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GLUCOCORTICOID TYPE II RECEPTORS OF THE SPINAL CORD SHOW LOWER AFFINITY THAN HIPPOCAMPAL TYPE II RECEPTORS: BINDING PARAMETERS OBTAINED WITH DIFFERENT EXPERIMENTAL PROTOCOLS

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Summary—We have used three experimental protocols to determine binding parameters for type I and type II glucocorticoid receptors in the spinal cord and hippocampus (HIPPO) from adrenalectomized rats. In protocol A, 0.5–20 nM [^{3}H]dexamethasone (DEX) was incubated plus or minus a 1000-fold excess of unlabeled DEX, assuming binding to a two-site model. In protocol B, [^{3}H]DEX competed with a single concentration of RU 28362 (500 nM), whereas in protocol C, we used a concentration of RU 28362 which varied in parallel to that of [^{3}H]DEX, such as 500 ×. Results of protocols A and C were qualitatively similar, in that: (1) B_{max} for type I receptors favored the HIPPO, while the content of type II sites was comparable in the two tissues; (2) K_d was consistently lower for type I than for type II sites in both tissues; and (3) type II receptors from the spinal cord showed lower affinity than their homologous sites from HIPPO. This last result was also obtained when using protocol B. In contrast, protocol B yielded binding data indicating that type II sites were of similar or higher affinity than type I sites. Computer simulation of the binding protocols demonstrated that protocols A and C were the most theoretically reliable for estimating the K_d and B_{max} of type I sites, and the predicted error was smaller for protocol C, in comparison with protocol B. We suggest that the noted differences in the K_d of type II receptors between the spinal cord and HIPPO could account for a difference in sensitivity of the two systems in the physiological adrenal hormone range.

INTRODUCTION

The central nervous system (CNS) is a recognized target of steroid hormone action. In the case of adrenal corticosteroids, two classes of receptors have been identified, and in agreement with terminology proposed for the kidney, they have been called type I and type II sites [1, 2]. Type I receptors show preference for natural hormones, such as corticosterone (CORT), and are highly concentrated in the limbic system, particularly the hippocampus (HIPPO), with moderate to low expression in other CNS regions, including the anterior and posterior horns of the spinal cord [2–5]. Type II receptors, however, are more widely distributed in neurons and glial cells throughout the CNS, and show preference for the synthetic corticoid dexamethasone (DEX), as concluded mainly from *in vivo* studies [5]. Type II sites are highly concentrated in HIPPO, some hypothalamic nuclei, cerebral cortex, as well as in the gray and white matters of the spinal cord [2, 3, 6].

In vitro binding assays have revealed that the prevalent receptor in the spinal cord was the type II receptor [7]. This binding site showed a number of similarities but also some differences when compared with the homologous HIPPO receptor, mainly when considering its regulation [8, 7]. Preliminary work also suggested that type II receptors of the spinal cord showed reduced affinity towards [^{3}H]DEX in comparison with HIPPO [9], using competition with the pure glucocorticoid RU 28362 to resolve the two receptor systems.

The present study was therefore undertaken to compare the type I and type II receptors in the two tissues. To this end, we have determined the relationship between three commonly used procedures for assessing binding to a population of two different binding sites in order to establish the validity of B_{max} and K_d estimates and the conditions of competitor concentration under

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which each protocol is most or least valid. The results indicated that in HIPPO and the spinal cord, [^{3}H]DEX was bound with higher affinity by type I than by type II receptors. Furthermore, type II receptors of the spinal cord showed reduced affinity, when compared with HIPPO receptors.

MATERIALS AND METHODS

Chemicals

[6,7-^{3}H]DEX (sp. act 45.8 Ci/mol) was purchased from New England Nuclear (Boston, MA, U.S.A.), RU 28362 was a gift from Dr D. Philibert (Roussel-Uclaf, France). Sephadex LH-20 was purchased from Sigma (St Louis, MO, U.S.A.). All other chemicals used were reagent grade.

Experimental animals

Adult male Sprague–Dawley rats were bilaterally adrenalectomized 3–5 days before the experiment. During this time they were given 0.9% NaCl in the drinking water and rat chow *ad libitum*. Ether-anesthetized animals were perfused intracardially with 0.9% NaCl; after dorsal laminectomy the spinal cord was removed, whereas the HIPPO was dissected out from the brain [7, 8]. These procedures were performed by two independent workers, in order to minimize time elapsed from tissue extraction until homogenization.

Labeling of type I and type II receptors

Spinal cords and HIPPO were homogenized in 2–3 vol of TEMGMo (10 mM Tris pH 7.4, 1.5 mM EDTA, 2 mM mercaptoethanol, 10% glycerol and 20 mM sodium molybdate) and the homogenate was centrifuged at 105,000 *g* for 60 min at 0–4°C. The resulting cytosol was divided into 0.2 ml aliquots and incubated with 0.5–20 nM [6,7-^{3}H]DEX with or without competition with the glucocorticoid receptor marker RU 28362 [10]. For assessment of non-specific binding, a 1000-fold molar excess of DEX was added to duplicate tubes containing different concentrations of [^{3}H]DEX.

The incubates were left for 20 h at 0–4°C, after which time bound and free hormones were separated on Sephadex LH minicolumns. The column eluates containing bound hormone were collected and their radioactivity content was determined by liquid scintillation spectrometry. Results were expressed as fmol specifically bound [^{3}H]DEX/mg protein [11].

The procedures used for determination of type I and type II receptors using [^{3}H]DEX have been reported previously [3, 7, 12, 13]. Briefly, [^{3}H]DEX ($\pm$1000-fold excess non-radioactive hormone) was incubated with or without RU 28362. In the absence of RU 28362 [^{3}H]DEX was bound to both receptor types. With RU 28362, the labeled DEX was bound predominantly to type I receptors, whereas type II sites were considered those suppressed by RU 28362. Therefore, three experimental protocols were devised for determination of the binding parameters of glucocorticoid receptors. In protocol A, [^{3}H]DEX was incubated without RU 28362: under these conditions, a two-site model better fitted the actual binding data, as shown by Burgisser [14]. In protocol B, we added a single, high concentration of RU 28362 (500 nM) to tubes containing 0.5–20 nM [^{3}H]DEX. This method resulted in a variable ratio of competitor/ligand, ranging from 1000 at the lowest [^{3}H]DEX point to a minimum of 25 at the highest radioactive ligand concentration. In the third method, or protocol C, we used a concentration of RU 28362 which varied in parallel to that of [^{3}H]DEX, such as 500×. Validation of these protocols was discussed previously [12].

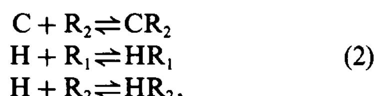
Statistical and computer-assisted analysis

Data were analyzed according to Student's *t*-test, or by one-way analysis of variance (ANOVA), followed by *post-hoc* comparisons with the Duncan test. Binding parameters from saturation experiments were calculated by the limiting-slopes method of Rodbard *et al.* [15] or the direct lineal method of Cressie and Keightley [16], depending on whether two models or a single model fitted the experimental data. For determination of K_d we used:

$$K_{d_2} = \frac{B_{\max_2} \times F}{B_t - \dfrac{B_{\max_1} \times F}{(K_{d_1} + F)}} - F, \qquad (1)$$

where K_{d_1}, K_{d_2}, $B_{\max_1}$ and $B_{\max_2}$ correspond to the dissociation constants and maximum number of sites for the type I and type II sites, F is free hormone and B_t bound [^{3}H]DEX. This equation was derived from the equation of Keller *et al.* [17]. In order to calculate K_{d_2}, we used the values for K_{d_1} and $B_{\max_1}$ obtained from protocols B or C, and $B_{\max_2}$ from protocol A. K_{d_2} was then the average of data yielded by equation (1), using each pair of F and B_t values produced by protocol A.

Additionally, in computer-assisted analysis, simulated data were fed to the computer considering a system with two binding sites of different affinity, resolved by differential competition with RU 28362, as exemplified above for protocols B and C. The system was defined by:



$$\begin{aligned} C + R_2 &\rightleftharpoons CR_2 \\ H + R_1 &\rightleftharpoons HR_1 \\ H + R_2 &\rightleftharpoons HR_2, \end{aligned} \quad (2)$$

where C represents RU 28362, R_1 and R_2 the type I and type II receptors, H is [^{3}H]DEX and CR_2, HR_1 and HR_2 the competitor–receptor, [^{3}H]DEX–type I complex and [^{3}H]DEX–type II complex, respectively. Formation of CR_1 is unlikely, as RU 28362 in the concentration used did not bind to type I sites [10]. The equation representing [^{3}H]DEX binding to both receptors is given by:

$$B_t = \frac{F \times B_{max_1}}{K_{d_1} + F} + \frac{F \times B_{max_2}}{K_{d_2} + \frac{K_{d_2}}{K_{d_3}}[C]_f + F}, \quad (3)$$

where B_t is total bound [^{3}H]DEX (i.e. to both type I plus type II sites), $[C]_f$ is the concentration of unbound RU 28362, K_{d_3} is the dissociation constant for RU 28362, $F \times B_{max_1}/K_{d_1} + F$ (also named B_1) representing binding to type I sites, and $F \times B_{max_2}/K_{d_2} + K_{d_2}/K_{d_3}[C]_f + F$ (also called B_2) representing binding to type II sites. According to results previously reported, $K_{d_2}/K_{d_3} = 1.7$ [10]. Protocol B was simulated by replacing $[C]_f$ with the expression 500 nM − B_{max_2} and protocol C by replacing $[C]_f$ with the expression $500 \times F + 500 \times B_1 - B_{max_2}$ (see Results).

RESULTS

In protocol A, we incubated cytosol from the spinal cord and HIPPO with 0.5–20 nM [^{3}H]DEX without RU 28362. As shown in Fig. 1, curvilinear Scatchard plots were obtained with this method, consistent with a two-site system: therefore, the limiting-slopes method of Rodbard *et al.* [15] was considered appropriate for determination of B_{max} and K_d for each receptor type. First, we observed that B_{max} for type I receptors favored the HIPPO ($P < 0.01$ vs spinal cord), while the content of type II sites was comparable in the two tissues, as expected from previous reports [7] (Table 1). While both receptors showed appreciable affinity for [^{3}H]DEX, K_d was consistently lower for type I than for type II sites in both HIPPO and the spinal cord ($P < 0.05$ by ANOVA followed by Duncan's multiple range test).

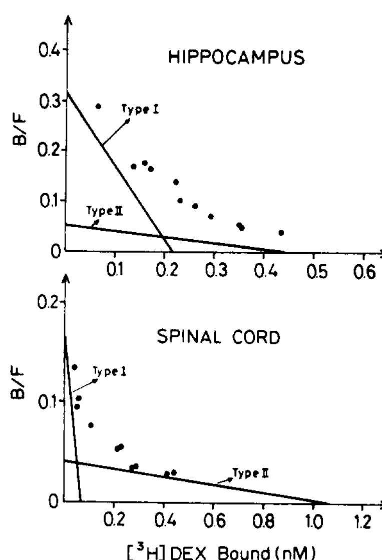


Fig. 1. Scatchard plot analysis of [^{3}H]DEX binding in cytosol from HIPPO and spinal cord of adrenalectomized rats. Cytosol was incubated in buffer TEMGMo during 20 h at 0–4°C with 0.5–20 nM radioactive DEX (±1000× non-radioactive DEX), conditions designed as protocol A. Binding parameters from curvilinear plots were calculated according to the limiting-slopes method of Rodbard *et al.* [15]. The figure is representative of 6 experiments. For Type I receptors in HIPPO, K_{d_1} measured 0.67 nM and B_{max_1} 73 fmol/mg protein, while in the spinal cord the corresponding values were K_{d_1} 0.44 nM and B_{max_1} 12.6 fmol/mg protein. For Type II receptors in HIPPO, K_{d_1} measured 8.4 nM and B_{max_1} 153.1 fmol/mg protein, while in the spinal cord the corresponding values were K_{d_1} 26 nM and B_{max_2} 190 fmol/mg protein, respectively.

Table 1. Binding parameters for type I sites and type II sites in the spinal cord and HIPPO, obtained with protocol A

	Type I receptors		Type II receptors		
Tissue	B_{max_1} (fmol/mg/protein)	K_{d_1} (nM)	B_{max_2} (fmol/mg protein)	K_{d_2} (nM)	K_{d_1}/K_{d_2}
Spinal cord	12 ± 3[a]	1.5 ± 0.6	120 ± 43	17 ± 4[b]	0.09
HIPPO	57 ± 13	0.36 ± 0.01	165 ± 31	5.2 ± 0.9	0.07

Cytosol from the spinal cord and HIPPO was incubated with 0.5–20 nM [^{3}H]DEX with or without 1000× unlabeled DEX. Binding parameters from curvilinear Scatchard plots were obtained by the limiting-slopes method of Rodbard *et al.* [15], assuming a two-site model. The figures represent the mean ± SE of $n = 6$ experiments.

[a]$P < 0.01$ vs B_{max_1} from HIPPO; [b]$P < 0.01$ vs K_{d_2} from HIPPO (by Student's *t*-test).

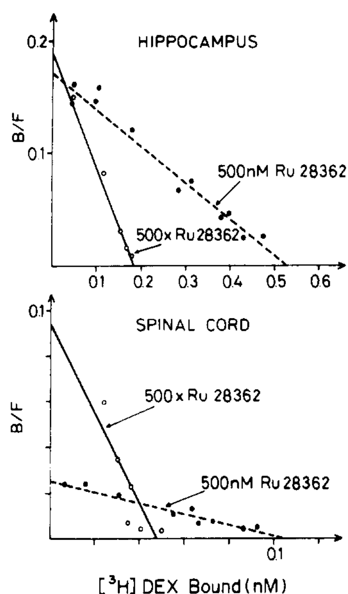


Fig. 2. Binding of [^{3}H]DEX to glucocorticoid receptors in the presence of RU 28362. Cytosol from HIPPO and the spinal cord was incubated with 0.5–20 nM [^{3}H]DEX in the presence of 500 nM RU 28362 (protocol B, ----) or 500 × RU 28362 (——). Binding parameters for this representative experiment, calculated according to Cressie and Keightley [16] were as follows. *Protocol B*—HIPPO K_{d_1} 3.1 nM and B_{max_1} 180 fmol/mg protein; spinal cord 4.3 nM and 18.7 fmol/mg protein, respectively. *Protocol C*—HIPPO K_{d_1} 0.9 nM and B_{max_1} 54 fmol/mg protein; spinal cord 0.44 nM and 10.5 fmol/mg protein, respectively.

Thirdly, and most important for the purposes of this work, the K_d of type II sites of the spinal cord was significantly higher than the K_d from HIPPO ($P < 0.01$, Table 1). This 3-fold difference in K_d values suggested heterogeneity for this receptor species between these tissues.

In protocol B, we competed [^{3}H]DEX with a single, high concentration of RU 28362 (500 nM). This assay linearized the Scatchard plot (Fig. 2) but in contrast to binding data from protocol A, type II sites displayed similar or higher affinity than type I sites. This tendency was observed for both HIPPO and the spinal cord, but it remained non-significant (Table 2). Despite the noted differences in K_d values for type I and type II sites produced by both protocols, the binding capacity obtained under protocol B was qualitatively comparable with the values shown in Table 1 for protocol A: type I sites were more abundant in HIPPO than in the spinal cord ($P = 0.05$), whereas type II sites did not not differ in the two tissues (Table 2).

In protocol C, RU 28362 was also added to occupy type II receptors, but in contrast to protocol B, the competitor/ligand ratio was kept constant at 500 × . This procedure linearized the Scatchard plot (Fig. 2), and binding data obtained according to Cressie and Keightley [16] are summarized in Table 2. With protocol C, type I sites of the spinal cord and HIPPO showed higher affinity than type II sites, resembling data from protocol A ($P < 0.01$ by ANOVA followed by the Duncan's multiple range test). After addition of 500 × RU 28362, the K_d of type II receptors from the spinal cord again showed lower affinity than their homologous sites from HIPPO ($P < 0.05$), although the differences were not as pronounced as those obtained under protocol A. The binding capacity for type I sites again favored HIPPO ($P < 0.001$), although values for B_{max_2} of the spinal cord were in the lower range of those measured under protocol A, amounting to 55% of those present in HIPPO (Table 2).

In order to understand why K_d values were dependent on the competition protocol applied, we have speculated that 500 nM RU 28362 (protocol B) would compete better at the beginning of the [^{3}H]DEX concentration curve, but less effectively at the end, whereas the competitive effectiveness should be the same after addition of 500 × RU 28362. This hypothesis was tested using a computer-simulated experiment, in which the values for K_d and B_{max} were pre-

Table 2. Binding parameters for type I sites and type II sites in the spinal cord and HIPPO, obtained with protocols B and C

		Type I receptors		Type II receptors	
Tissue	Protocol	B_{max_1} (fmol/mg protein)	K_{d_1} (nM)	B_{max_2} (fmol/mg protein)	K_{d_2} (nM)
Spinal cord	B	20 ± 7.9[a]	3.9 ± 0.7	91 ± 48	2 ± 1.1
HIPPO	B	119 ± 35	2.0 ± 0.6	76 ± 28	0.7 ± 0.17
Spinal cord	C	9 ± 1.5[b]	0.8 ± 0.16	80 ± 17[c]	7 ± 1.2[d]
HIPPO	C	55 ± 2.1	0.7 ± 0.12	145 ± 28	4 ± 1.4

Cytosol from the spinal cord and HIPPO was incubated with 0.5–20 nM [^{3}H]DEX (±1000 × unlabeled DEX) in the presence or absence of RU 28362. In protocol B, 500 nM RU 28362 was added to each [^{3}H]DEX concentration, whereas in protocol C the amount of competitor varied 500 × with respect to [^{3}H]DEX. Binding parameters from single line Scatchard plots were obtained according to Cressie and Keightley [16] using a subtraction method for calculation of binding data for each receptor type. The figures represent the mean ± SE of $n = 3$ experiments (protocol) and $n = 4$–5 experiments (protocol C).
[a]$P = 0.05$ vs B_{max_1} from HIPPO; [b]$P < 0.001$ vs B_{max_1} from HIPPO; [c]$P < 0.01$ vs B_{max_2} from HIPPO; [d]$P < 0.05$ vs K_{d_2} from HIPPO.

defined, and in equation (3) we replaced $[C]_f = 500\ \text{nM} - B_{max_2}$ (simulating protocol B) or by $[C]_f = 500 \times B_1 - B_{max_2}$ (simulating protocol C). The values obtained demonstrated that the intrinsic errors of the assays localized at the beginning of bound [^{3}H]DEX for protocol B, with deviations from the theoretical curve amounting to 2.5%, with a minimal error at the end of the curve (0.5%) for protocol C. Therefore, lower K_d values for type I sites would be expected when using protocol C rather than protocol B, as found in the present experiments.

Furthermore, comparison of saturation analysis for type I sites measured after competition with 500 nM or 500× RU 28362, demonstrated a lower number of type I sites of higher affinity when using 500× agonist, in comparison with the fixed competitor method (Fig. 2). Taking into account that the error predicted by computer simulation was lower for protocol C, it is clear that this binding assay would reflect more precisely the number of binding sites of the cytosol fraction under study.

Finally, computer simulation was also used, based on binding parameters from protocol A, to establish the effects of varying the concentrations and ratios of RU 28362, e.g. 100 nM or 100×; 250 nM or 250×; 500 nM or 500× and 1000 nM or 1000×, on estimates of K_d and B_{max} of type I sites. Figure 3 shows results of experiments designed to test whether protocols B and C systematically varied or approximated the normalized binding data from protocol A. At low RU 28362 quantities (100–250), protocol B greatly deviated from the theoretical values of protocol A, both for K_d (graphs I and II for the spinal cord and HIPPO, respectively, Fig. 3) as well as for B_{max} (graphs III and IV for the same tissues). At higher quantities of competitor the tendency of both protocols was to approximate the normalized values of protocol A. However, better coincidence was visualized when the conditions of protocol C were simulated, both for K_d and B_{max} values.

DISCUSSION

The present study was designed to investigate whether the spinal cord and HIPPO type II

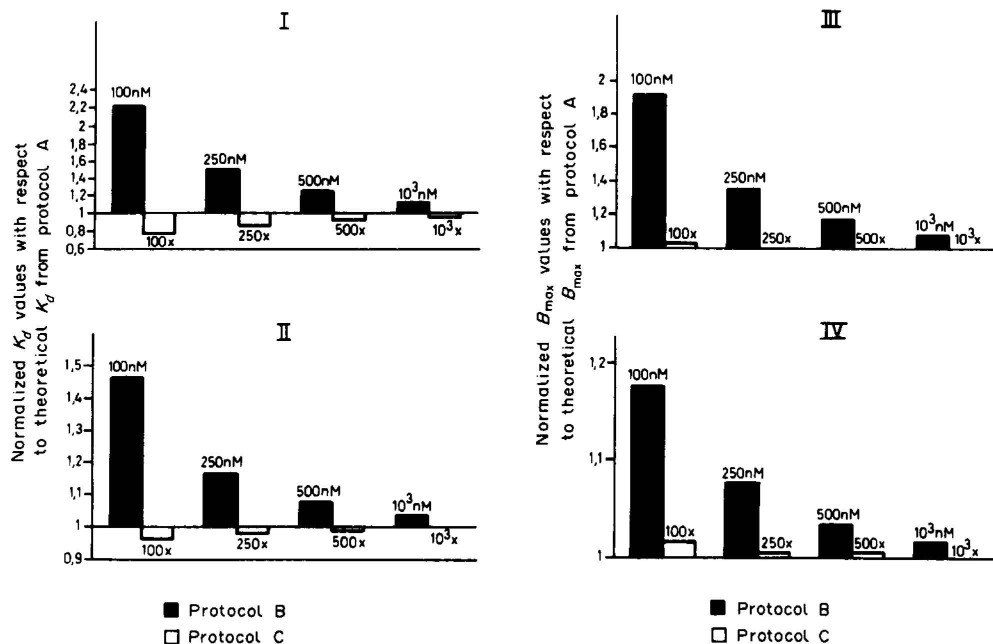


Fig. 3. Computer simulation experiments to test deviations of binding parameters of type I sites yielded by protocols B and C, with respect to protocol A. Graphs I and II represent normalized K_d [1: theoretical K_d converted from protocol A, equation (3)] in the spinal cord (graph I) and HIPPO (graph II), whereas graphs III and IV represent normalized B_{max} [1: theoretical B_{max} converted from protocol A, equation (3)] in the spinal cord (graph III) and HIPPO (graph IV). The simulated experiments demonstrated the effects of varying the concentration of RU 28362, 100–1000 nM (protocol B, ■) or 100–1000× (protocol C, □), on [^{3}H]DEX binding to type I sites. As the concentration of RU 28362 increases, both protocols B and C systematically approximate the normalized values of protocols A, although approximations are greater for protocol C. At the lowest competitor concentrations (100–250), protocol B deviates greatly from the values of protocols C and A. Identical behavior was obtained for K_d (graphs I and II) as well as for B_{max} (graphs III and IV), in spite of the fact that deviations of both parameters were more pronounced for protocol B in the spinal cord than in HIPPO.

receptors showed different affinities, as preliminary results suggested [9].

In our work, application of the limiting-slopes method for estimation of binding parameters of the high and low affinity sites (protocol A) established that type I receptors were the higher affinity form for [^{3}H]DEX. The method employed, however, may be questioned on the basis that curvilinear Scatchard plots are less reliable for subsite measurement, while steroid competition would provide more accurate data [14]. In our hands, competition assays yielded binding parameters which varied according to the method of addition of RU 28362: the fold-addition (protocol C) and the fixed method (protocol B) showed discrepancies regarding K_d values, but consensus regarding B_{max} values. In this context, both protocols demonstrated that the binding capacity of type I sites strongly favored HIPPO, while type II sites were more comparable in both tissues. After computer simulation suggested that addition of 500 × RU 28362 would lower the intrinsic error of the method, we experimentally tested this possibility and found that K_d of type I receptors was lower than K_{d_2} in both CNS areas. Additionally, computer simulation of protocol C also demonstrated that estimates of K_d and B_{max} for type I sites, using a range of RU 28362 from 100 × to 1000 ×, adjusted better to the binding parameters of protocol A, while protocol B greatly deviated at low competitor concentrations. Thus, protocols A and C, both of which are the most theoretically reliable in estimating the K_d of type I sites, show they have higher affinity for [^{3}H]DEX than do type II sites. It should be stated that interpretation of the results shown in Fig. 3, assumes that RU 28362 does not bind to type I receptors. However, high concentrations of RU 28362 can interact with type I sites [12, 13, 18], making it advisable not to use competitor concentrations above 500 × or 500 nM.

The high affinity binding of [^{3}H]DEX to type I receptors is surprising because, judged from hormone uptake studies, [^{3}H]DEX interacted poorly with type I receptors [5, 6, 19] and DEX administration did not activate type I sites very efficiently *in vivo* [13]. The *in vitro* results are in agreement with the recent reports by Luttge *et al.* [12, 18] and by Allen *et al.* [20] who demonstrated that the K_d of type I receptors for [^{3}H]DEX was nearly as low as that for aldosterone, a typical type I agonist, and considerably lower than the K_d of type II sites. These observations, coupled with the present report, imply that DEX's difficulty in occupying type I sites *in vivo* is not due to reduced affinity for these receptors, but that other mechanisms should be explored to explain mediation of DEX action in CNS by receptor subtypes.

The present study also demonstrated that using the conditions specified for protocol A, the K_d of type I receptors of HIPPO was 3.5-fold lower than K_{d_2} for the spinal cord. The 500 × competition assay (protocol C) also disclosed that type II, but not type I receptors of the spinal cord, were of lower affinity than their HIPPO counterpart. These results supported our preliminary data that the spinal cord expressed a reduced affinity type II receptor [9] and it could account for a difference in sensitivity of the two systems in the physiological hormone range.

Thus, the high sensitivity of HIPPO cells to circulating glucocorticoids correlates with their participation in feedback mechanisms, in particular the fine tuning of the system [2, 4, 5, 21, 22], and with changes in cell function and number. For example, endogenous levels of glucocorticoids induce neuronal loss in HIPPO of aging rats, a phenomenon prevented by prior ADX [23, 24], while high levels of exogenous glucocorticoids lead to autologous receptor down-regulation [25] and are neurotoxic to the cells of the CA_2 and CA_3 fields [6].

In the spinal cord, glucocorticoids induce the enzymes glycerol-phosphate dehydrogenase and ornithine decarboxylase [26], enhance synaptic transmission and neuronal excitability, regulate neurotransmitter content and lipid peroxidation and shorten recovery time after injury [27–29]. Commonly, these beneficial actions of adrenal hormones required doses in the upper physiological or pharmacological range [30, 31], which would support mediation by a type II receptor of low affinity. In comparison with HIPPO, there are no reports of neuronal loss in the spinal cord after prolonged exposure to adrenal cortical hormones. On the contrary, pharmacological amounts often result in fast recovery from spinal cord injury and in clinical improvement of patients suffering from degenerative spinal cord diseases [31, 32].

We have already demonstrated that the glucocorticoid type II receptors of the spinal cord and HIPPO presented some biochemical differences [7, 8, 33, 34]. For example, the spinal cord receptor showed increased binding to DNA-cellulose compared with the HIPPO receptor

during heat-induced receptor transformation [7]. Secondly, the spinal cord receptor showed resistance to the action of the enzyme RNAse A, which increases DNA-cellulose binding of the HIPPO receptor [7]. Thirdly, addition of EDTA, which reduces binding of the steroid-receptor complex to DNA-cellulose by chelation of zinc ions, produced a greater inhibition of type II receptors from HIPPO than from the spinal cord (D. F. Moses and A. F. De Nicola, unpublished). Thus, type II receptors from the spinal cord and HIPPO might be structurally and functionally dissimilar. Different molecular forms of glucocorticoid receptors have been observed in melanoma cells [35] bovine tissues [36] rat liver nuclei [37] and HeLa S_3 cells [38]. However, the relationship between these various receptor forms remains obscure.

Finally, there are reasons to support the presence of a high affinity type II receptor in HIPPO, considering the positive coupling between type I and type II receptor regulation. An attractive hypothesis would be the up-regulation of type II receptor affinity after ligand occupation of type I sites; this possibility is supported by the observations of McEwen *et al.* [6] and Spencer *et al.* [13] on the ability of low basal levels of CORT to occupy both type I and type II receptors preferentially in HIPPO. This mechanism may be unique to HIPPO, due to the stoichiometric ratio existing between type I and type II receptors in this tissue, unlike the rest of the CNS regions.

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