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BINDING OF GLUCOCORTICOID HORMONES IN BOVINE HYPOTHALAMIC AND PITUITARY CYTOSOL

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SUMMARY

The existence of macromolecules which bind dexamethasone and corticosterone with high affinity to a small number of binding sites in the cytosol of bovine hypothalami and pituitary has been demonstrated. The dexamethasome–receptor complex had a sedimentation coefficient of 6·4S in low salt and 5·5S in high salt conditions, as determined by sucrose–glycerol density gradient analyses. The receptors possessed specificity for glucocorticoid hormones and were destroyed by pronase and N-ethylmaleimide but not ribonuclease or deoxyribonuclease. Based on evidence from Sephadex gel filtration, ammonium sulfate fractionation and steroid competition studies, corticosterone appeared to be bound to the dexamethasone-binding cytosol protein.

INTRODUCTION

The primary site of the feedback effect of glucocorticoid hormones on ACTH secretion remains a matter of controversy. It is likely, however, that feedback is exerted to some extent at the level of both the hypothalamus and pituitary and also involves other neural areas. Evidence favouring one or other site has been obtained from studies on the effects of local injection, implantation or application of synthetic or natural glucocorticoids on ACTH release or CRF release or action, and effect of removal or destruction of the hypothalamus [1]. Electrophysiologic studies have demonstrated an effect of glucocorticoids on single cell firing in the hypothalamus [2, 3].

Results of studies on distribution of radioactive glucocorticoid hormones have differed among species. Thus in the dog, cortisol was concentrated in the hypothalamus in preference to the pituitary or cerebral cortex [4], but in the pig, higher concentrations were found in the pituitary [5]. The adrenalectomized rat took up corticosterone mainly in the hippocampus with lesser amounts being localized in the pituitary and hypothalamus [6]. In another study, tritiated corticosterone was found in the highest concentration in the pituitary after systemic injection [7].

Specific protein receptors appear to be necessary to bind and transfer steroid hormones into nuclei where feedback and other effects are probably mainly exerted. If glucocorticoids suppress ACTH secretion by an action on both the hypothalamus and pituitary, it is likely that specific glucocorticoid receptors exist in both these structures. Glucocorticoid receptors have been characterized in the brain [8, 9] and pituitary [10–12], but reports on glucocorticoid binding in the hypothalamus have been limited [9, 13, 14].

Since the target site of action of a particular concentration of glucocorticoid hormone is likely to be affected or determined by the nature and number of available binding sites in these tissues, it was of interest to characterize and compare glucocorticoid receptors in both tissues in the same species and attempt to determine the relative amount and importance of these sites. Initial studies on binding of dexamethasone in bovine pituitary cytosol have been described elsewhere [12]. In this report, the evidence for the existence of a receptor for dexamethasone in the cytosol of bovine hypothalamus is presented. Since many investigations on feedback in the past have involved the use of corticosterone, and the receptors for synthetic and natural glucocorticoids may differ in some tissues, it was also of interest to study the binding of corticosterone in these tissues.

EXPERIMENTAL

Chemicals

[1,2,4-^{3}H]-Dexamethasone (16 Ci/mmol) and [1,2,4-^{3}H]-triamcinolone acetonide (10·7 Ci/mmol) were purchased from Schwarz–Mann and [1,2-^{3}H]-corticosterone (50 Ci/mmol) and [1,2-^{3}H]-hydrocortisone (51·7 Ci/mmol) from New England Nuclear. The source of other chemicals used has been previously described [12].

Preparation of cytosol

Bovine tissues were obtained from intact adult male and female animals at a local slaughterhouse. They were transported to the laboratory in ice-cold Krebs–Ringer bicarbonate containing 11 mM D-glucose and used within about 3 h of removal. The tissues were rinsed in normal saline and homogenized at 2°C in buffer A (0·05 M Tris–HCl, 0·001 M Na_2EDTA, 0·012 M monothioglycerol, pH 7·5, about 2 ml/g) using a glass Teflon–glass homogenizer. Cytosol was

obtained after centrifugation of the homogenate at 27,000 *g* for 10 min and then 200,000 *g* for 30 min. The cytosol protein concentration was determined by the method of Lowry[15].

Filter binding assays

Binding of tritiated steroid was measured by DEAE–cellulose filter assays [16]. Cytosol (0·2 ml) was incubated at 2°C with 1×10^{-8} M [^{3}H]-dexamethasone with or without a 1000-fold excess of non-radioactive dexamethasone for about 1 h. Aliquots (50 μl unless otherwise stated) were filtered in triplicate and filters washed with buffer B (0·02 M Tris–HCl, 0·0015 M Na_2EDTA, pH 7·9). Filters were counted in 5 ml of toluene containing 4·2% (v/v) Liquifluor with an efficiency of 27%. Specific binding was calculated as the difference between values obtained in samples containing only tritiated dexamethasone and those of samples containing excess non-radioactive dexamethasone.

Protamine sulfate assay for binding of corticosterone [9]

Cytosol was incubated with [^{3}H]-corticosterone with or without a 1000-fold excess of non-radioactive corticosterone in duplicate tubes. To 0·5 ml cytosol was added 2 ml of protamine sulfate, 2·88 mg/ml in 0·01 M Tris–HCl, 1 mM EDTA, pH 7·5. The mixture was allowed to stand for 10 min at 2°C, then centrifuged at 800 *g* for 10 min. The precipitate was dissolved in 0·5 ml of Soluene (Packard) and the radioactivity counted in 10 ml of Liquifluor-toluene. Duplicate (0·5 ml) aliquots of the supernatant were counted to determine unbound radioactivity in 5 ml of Aquasol with a counting efficiency of 16%.

Sucrose density gradient centrifugation analysis

Sucrose density gradient centrifugation studies were performed by layering 0·3 ml of cytosol previously incubated with 1×10^{-8} M [^{3}H]-dexamethasone on 5–20% linear sucrose density gradients containing 10% glycerol and 0·01 or 0·4 M KCl in buffer C (0·01 M Tris–HCl, 0·001 M Na_2EDTA, 0·012 M monothioglycerol, pH 7·5). The gradients were centrifuged for 16 h at 297,000 *g* in a Beckman L265B ultracentrifuge using a Spinco SW56 rotor at 2°C. Fractions of 8 drops were collected from the bottoms of the tubes and counted in 5 ml of Aquasol with an efficiency of 17%.

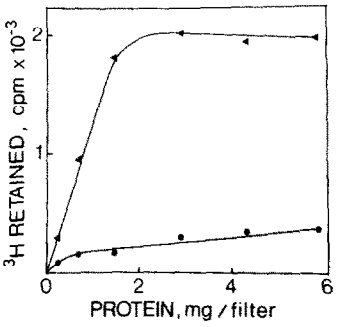

Fig. 1. Relation of cytosol protein concentration and retention of bound dexamethasone on DEAE–cellulose filters. Bovine hypothalamic cytosol was incubated with 1×10^{-8} M [^{3}H]-dexamethasone with (●) or without 10^{-5} M non-radioactive dexamethasone for 1 h at 2°C. Binding was then determined with the filter assay using different volumes of cytosol. ▲: difference between values obtained in the presence and absence of non-radioactive steroid, ●: non-specific.

RESULTS

Binding of dexamethasone in hypothalamic cytosol

When bovine hypothalamic cytosol was incubated at 2°C with [^{3}H]-dexamethasone, specific binding was readily demonstrated using the DEAE–cellulose filter assay [16]. Binding was not due to serum contamination of the cytosol preparation, as demonstrated by failure to detect binding of dexamethasone to bovine serum under the same assay conditions. The amount of dexamethasone retained by the filters was linearly related to the amount of cytosol protein up to about 2 mg protein (Fig. 1). Nonspecific binding was determined by incubation of cytosol with a large excess of nonradioactive dexamethasone in addition to the radioactive steroid, and accounted for only a small percentage (14%) of total binding.

When hypothalamic cytosol was incubated with increasing concentrations of dexamethasone, saturation was reached at about 3×10^{-8} M steroid. A Scatchard plot of the data disclosed the presence of a single set of binding sites for the steroid concentration used, and a Kd of $1{\cdot}2 \times 10^{-8}$ M (Fig. 2). The number of sites was $2{\cdot}8 \times 10^{-13}$ mol per mg of cytosol protein.

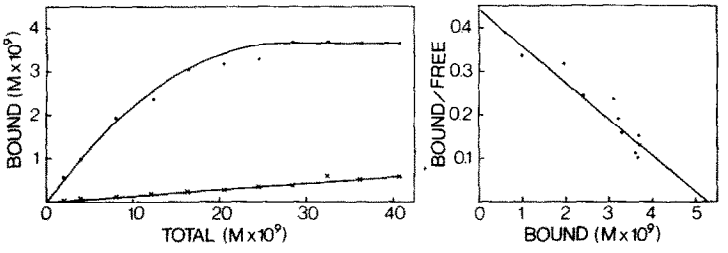

Fig. 2. Scatchard analysis of dexamethasone binding to hypothalamic cytosol. Bovine hypothalamic cytosol (18·4 mg protein per ml) was incubated with varying amounts of tritiated dexamethasone with or without 1×10^{-5} M non-radioactive dexamethasone for 1 h at 2°C. Binding was determined by filter assay. ●; difference between binding in the presence and absence of non-radioactive steroid, x; non-specific.

Sucrose density gradient analysis of dexamethasone binding

The sedimentation coefficient of most glucocorticoid receptors has been found to be about 7 S in low salt and about 4 S in high salt conditions. When bovine hypothalamic cytosol was incubated with [^{3}H]-dexamethasone and layered on 10–30% sucrose density gradients containing 10% glycerol, a single peak of radioactivity was obtained (Fig. 3A). In the presence of 0·01 M KCl, the sedimentation coefficient was 6·4 S, while in 0·4 M KCl, the coefficient was 5·5 S. (In 0·05 M KCl and 0·1 M KCl, the coefficients were 6·0 and 5·5 S, respectively.)

Bovine pituitary cytosol has also been found to contain specific glucocorticoid receptors [12]. To compare the hypothalamic and pituitary receptors, bovine pituitary cytosol was incubated with dexamethasone and analyzed on similar sucrose density gradients. In the case of the pituitary, the sedimentation values were 6·4 S and 5·0 S in 0·01 and 0·4 M KCl, respectively (Fig. 3b). Incubation of bovine serum with [^{3}H]-dexamethasone did not produce a peak of radioactivity on identical sucrose gradients.

Competition for hypothalamic cytosol binding of [^{3}H]-dexamethasone

The peak of bound [^{3}H]-dexamethasone was reduced by incubation of cytosol with a 10-fold excess of non-radioactive corticosterone, cortisol or dexamethasone in addition to the radioactive dexamethasone prior to centrifugation (Fig. 4). By summation of the areas under the peaks, it was estimated that bound [^{3}H]-dexamethasone was reduced to roughly 65·7, 37·8 and 23·7% of the control value by addition of a 10-fold excess of cortisol, corticosterone, and dexamethasone, respectively. The differences in binding of the glucocorticoids were not due to differences in steroid degradation. The steroids in dichloromethane extracts of cytosol preparations were found to be essentially unmetabolized when analyzed by thin-layer chromatography on silica gel coated plates in the systems chloroform–ethanol, 9:1 v/v, and methylene chloride–acetone, 7:3 v/v.

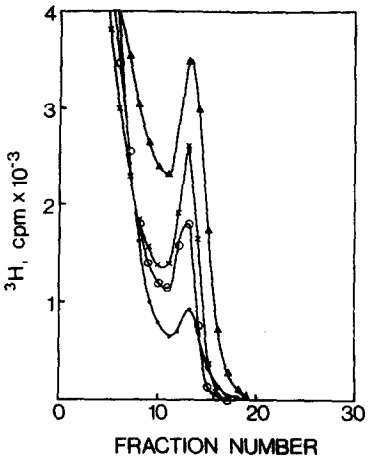

Fig. 4. Sucrose density gradient centrifugation analysis of bound [^{3}H]-dexamethasone. Bovine hypothalamic cytosol was incubated with 1×10^{-8} M [^{3}H]-dexamethasone with or without (control) a 10-fold excess of non-radioactive dexamethasone, corticosterone or cortisol in buffer C containing 0·4 M KCl for 15 min at 2°C. Aliquots were then layered on linear 5–20% sucrose density gradients containing 10% glycerol and 0·4 M KCl and the gradients centrifuged at 297,000 *g* for 16 h. △: control, ●: + dexamethasone, ○: + corticosterone, x: + cortisol.

The inhibitory effect of cortisol (and corticosterone and dexamethasone) was more marked at higher concentrations. Thus, when cortisol was added at a concentration 25 times that of tritiated dexamethasone, binding of the radioactive steroid was reduced to 40·5% of the control value in the absence of the competitor (Table 1). At similar relative concentrations, progesterone, desoxycorticosterone and aldosterone had a small inhibitory effect, but binding of [^{3}H]-dexamethasone was not reduced by estradiol, testosterone, or dihydrotestosterone.

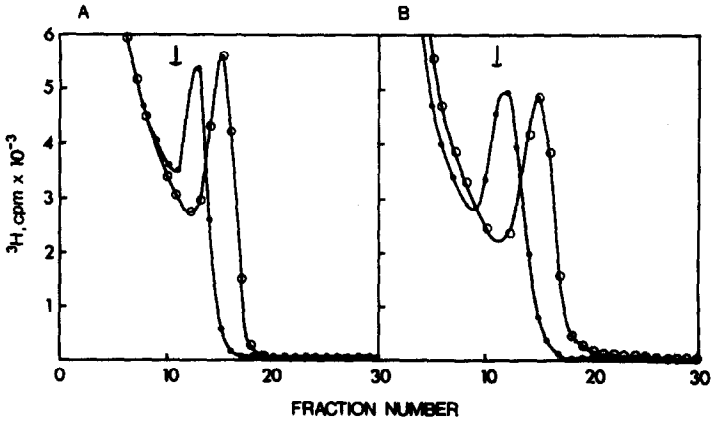

Fig. 3. Comparison of bovine pituitary and hypothalamic cytosol complexes by sucrose density gradient centrifugation analysis. Pituitaries and hypothalami were homogenized to prepare cytosol as described in Methods. Aliquots were incubated with 1×10^{-8} M [^{3}H]-dexamethasone for 30 min and layered on 5–20% sucrose density gradients containing 10% glycerol. The arrow indicates the position of migration of BSA. ○: 0·01 M KCl, ●: 0·4 M KCl, A: hypothalamus, B: pituitary.

Table 1. Effect of nonradioactive steroids on binding of [^{3}H]-dexamethasone by bovine hypothalamic cytosol

Non-radioactive steroid	[^{3}H] Bound (% control ± S.E.M)
Dexamethasone	22·6 ± 7·4
Cortisol	40·5 ± 5·4
Progesterone	80·8 ± 4·0
Desoxycorticosterone	84·6 ± 3·4
Aldosterone	92·9 ± 11·3
Estradiol-17B	94·6 ± 0·8
Testosterone	105·6 ± 5·2
Dihydrotestosterone	103·8 ± 1·4

Cytosol was incubated with 1×10^{-8} M [^{3}H]-dexamethasone alone (control) or with a 25-fold excess of nonradioactive steroid in addition for 1 h at 2°C. Specific binding of [^{3}H]-dexamethasone was then determined by charcoal assay.

Chemical nature of hypothalamic receptor

The nature of the binding moiety was studied by incubating cytosol with enzymes and N-ethylmaleimide before or after incubation with dexamethasone. The hypothalamic cytosol receptor was at least partially composed of protein since binding activity was destroyed by treatment with pronase (Table 2). DNase and RNase had no significant effect on binding. The importance of intact sulfhydryl groups for binding was demonstrated by a reduction in binding on addition of 3 mM N-ethylmaleimide (NEM). Prior binding of steroid and receptor had some protective effect against the action of pronase and NEM.

Binding of corticosterone

In rat liver cytosol, there appear to exist 3 glucocorticoid binding proteins, 2 of which bind only the natural glucocorticoids and one of which binds both synthetic and natural glucocorticoid hormones [17]. Two distinct glucocorticoid receptors with differing affinities for dexamethasone and corticosterone have been described in rat kidney cytoplasm [18]. When bovine pituitary or hypothalamic cytosol was incubated with tritiated corticosterone and cortisol, specific binding of both natural glucocorticoids was detected. Cortisol binding was not studied in detail because of difficulty in distinguishing binding of the steroid to cytoplasmic and blood components.

Corticosterone binding to tissue proteins could, however, be demonstrated by protamine sulfate assay [9]. Using this assay, it was determined that the corticosterone binding sites in the hypothalamus were limited in number, $4{\cdot}6 \times 10^{-13}$ (4·3–4·9) mol/mg of cytosol protein, and characterized by an apparent dissociation constant of $5{\cdot}3 \times 10^{-8}$ (5·0–5·6) M at 2°C (Fig. 5).

To determine whether the natural glucocorticoids were bound to the same protein as the synthetic steroid, ammonium sulfate fractions of pituitary cytosol were prepared. The 50–60% ammonium sulfate fraction contained the greatest binding activity for all these glucocorticoids and triamcinolone acetonide, another synthetic glucocorticoid hormone (Table 3),

Table 2. Effect of enzymes and NEM on [^{3}H]-dexamethasone binding

Treatment	[^{3}H] Bound (% control) A	B
Pronase	19·2	0·1
DNase	97·4	109·3
RNase	94·8	95·8
NEM	39·0	19·5

A: Bovine hypothalamic cytosol was prepared by homogenizing tissue in 0·05 M Tris, pH 8, and incubated with $2{\cdot}5 \times 10^{-8}$ M [^{3}H]-dexamethasone with or without $2{\cdot}5 \times 10^{-6}$ M non-radioactive dexamethasone for 30 min at 2°C. Pronase, 1 mg per ml, DNase, 1 mg per ml, RNase, 1 mg per ml, and NEM, 3 mM, were then added and samples incubated at 37°C for 60 min. The mixtures were chilled in ice for 30 min before assessment of specific binding by filter assays. $MgCl_2$, 1 mM, was added to the DNase-treated samples and a control sample to which binding of this sample was compared. The control value was 11,528 c.p.m. bound.

B: Pronase, DNase, RNase and NEM were added to cytosol samples at the same concentrations as in experiment A and the samples incubated at 37°C for 60 min before chilling on ice for 30 min. [^{3}H]-dexamethasone and non-radioactive dexamethasone were then added and the samples incubated at 2°C for 30 min before assay. Each figure represents the mean of 3 determinations corrected for non-specific binding. The control value was 13,648 c.p.m. bound.

and was 5-fold enriched in the binding protein. There was little glucocorticoid binding activity in the other ammonium sulfate fractions.

The binding of tritiated corticosterone was (in most part) not due to contaminating serum proteins in the cytosol. This was demonstrated by studying the exchange of [^{3}H]-corticosterone bound in cytosol and serum with non-radioactive corticosterone and dexamethasone, When serum or pituitary cytosol was incubated with [^{3}H]-corticosterone, specific binding was detected by protamine sulfate assay in both preparations after 1 h. The tritium bound to serum proteins was displaced by addition of nonradioactive corticosterone in excess, but not dexamethasone (Table 4). In contrast, [^{3}H]-corticosterone bound to cytosol protein exchanged with both corticosterone and dexamethasone within the 2 h incubation period. Thus, the cytosol receptor appeared to be different from the serum corticosterone binding protein and corticosterone was bound in the cytosol preparation to a glucocorticoid receptor which also bound dexamethasone. As in previous experiments, dexamethasone was not bound by serum proteins. In addition, the greatest concentration of corticosterone and cortisol binding protein was found in the 60–90% ammonium sulfate fraction of bovine serum and not the 50–60% fraction as in the case of cytosol.

Sephadex gel filtration analysis

When [^{3}H]-dexamethasone receptor complexes were partially purified by ammonium sulfate precipitation and chromatographed on a Sephadex G200 column equilibrated and eluted with buffer containing

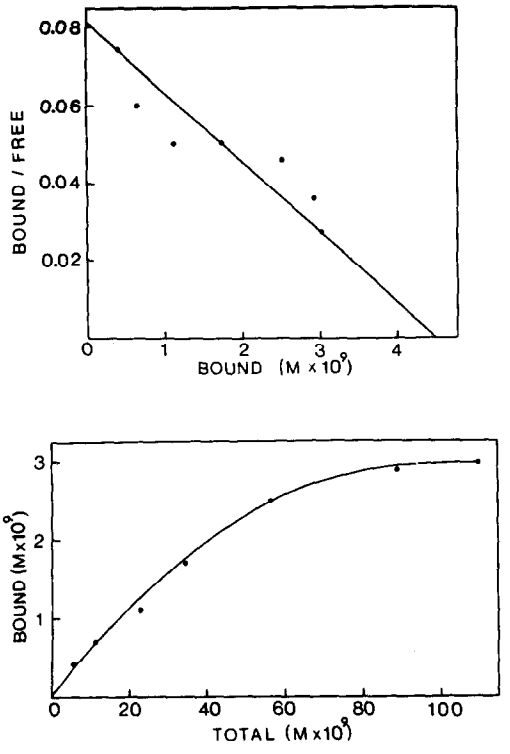

Fig. 5. Relation of binding of corticosterone to steroid concentration. Bovine hypothalamic cytosol was incubated with varying amounts of [^{3}H]-corticosterone with or without 1×10^{-5} M non-radioactive corticosterone in duplicate tubes at 2°C for 30 min before determination of binding by the protamine sulfate assay as described in Methods. Values obtained in the presence of excess corticosterone were subtracted to obtain specific binding. The cytosol protein concentration was 9·2 mg per ml. ●: specific, x: non-specific.

0·1 M KCl, the bound dexamethasone was slightly retained by the column, emerging a few fractions behind the void volume (Fig. 6). The elution volume corresponded to that of a protein with a molecular weight of about 160,000. There was no peak of binding activity in the region corresponding to the elution volume of bovine serum albumin (or CBG). A similar preparation labelled with [^{3}H]-corticosterone contained a smaller peak of bound hormone which coincided in position with the dexamethasone peak, again tending to support identity of the binding protein for the natural and synthetic glucocorticoids.

DISCUSSION

Specific high affinity, low capacity binding of glucocorticoids has been demonstrated in bovine hypothalamic cytosol. This finding was not unexpected in view of previous indirect evidence favouring the presence of such receptors. In another investigation we have found specific dexamethasone receptors in the cytosol of bovine pituitaries [12]. The complex of the pituitary cytosol binding protein and [^{3}H]-dexamethasone sedimented as a single peak with a coefficient of 7 S in 0·01 M KCl, 5·5 S in 0·1 M KCl and 4·9 S in 0·4 M KCl in 5–20% or 10–30% linear sucrose density gradients.

Table 3. Specific binding of glucocorticoids to ammonium sulfate fractions of bovine pituitary cytosol

Steroid	% A.S.	[^{3}H]-Glucocorticoid bound (femtomol/mg protein) 0–30	30–50	50–60	60–90
Dexamethasone		8·8	33·1	154·6	1·7
Triamcinolone acetonide		1·6	0·9	39·0	0·5
Corticosterone		0·7	2·1	17·0	3·7
Cortisol		3·8	12·6	68·4	12·0

Ammonium sulfate fractions were prepared by addition of solid ammonium sulfate to pituitary cytosol and centrifugation at 39000 *g* for 10 min after incubation at 2°C for 30 min. The precipitates were dissolved in buffer A, and aliquots of the dissolved pellets incubated with 1×10^{-8} M tritiated dexamethasone, triamcinolone acetonide, corticosterone or cortisol, with or without a 1000-fold excess of the non-radioactive form of the same steroid. After 1 h of incubation at 2°C, specifically bound steroid was determined using the filter assay. Protein concentrations of the dissolved precipitates were determined by the Lowry reaction. Each figure represents the mean of 3 determinations. % A.S. = percentage ammonium sulfate saturation.

The bovine hypothalamic–cytosol complex formed under similar conditions was more difficult to demonstrate by this technique and the use of glycerol was required to obtain good results. Using 10% glycerol and similar (5–20%) sucrose concentrations the sedimentation coefficient of the hypothalamic cytosol–receptor–dexamethasone complex was 6·4 S in 0·01 M KCl, and 5·5 S in 0·1 M and 0·4 M KCl. It is possible that glycerol stabilized the structure so that there was relatively less change in sedimentation behaviour of the complex in different salt concentrations. To allow comparison of the sedimentation behaviour of the bovine pituitary and hypothalamic cytosol receptors under identical conditions, pituitary cytosol was analyzed in this study in 5–20% sucrose gradients containing 10% glycerol. The sedimentation coefficients (6·4 S and 5·0 S) obtained in high and low salt conditions were very similar to those of the hypothalamic dexamethasone–receptor complex.

Table 4. Exchange of [^{3}H]-corticosterone with non-radioactive steroid

Steroid	^{3}H Bound (c.p.m.) Cytosol	Serum
None	4379	1183
Corticosterone	0	100
Dexamethasone	621	1024

Bovine pituitary cytosol and serum were diluted with homogenization buffer to similar protein concentrations and incubated with 5×10^{-9} M [^{3}H]-corticosterone with or without a 1000-fold excess of nonradioactive corticosterone for 1 h at 2°C. Non-radioactive corticosterone or dexamethasone, 1×10^{-5} M, was then added, and specific binding of the labeled steroid determined by protamine sulfate precipitation 2 h later. Each result represents the mean of 2 determinations.

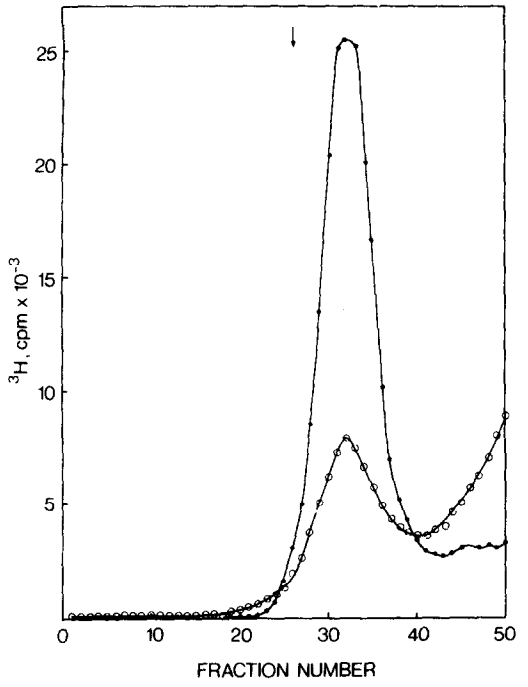

Fig. 6. Sephadex G200 gel filtration of bovine pituitary cytosol incubated with [^{3}H]-dexamethasone and [^{3}H]-corticosterone. Equal volumes of bovine pituitary cytosol were incubated with 5×10^{-9} M [^{3}H]-dexamethasone and [^{3}H]-corticosterone at 2°C. A 40–70% ammonium sulfate fraction was prepared from the cytosol and dissolved in 1 cc of 0·01 M Tris–HCl, pH 7·5, 1 mM EDTA, 0·012 M monothioglycerol, 0·1 M KCl. The samples were applied to a Sephadex G200 column (2·5 × 35 cm.) equilibrated with the same buffer and 2·5 ml fractions collected. Aliquots (0·5 ml) were counted for radioactivity in 5 ml Aquasol. The void volume (arrow) was determined with Dextran blue 2000 and the column standardized with aldolase, BSA, ovalbumin, chymotrypsinogen, and ribonuclease. ●: [^{3}H]-dexamethasone, ○: [^{3}H]-corticosterone.

The pituitary receptor had a Kd for dexamethasone binding of $1{\cdot}6 \times 10^{-8}$ ($\pm 0{\cdot}2$) M, and the number of binding sites was $5{\cdot}0 \times 10^{-13}$ ($\pm 0{\cdot}8$) mol per mg of cytosol protein (mean ± SEM of 7 experiments) [12]. In the case of hypothalamic cytosol, the Kd was $1{\cdot}4 \times 10^{-8}$ ($\pm 0{\cdot}2$) M, and the number of sites $2{\cdot}6 \times 10^{-13}$ ($\pm 0{\cdot}3$) mol per mg of cytosol protein (based on 4 experiments). Thus, the dexamethasone binding proteins in the two tissues were quite similar in binding affinities and capacities. Whether the receptors in the two organs are different in other respects and in their function will require further investigation to ascertain.

Dexamethasone, the most potent glucocorticoid hormone available, was used in most binding studies in preference to corticosterone because of lack of detectable binding to serum components and greater stability of the dexamethasone–receptor complex. Since dexamethasone is a synthetic hormone, it was important to demonstrate that natural glucocorticoid hormones interacted with the same site. The natural glucocorticoid hormones appeared to be bound to the dexamethasone binding sites but they did not completely displace dexamethasone at concentrations 25 or 100 times that of [^{3}H]-dexamethasone. The cytosol receptors appeared to bind dexamethasone with greater affinity than corticosterone. The possibility of their binding to other cytosol proteins specific for only the natural glucocorticoid hormones, such as exist in rat liver [17] or to other proteins with low affinity for dexamethasone, as in rat kidney [18], has not been definitively excluded, but appears unlikely. The differences in binding of the various glucocorticoids were not attributable to steroid degradation *in vitro*.

It should be noted that these studies were performed on tissues obtained from intact animals, since it was not possible to obtain specimens from adrenalectomized cattle. The number of binding sites has possibly been underestimated due to masking of some binding sites by endogenous steroid present in these cytosol preparations. In other systems it has been noted that adrenalectomy is accompanied by an increase in the concentration of glucocorticoid binding sites in the cytosol [1, 13, 17]. It is possible that the number of sites in bovine hypothalamic and pituitary cytosol would be greater, and the apparent dissociation constants different, in adrenalectomized animals.

In this preliminary report the evidence for the presence of specific glucocorticoid receptors in bovine hypothalamic cytosol has been documented. These receptors may be functionally significant in the biologic effects of glucocorticoids on hypothalamic activity. In the controversy of site of action of glucocorticoids in the regulation of ACTH secretion, most investigators in the past have favoured the hypothalamus as the main site of feedback action [1]. Implantation of dexamethasone or cortisol in the median eminence reduced plasma and adrenal corticosterone levels [20]. It is probable that corticosteroids inhibit CRF release from hypothalamic storage sites or synaptosomes in the median eminence. Thus, intraventricularly injected dexamethasone was bound preferentially to hypothalamic particles with characteristics of synaptosomes [21]. Studies with synaptosomes which release CRF [22] should help to elucidate whether feedback inhibition of ACTH by glucocorticoids is mediated through inhibition of release or inhibition of synthesis of CRF.

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