CBG-like protein was taken to equal the fraction of specific corticosterone binding quenched by excess cortisol-17\(\beta\) acid. Corticosterone receptor binding was calculated by subtracting the CBG-like binding activity from specific corticosterone binding. Finally, dexamethasone binding to the glucocorticoid receptor was taken to equal the fraction of \(^{3}H\)corticosterone binding quenched by excess radioinert dexamethasone. The distribution of dexamethasone binding thus obtained follows the pattern: cortex > hippocampus > pituitary > hypothalamus, in agreement with the results depicted in Table I. Furthermore, the distribution of CBG-like binders and corticosterone receptor binding relative to the distribution of dexamethasone binding from several such experiments is shown in Fig. 5 in the form of histograms. It is evident from Fig. 5 that CBG-like binders are mainly located in the pituitary and that the distribution of corticosterone-specific binding is identical to dexamethasone-specific binding in all brain areas, and somewhat higher in the pituitary.

**Glucocorticoid Receptor Properties**—Brain glucocorticoid receptor migrated in linear sucrose gradients in isotonic buffer \(P\) as one peak of radioactivity behind the IgG peak (7.1 S) with a sedimentation coefficient of 6 S, thus resembling rat liver glucocorticoid receptor in its hydrodynamic behavior (20).

The chromatographic behavior of the brain glucocorticoid receptor was investigated in order to compare it with the extensively analyzed chromatographic properties of the rat liver glucocorticoid receptor (13, 16). Of particular interest to us were the possible changes in receptor characteristics during activation of the protein. Crucial in these studies was the addition of 10 \(\mu M\) sodium molybdate to the buffer, which resulted in stabilization of the unactivated glucocorticoid receptor (23), thus permitting a study of the activation process. Anion exchange chromatography of brain and liver cytosolic receptors, labeled with \(^{3}H\)dexamethasone in isotonic buffer \(P\) in the presence of 10 \(\mu M\) sodium molybdate, showed extensive similarities of the two receptor systems (Fig. 6). The bulk of specifically bound \(^{3}H\)dexamethasone eluted at 0.3 M NaCl for both liver and brain, thus indicating one class of binding

### Table I

**Dissociation constants and binder concentration for dexamethasone binding in pituitary, hippocampus, hypothalamus, cortex, and brain stem white matter cytosol**

<table>
<thead>
<tr>
<th></th>
<th>Hippocampus</th>
<th>Hypothalamus</th>
<th>Cortex</th>
<th>Pituitary</th>
<th>Brain stem white matter</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Equilibrium dissociation constant,</strong> (10^6 \times K_d) ((M))</td>
<td>3.24 (\pm) 1.67 (4)</td>
<td>3.52 (\pm) 1.86 (4)</td>
<td>3.50 (\pm) 2.45 (4)</td>
<td>5.8 (\pm) 2.1 (3)</td>
<td>3.1 (1)</td>
</tr>
<tr>
<td><strong>Concentration of binding sites,</strong> (N_{max}) ((fmol/mg protein))</td>
<td>400 (\pm) 100 (4)</td>
<td>364 (\pm) 34 (4)</td>
<td>597 (\pm) 44 (4)</td>
<td>417 (\pm) 34 (4)</td>
<td>180 (1)</td>
</tr>
</tbody>
</table>
sites in the “unactivated” state. Heat activation of the brain glucocorticoid receptor at 25 °C for 30 min with subsequent addition of sodium molybdate to an final concentration of 10 mM, to stop the activation process (23), resulted in a shift of the bulk of bound [3H]dexamethasone to a lower salt elution (0.15 M NaCl). This behavior paralleled the chromatographic activation pattern of the liver glucocorticoid receptor also shown in Fig. 6. Corticosteroid binder IB was detected in the buffer wash with both liver and brain activated dexamethasone receptors (Fig. 6).

Brain corticosterone receptor was prepared by incubating cytosol with a saturating concentration of [3H]corticosterone (50 nM) in the presence of excess cortisol-17β acid (15 μM) to inhibit corticosterone binding to CBG-like binders. Fig. 6 shows that rat brain corticosterone and dexamethasone receptors prepared in the presence of stabilizing sodium molybdate are indistinguishable by anion exchange chromatography. However, upon heat activation at 25 °C for 30 min, brain corticosterone receptor was readily converted to the form appearing in the buffer wash, a property also exhibited by brain dexamethasone receptor, although to a lesser extent.

As can be seen from Fig. 6, after activation of the brain receptor for 30 min at 25 °C, the complex found in significant amounts with [3H]dexamethasone is the 0.15 M NaCl form, together with lower percentage (15%) of the buffer wash form of the complex. However, brain corticosterone receptor labeled with [3H]corticosterone gives rise almost exclusively to the form appearing in the buffer wash, when heat-activated under the same conditions. The ratio of the buffer wash to the 0.15 M NaCl form is thus low for dexamethasone and high for corticosterone. A comparative study of the activation pattern of the receptor with either glucocorticoid for hippocampus, hypothalamus, and cortex yielded the same ratio of the two activated forms, typical of the glucocorticoid occupying the site (figure not shown).

**DISCUSSION**

Data are presented in this paper on the properties and the distribution in various brain parts and in the pituitary of both corticosterone and dexamethasone glucocorticoid binding sites. The accumulated evidence points to one class of binding sites for the two glucocorticoids since 1) their distribution in the brain and the pituitary is identical, 2) both steroids compete effectively for all the binding sites, and 3) in equilibrium and kinetic experiments with both steroids only one class of sites is detected. Furthermore, brain and liver receptors show the same binding, chromatographic, and hydrodynamic characteristics and the liver receptor is not known to exhibit any apparent heterogeneity. On the other hand, small contamination by plasma or the presence of CBG-like molecules tightly associated with brain and pituitary cells (21) would have obviously introduced an apparent heterogeneity of corticosterone binding sites, thus interfering with possible true corticosterone receptor heterogeneity. The use of cortisol-17β acid, effectively competing for the remaining CBG corticosterone binding, helped us to overcome the difficulty. Typically [3H]corticosterone binding not competed for by excess dexamethasone is shown to be completely abolished in the presence of excess cortisol-17β acid (Fig. 1a). In a parallel experiment, the acid was shown to be inactive for the glucocorticoid receptor binding sites up to a concentration of 10 μM, whereas 17β-estradiol and testosteron, known glucocorticoid anti-inducers, managed to displace more than two-thirds of corticosterone-specific binding (Fig. 1a).

Hence, the specificity of [3H]corticosterone for its receptor is established in the presence of cortisol-17β acid. This allowed the comparison of dexamethasone and corticosterone receptor distribution in the brain and the pituitary by Scatchard analysis. Linear plots, typical of one class of binding sites, were invariably seen with both steroids in the presence of stabilizing sodium molybdate in isotonic phosphate media. Furthermore, one class of binding sites for corticosterone is independently established by its dissociation kinetics. A first order linear plot is evidenced throughout the kinetic study (Fig. 3). Maximum stability for both the occupied and the unoccupied receptor is necessary in order to avoid curvilinear Scatchard plots. These plots may be the result of a gradual increase of the “activated” receptor form possessing different binding properties, or of the free receptor inactivation process (i5), leading to lower Kd estimates and erroneous receptor numbers. The dissociation constants obtained for dexamethasone and corticosterone binding to brain cytosol are in good agreement with values reported elsewhere (2, 15, 17).

A detailed analysis of [3H]corticosterone binding in the brain and the pituitary in the presence of an excess of cortisol-17β acid, dexamethasone, or corticosterone revealed the regional distribution of all known binders, i.e. dexamethasone receptor, corticosterone receptor, and CBG-like binders, in the brain and the pituitary. Pituitary is extremely rich in CBG-like molecules binding circulating corticosteroids and consequently acting as a buffer in regulating free intracellular corticosterone concentration and retarding corticosterone binding to the receptor. The significance of this for endocrine regulation is not well understood. On the other hand, the various brain regions contain relatively low levels of CBG-like binder(s). It is also shown that both corticosterone and dexamethasone receptors followed the pattern: cortex > hippocampus > pituitary > hypothalamus. An analogous pattern of cell nuclear corticosterone retention: hippocampus = pituitary > hypothalamus, that parallels our cytoplasmic receptor levels, has been reported from in vitro studies (6), thus suggesting a closely related activation process for the mobilization of the receptor into the cell nucleus for all these brain areas.

Whereas this pattern of receptor distribution holds true in in vitro studies, where a high steroid concentration can be applied and maintained, it is not seen in in vivo uptake experiments with a single dose of injected [3H]corticosterone or [3H]dexamethasone. Extensive investigation (1, 4, 5) showed that [3H]corticosterone was localized mainly in the rat brain hippocampus, whereas [3H]dexamethasone was localized preferentially in the pituitary. The argument was therefore raised as to what extent the in vivo localization patterns reflected the presence in the brain of two classes of glucocorticoid binding sites, differing in their distribution and properties (8). Alternatively, the in vivo results could be due to in situ manifestation of only one class of sites under the influence of a specific cell milieu. We therefore proceeded with a study of the activation properties of both corticosterone and dexamethasone receptors from different brain parts and further compared brain receptor with rat liver glucocorticoid receptor.

Anion exchange chromatography of brain receptor with either dexamethasone or corticosterone in the presence of 10 mM sodium molybdate revealed only one population of binding sites eluting at 0.3 M NaCl, in agreement with the kinetic and equilibrium data discussed above. Furthermore, both complexes resembled the unactivated rat liver glucocorticoid receptor of Marcovic et al. (13) in its chromatographic properties. Chromatography of the heat-activated brain dexamethasone receptor followed a pattern similar to that shown for the liver receptor, whereas brain corticosterone receptor was almost exclusively converted to the IB form under the same conditions. The properties of the activated corticosterone receptor could be taken to imply that although cytosolic
glucocorticoid receptors belong to one class of binding sites and are indistinguishable by number of techniques, the activated form of the complex depends on the glucocorticoid occupying the site. This is in agreement with the proposed model of receptor as an allosteric protein (18), and a dependence of activated receptor complex forms on steroid structure in rat thymus cells (22). However, this alone would not be sufficient to explain the preferential labeling of hippocampus cell nuclei by injected [3H]corticoosterone, since a comparative study of the activated form(s) of the receptor from hippocampus, hypothalamus, and cortex revealed the same pattern.

An additional level of regulatory activity closely related to the cell milieu may be operating in the brain. A down-regulation of the glucocorticoid receptors in gliomas and normal rat brain may be operating in the brain. A down-regulation on steroid penetration into the brain through the blood-brain barrier (24) could account for the differences between in vitro and in vivo nuclear uptake patterns (1, 4, 5). Such a regulatory activity could account for the differences between in vivo nuclear uptake patterns (1, 4, 5) and in vitro nuclear uptake patterns (6), which are basically in agreement with our cytoplasmic receptor levels.

REFERENCES
2. Stevens, W., Reed, D. J., and Gasser, B. I. (1975) J. Steroid Biochem. 6, 521-527


SUPPLEMENTARY TO

The distribution and properties of the glucocorticoid receptor from rat brain and pituitary. By: W. S. de Kloet, R. J. K. K. K, P. L. Litwack

MATERIALS AND METHODS

Corticosteroids and Receptors

[1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24] Glucocorticoid binders in Rat Brain and Pituitary.
Kinetics of C3H3 corticosterone dissociation from rat brain glucocorticoid receptors were studied using rat brain cytosol in the presence of excess cortisol-11β-acetate. To determine the dissociation rate constant, 125I-labeled corticosterone was added into the incubation mixture and the rate of ECA corticosterone dissociation was followed as described in the "Materials and Methods". Rate constants were mean ± S.E. for separate experiments. Standard deviations smaller than the diameter of the symbol used are not shown.