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BINDING OF NATURAL AND SYNTHETIC GLUCOCORTICOIDS IN RAT BRAIN

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SUMMARY

The protein(s) from rat brain cytosol which bind corticosterone are selective, stereospecific and have a high affinity ($K_{dissoc.} = 3{\cdot}8 \times 10^{-9}$ M) and low capacity ($n = 4{\cdot}7 \times 10^{-13}$ mol/mg protein) for [1,2-^{3}H]-corticosterone. These or similar molecules also bind [1,2,4-^{3}H]-dexamethasone but have a lower capacity ($n = 2{\cdot}4 \times 10^{-13}$ mol/mg protein), lower affinity ($K_{dissoc} = 2{\cdot}5 \times 10^{-9}$ M) and were less selective for this synthetic glucocorticoid than for corticosterone.

The amount of [1,2-^{3}H]-corticosterone bound by brain receptor proteins has a diurnal pattern which is the mirror image of the circulating levels of endogenous corticosterone in blood plasma. This cyclic variation in binding was abolished by adrenalectomy. Thus, as the plasma concentration of endogenous corticosterone increases, the number of specific receptor sites in the cytosol of whole brains filled by endogenous corticosterone also is increased quantitatively.

Following the bilateral removal of the adrenal glands, the amount of [1,2-^{3}H]-corticosterone bound/mg protein to brain cytosol protein rapidly increases during the first 24 h after adrenalectomy and approaches values seen at 3 days. During the same period the plasma corticosterone concentration is rapidly decreasing.

By 30 days after adrenalectomy the amount of [1,2-^{3}H]-corticosterone bound per mg of protein has increased over those values measured at 1 day after removal of the adrenals. At 60 and 120 days post-adrenalectomy, the amount of [1,2-^{3}H]-corticosterone bound is near control values (3 day) but decreases below this value by 180 days after removal of the adrenals.

In newborn rats (up to 50 g), the amount of [1,2-^{3}H]-corticosterone bound/mg receptor protein increases with increasing weight. However, from 195 g to 450 g the amount of [1,2-^{3}H]-corticosterone bound/mg protein decreases with increasing weight.

The increase in binding of corticosterone to brain protein of new born rats has been correlated with other maturational events.

INTRODUCTION

A protein which binds corticosterone has been isolated from the cytosol of rat brain [1, 2]. This protein, which has been determined not to be CBG, has the properties of a "receptor", *i.e.*, selectivity for corticosterone, stereospecificity, and a high affinity and limited capacity for this steroid [2, 3]. Although found in various areas of the brain, the corticosterone receptor appears to be concentrated in limbic structures—particularly hippocampus [2, 4, 5]. The physiological significance of these observations has not yet been established; however, the possibility exists that the interaction of corticosterone with specific brain receptor protein in some way contributes to the regulation of ACTH. In the studies described below, the binding of [^{3}H]-corticosterone by the cytosol from rat brain was compared to that of two potent synthetic steroids—[^{3}H]-dexamethasone and [^{3}H]-triamcinolone-acetonide. In addition, the effect of age and adrenalectomy on the binding of [^{3}H]-corticosterone to brain receptor protein was investigated.

EXPERIMENTAL

Steroids

The radioactive hormones used in these experiments were [1,2-^{3}H]-corticosterone (S.A. = 30 Ci/mmol), [1, 2, 4-^{3}H]-dexamethasone (1,4-pregnadiene-9α-fluoro-16α methyl-11β, 17α-21-triol-3,20-dione) (S.A. = 30 Ci/mmol) (New England Nuclear Corp.) and [1, 2, 4-^{3}H]-triamcinoloneacetonide (1, 4-pregnadiene-9α-fluoro-11β,16α,17α,21-tetrol-3,20-dione-16, 17 cyclic acetal) (S.A. = 10·9 Ci/mmol) (Schwartz-Mann).

The non-radioactive hormones used were 17β-estradiol (Schering), progesterone, cortisol, corticosterone (Calbiochem), dexamethasone and triamcinolone-acetonide (Sigma) and 11α-corticosterone.* Purity of all steroids was determined by paper chromatography [6].

General

Male Sprague–Dawley rats adrenalectomized *via* the dorsal approach and weighing between 195 and 450 g were used in these studies. At the time of the experiment the animals were anesthetized with pentobarbitol sodium (50 mg/kg), intraperitoneally, and exsanguinated *via* the abdominal aorta. The animals were flushed with ice-cold isotonic sucrose through the aorta until the fluid recovered contained less than 2% blood. After removal of the brain (minus olfactory lobes), the brain was homogenized in 5 vol. of ice-cold Tris (0·01 M)–EDTA (0·0015 M) buffer, pH 7·4. The homogenate was centrifuged at 105,000 *g* for 60 min. The resulting supernatant (cytosol) was removed and was analyzed for capacity to bind radioactive steroid

* Courtesy Dr. Hans Reich.

by either a charcoal adsorption method or by gel chromatography. The radioactive steroid was added at either the time of homogenization or to the fresh cytosols. In both instances the final concentrations of radioactive hormone in the cytosol was in the range of 10^{-8} to 10^{-9}M. After the radioactive hormone was added to the cytosols, the mixture was incubated at 4°C for time periods ranging from 30 to 90 min. In some experiments the cytosols were labeled with radioactive hormone, frozen in dry ice-acetone and lyophilized overnight. Lyophilized cytosols could be stored for up to 30 days without a significant loss of bound hormone. The specific details related to each experiment are described below.

The effect of age

In the experiments designed to study the effect of the time after adrenalectomy on the amount of [^{3}H]-corticosterone bound to brain cytosol proteins, a group of 65 rats were adrenalectomized during a one month period, and randomly assigned to experimental groups (days post-adrenalectomy) for autopsy. The animals were sacrificed in groups of three over a period of six months and the amount of [^{3}H]-corticosterone bound by the cytosol of each rat brain was determined. The weight of the animal at sacrifice was noted and subsequently was used to estimate its age. The control animals for this experiment were 28 rats of different body weights, sacrificed three days after adrenalectomy.

Newborn rats were adrenalectomized and 24 h later were anesthetized with ether (between 12 noon and 1:30 p.m. of the following day). Blood was collected from the heart for assay of corticosterone. The animals then were perfused with cold 0·25 M sucrose in Tris buffer through the heart to reduce the amount of residual blood (CBG) in the brain. As many as four brains were pooled, weighed and homogenized in 5 vol. ice-cold Tris–EDTA buffer and cytosols prepared as described above. One ml aliquots of cytosols were incubated with [^{3}H]-corticosterone in the concentration of 10^{-8} M for 90 min at 4°C. The amount of [^{3}H]-corticosterone bound to the protein was determined using the charcoal adsorption method.

Gel chromatography

Chromatography was performed by gel filtration on Sephadex G-25 (Pharmacia, Uppsala, Sweden). The buffer used for elution was the Tris–EDTA buffer used for homogenization. Binding was determined by measuring the amount of radioactivity per mg of protein excluded from the gel phase. Protein concentration was determined by the method of Lowry *et al.* [7].

Charcoal adsorption method

Activated charcoal (Norit A, Sigma) was coated with Dextran T-70 (Pharmacia, Uppsala, Sweden) in Tris–EDTA buffer. A 10% suspension of charcoal was made in a 1% solution of Dextran T-70. Fifty μl of the charcoal-Dextran mixture was added to 0·4 ml of cytosol and after 4–6 min centrifuged at 12,000 *g* for 2·5 min. Following centrifugation, the supernatant was sampled for radioactivity and protein. The data are expressed as disintegrations per min per mg of protein. Non-specific binding was determined by incubating cytosols with [^{3}H]-hormone in the presence of a 1000 times M concentration of non-radioactive hormone.

Assays for blood corticosterone

The amount of corticosterone circulating in the blood was determined fluorometrically according to the method of Verniko-Danellis *et al.* [8].

RESULTS

The selectivity and stereospecificity of the corticosterone receptor molecule can be demonstrated *in vivo* [1] and *in vitro* [3]. Molar concentrations of non-radioactive hormone 25 times that of the [^{3}H]-corticosterone were able to affect the binding of this hormone. Hormones such as estradiol-17β,dehydrocorticosterone and testosterone and the stereo-isomer of the natural hormone, 11α-corticosterone, had very little effect on the binding of [^{3}H]-corticosterone. Other hormones such as aldosterone and cortisol-21-acetate reduced the binding of [^{3}H]-corticosterone only slightly. Dexamethasone, a potent synthetic hormone, and 11-deoxycortisol, with no known biological activity, decreased the binding approximately 50%. Corticosterone and cortisol were most effective in reducing the amount of [^{3}H]-corticosterone bound. These studies demonstrated that the corticosterone receptor molecule in rat brain cytosol has selectivity and is stereospecific for the 11β-isomer [3].

The saturation of the receptor molecule with increasing concentrations of [^{3}H]-corticosterone are shown in Fig. 1. In this experiment the specific and non-specific binding of [^{3}H]-corticosterone were determined by using Dextran-coated charcoal. The receptor molecule becomes saturated at about a 1·5 × 10^{-8} M concentration of [^{3}H]-corticosterone. The non-specific binding of [^{3}H]-corticosterone increases

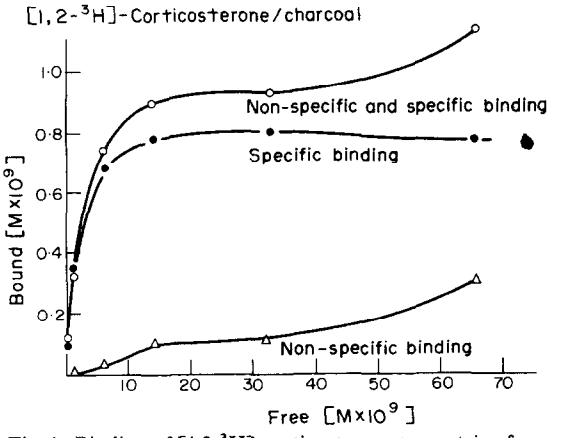


Fig. 1. Binding of [1,2-^{3}H]-corticosterone to proteins from cytosol of rat brain as a function of steroid concentration *in vitro*. Bound steroid was separated from free by charcoal adsorption of free steroid.

Table 1. The binding of [1,2,4-^{3}H]-dexamethasone, [1,2-^{3}H]-corticosterone and [1,2,4-^{3}H]-triamcinolone-acetonide to brain cytosol proteins

Radioactive Hormone	Fresh	Lyophilized
Dexamethasone	8885 ± 919	9861 ± 361*
Corticosterone	12692 ± 205	12264 ± 178
Triamcinolone Acetonide	7924 ± 1587	7029 ± 66

* d.p.m./mg protein. Mean ± standard deviation.
Radioactive hormones were added at time of homogenization. Data were corrected to a specific activity for each hormone of 30 ci/MM.

as the hormone concentration increases. In a similar study with dexamethasone, the receptor protein was saturated at a lower concentration of synthetic hormone.

The binding of [^{3}H]-dexamethasone, [^{3}H]-corticosterone and [^{3}H]-triamcinoloneacetonide were compared *in vitro* (Table 1). In this experiment brains were homogenized in the presence of 10^{-9} M concentration of either corticosterone, dexamethasone or triamcinoloneacetonide. Corticosterone was bound to a greater extent (per mg/protein) by brain cytosol proteins *in vitro* than either triamcinoloneacetonide or dexamethasone. Lyophilization had little or no effect on the binding of the steroid if the steroid–receptor complex is formed before lyophilization.

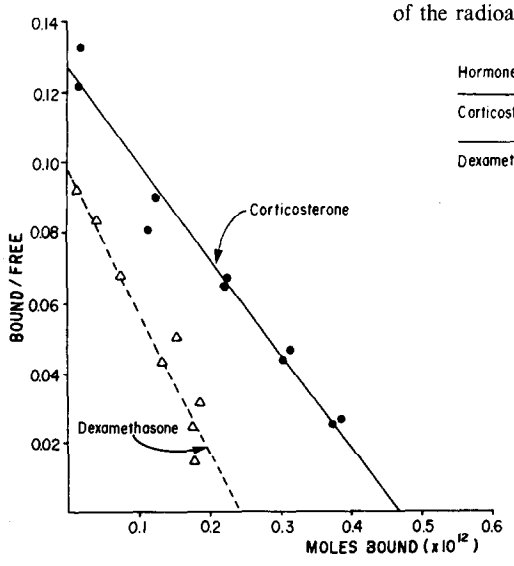
Since the amount of [^{3}H]-corticosterone and [^{3}H]-dexamethasone bound was different, it was important to determine whether the binding constants for [^{3}H]-corticosterone and [^{3}H]-dexamethasone were similar. Therefore, cytosols prepared from brains of adrenalectomized rats were pooled and 1 ml aliquots were incubated with either [^{3}H]-corticosterone or [^{3}H]-dexamethasone in concentrations from 10^{-8} to 10^{-10} M. In Fig. 2 the data obtained for the binding of [^{3}H]-corticosterone and [^{3}H]-dexamethasone are plotted as described by Scatchard[12]. The dissociation constants for corticosterone and dexamethasone differed by a factor of approximately 1·5. Also, a significant difference in the amount of corticosterone and dexamethasone bound per mg/protein was seen. Approximately two times as much corticosterone was bound per mg of protein under these conditions as was dexamethasone. The correlation coefficients for the fit of the line are very high.

The relative selectivity of the receptor molecule for [^{3}H]-dexamethasone then was compared to previous results obtained with [^{3}H]-corticosterone by incubating [^{3}H]-dexamethasone in the presence of non-radioactive hormones with brain cytosols for 30 min at 4°C (Table 2). In these experiments the amount of either [^{3}H]-hormone bound to the receptor molecule was not affected by the presence of 17β-estradiol (Table 2). However, progesterone reduced the binding of [^{3}H]-dexamethasone to 30% and [^{3}H]-corticosterone to 44% of the control value. The presence of non-radioactive dexamethasone reduced the amount of [^{3}H]-dexamethasone and [^{3}H]-corticosterone bound to 20% and 53% respectively of the control value.

Table 2. The effects of non-radioactive steroids on [1,2-^{3}H]-corticosterone and [1,2,4-^{3}H]-dexamethasone binding to brain cytosol proteins *in vitro*

Steroid*	[1,2-^{3}H]-Corticosterone n	% of Control	[1,2,4-^{3}H]-Dexamethasone n	% of Control
17β-Estradiol	2	96	8	82
11α-Corticosterone	4	77	-	--
Progesterone	4	44	8	30
Cortisol	2	25	8	33
Dexamethasone	4	53	8	20
Triamcinolone-acetonide	-	--	8	20
Corticosterone	4	16	8	26

* Non-radioactive steroids were added to each incubation flask in a molar concentration 25 times that of the radioactive hormone.



Hormone	K dissoc	n	r
Corticosterone	3.8×10^{-9}	$\frac{4.7 \times 10^{-13}\text{m}}{\text{mg Prot.}}$	0.98
Dexamethasone	2.5×10^{-9}	$\frac{2.4 \times 10^{-13}\text{m}}{\text{mg Prot.}}$	0.94

Fig. 2. Scatchard analysis of the binding of [1,2-^{3}H]-corticosterone and [1,2,4-^{3}H]-dexamethasone to proteins from cytosols of rat brain. Concentration of radioactive hormones was 3×10^{-10} M to $3{\cdot}5 \times 10^{-8}$ M.

Table 3. The binding of [1,2,4-^{3}H]-dexamethasone and [1,2-^{3}H]-corticosterone to brain cytosol proteins from rats treated with 0·1 mg non-radioactive hormones

Radioactive Hormone	Non-Radioactive Hormone			
	n	Dexamethasone	n	Corticosterone
[1,2-^{3}H]-Corticosterone	3	3927 ± 545*	3	1241 ± 25
[1,2,4-^{3}H]-Dexamethasone	3	1097 ± 152	3	657 ± 30

* dpm/mg protein. Mean ± standard deviation.

Non-radioactive corticosterone also was the most effective in preventing the binding of [^{3}H]-corticosterone and was very effective in blocking the uptake of [^{3}H]-dexamethasone. These data indicate that [^{3}H]-corticosterone and [^{3}H]-dexamethasone may share some, but not all, binding sites on the receptor protein *in vitro*.

These data from the *in vitro* experiment raised the question as to whether dexamethasone and corticosterone share the same binding sites *in vivo*. To answer this, adrenalectomized rats were pre-treated with 0·1 mg of either non-radioactive dexamethasone or non-radioactive corticosterone 30 min prior to sacrifice. At the time of sacrifice, cytosols were prepared and 1 ml aliquots of the cytosol from each pre-treated group were incubated with either 10^{-9}M [^{3}H]-corticosterone or 10^{-9} M [^{3}H]-dexamethasone for 15 min at 4°C. These *in vivo* results also indicate that some, but not all, receptor sites for [^{3}H]-corticosterone can be filled with dexamethasone whereas corticosterone probably fills all of the specific receptor sites for [^{3}H]-dexamethasone (Table 3).

In view of the described physical and biological properties of the receptor molecules, it is desirable to ascertain whether the binding of corticosterone to these molecules is in some way related to normal physiological processes. If the binding of [^{3}H]-corticosterone to brain cytosol protein is a part of a neuroendocrine control mechanism, then the amount of endogenous hormone in the brain should vary in response to the amount of corticosterone circulating in the blood. Therefore, the diurnal variation in the amount of [^{3}H]-corticosterone bound in rat brain cytosols from intact animals has been studied during a 24 h day [9]. The binding of [^{3}H]-corticosterone to brain cytosol was maximal at 1200 h with the minimum at 2000 h. The time of minimum binding of [^{3}H]-corticosterone to cytosol protein was coincident with the highest level of endogenous corticosterone in the blood, and with the extinguishing of the lights at 2000 h. Similar observations were made in the isolated hippocampus, hypothalamus and septum from rats treated in an identical manner. In adrenalectomized animals, the amount of [^{3}H]-corticosterone bound at 1200 and 2000 h was the same, *i.e.*, removal of the adrenal eliminated the diurnal variation in the amount of [^{3}H]-corticosterone bound.

The next experiments were designed to measure the quantity of [^{3}H]-corticosterone bound to brain cytosol protein during the first 72 h post-adrenalectomy. The amount of corticosterone bound, rapidly increased in the first 24 h (22,000 d.p.m. mg protein) post-adrenalectomy, and over the next 48 h, this value increased only slightly (Fig. 3). Concommitantly, the plasma corticosterone levels decreased during the first 12 h and remained constant for the rest of the experiment. As the concentration of endogenous corticosterone in blood decreased, the endogenous corticosterone bound to brain receptor protein dissociates, thus more receptor sites are available to be filled with [^{3}H]-corticosterone. When a similar study was extended to cover a period of six months after adrenalectomy, it was observed that after the first three to four days there is a gradual increase in the amount of [^{3}H]-corticosterone bound per mg protein. By 30 days post-adrenalectomy, the amount of [^{3}H]-corticosterone bound was considerably greater than at earlier times (Fig. 4). The shaded area represents the average amount of [^{3}H]-corticosterone bound in a control group of 28 animals of varying weights (ages) all three days post-adrenalectomy. By 60 days, the amount of corticosterone bound has decreased to control values and it remains at this level until 120 days. By 180 days post-adrenalectomy, the quantity of bound [^{3}H]-corticosterone was less than that in our control group. The plasma level of corticosterone

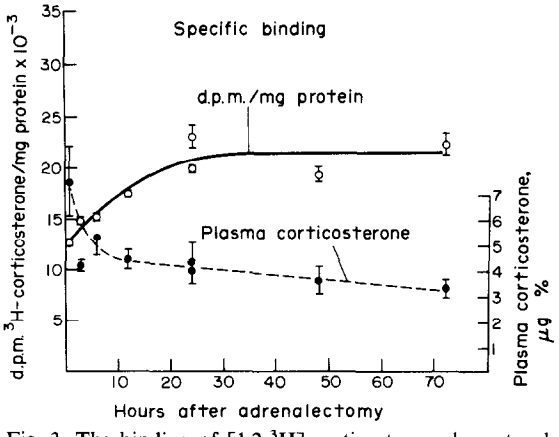


Fig. 3. The binding of [1,2-^{3}H]-corticosterone by cytosol protein from rat brain and plasma corticosterone at various times after bilateral adrenalectomy. Mean ± sem.

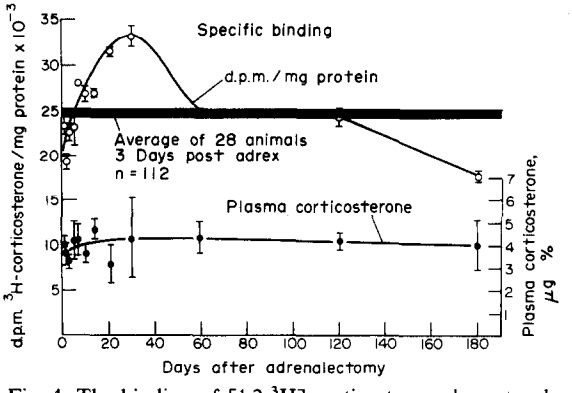


Fig. 4. The binding of [1,2-^{3}H]-corticosterone by cytosol protein from rat brain and plasma corticosterone at various times after bilateral adrenalectomy. Mean ± sem.

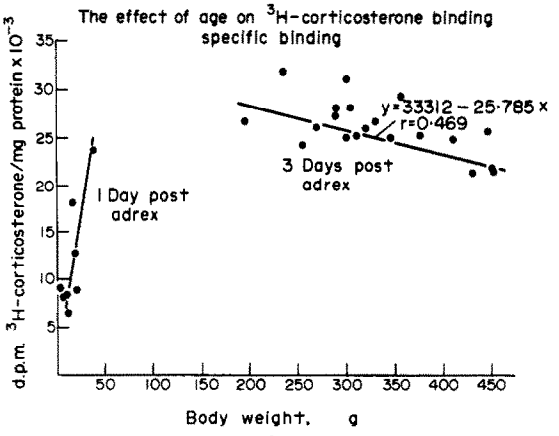


Fig. 5. The binding of [1,2-^{3}H]-corticosterone by cytosol protein from brains of adrenalectomized rats of different weights (age).

remained constant throughout this period, indicating that there was no regrowth of adrenal tissue during the 6 months experimental period.

If the data from the 28 control animals are plotted as a function of weight, a decrease in the amount of [^{3}H]-corticosterone bound by brain cytosol three days after adrenalectomy is seen as body weight increases. This is true in the animals weighting from 195–450 g (Fig. 5). In newborn rats studied one day after adrenalectomy, the opposite is true. As the animals increase in size, the capacity of brain protein to bind [^{3}H]-corticosterone also increases. Data have not been obtained for animals weighing between 50–190 g and accordingly must be obtained to determine when binding begins to decline to ascertain when this change can be correlated with other maturational events [10].

If data presented on the previous figure are re-plotted against days of age rather than body weight, a slightly different pattern emerges. During the first eight days the amount of [^{3}H]-corticosterone bound is quite low, but begins to increase by the 10th day, and by 20 days of age is very nearly up to the adult level (Fig. 6).

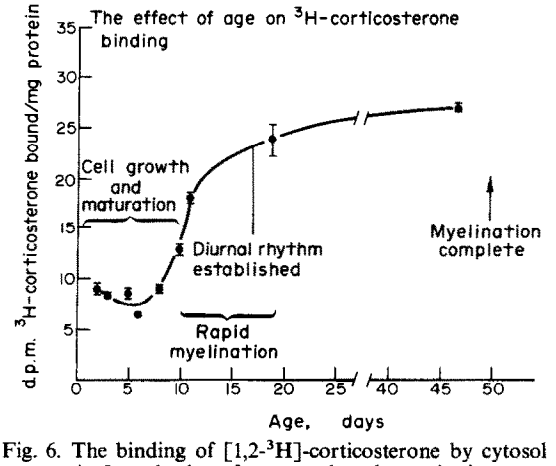


Fig. 6. The binding of [1,2-^{3}H]-corticosterone by cytosol protein from brains of young adrenalectomized rats.

DISCUSSION

The selectivity of the brain cytosol receptor has been demonstrated *in vitro*. Surprisingly, dexamethasone, a potent synthetic glucocorticoid with marked effects on the CNS, was not very effective in blocking the uptake of [^{3}H]-corticosterone. Progesterone was more effective than dexamethasone *in vitro* but previous studies *in vivo* have shown that progesterone did not prevent the binding of [^{3}H]-corticosterone to brain receptors [3]. The fact that 11α-corticosterone could not block binding of [^{3}H]-corticosterone demonstrates that the receptor protein also exhibits stereospecificity.

In *in vitro* experiments in which brain cytosol was incubated with different molar concentrations of [^{3}H]-corticosterone, the specific receptor sites were rapidly saturated at a concentration of approximately $1{\cdot}5 \times 10^{-8}$ M. The results were obtained with a charcoal adsorption method where specific and non-specific binding can easily be separated.

Although dexamethasone does bind to the receptor molecules, it does not fill all the available receptor sites for [^{3}H]-corticosterone. In kinetic studies the receptor molecule was saturated at a lower concentration of [^{3}H]-dexamethasone than [^{3}H]-corticosterone. Another potent synthetic hormone, [^{3}H]-triamcinolone-acetonide, had binding characteristics similar to [^{3}H]-dexamethasone. This is consistent with data reported by Chytil and Toft for [^{3}H]-triamcinolone-acetonide [11]. Results of Scatchard analysis [12], comparing [^{3}H]-dexamethasone and [^{3}H]-corticosterone binding show that about 50% less dexamethasone is bound per mg of protein than is corticosterone although the dissociation constants are similar.

In *in vitro* studies comparing the ability of [^{3}H]-corticosterone or [^{3}H]-dexamethasone to compete for binding sites with 25 times concentration of non-radioactive hormone, corticosterone was more effective in competing for receptor sites than was dexamethasone. These results together with those obtained by pre-injecting animals with either non-radioactive corticosterone or non-radioactive dexamethasone and measuring the binding of the respective [^{3}H]-hormone indicate that corticosterone can fill all of the dexamethasone receptor sites but that dexamethasone can fill only some of the corticosterone receptor sites. Even though triamcinolone-acetonide and dexamethasone are potent glucocorticoids, the receptor protein in rat brain cytosol is capable of binding more corticosterone than either of these synthetic glucocorticoids. This is an enigma which must be solved before the mechanism for the suppression of ACTH secretion by these hormones can be correlated with these results. Results reported by other laboratories indicate that there may be three classes of receptor molecules, one for [^{3}H]-corticosterone, one for [^{3}H]-aldosterone and one for [^{3}H]-dexamethasone [13].

The diurnal variation in the amount of circulating endogenous corticosterone is accompanied by a similar change in the amount of hormone bound by pro-

teins of the brain cytosol. This caused a cyclic variation in the amount of [^{3}H]-corticosterone bound which was inversely proportional to the circulating levels of endogenous corticosterone. Thus if the receptor sites for corticosterone in the brain are mostly filled with endogenous hormone, less [^{3}H]-corticosterone can be bound and the inverse would also be true. This relationship can be obliterated by the removal of the adrenal glands, and thus a constant high level of [^{3}H]-corticosterone binding is present during the 24 h period. These data suggest that there is no diurnal alteration in the amount of receptor protein or receptor sites present in the brain. It appears that the receptor protein in the brain does bind endogenous hormone and the amount bound is directly related to the amount circulating in blood [9].

Subsequent studies demonstrate that there is a rapid dissociation of endogenous steroid from the receptor protein following the removal of the adrenals (Fig. 3). These vacated sites can then be filled with [^{3}H]-corticosterone *in vitro* and thus the rate at which the endogenous hormone leaves the receptor sites can be measured. By 24 h, the amount of [^{3}H]-corticosterone bound by the receptor protein is nearly equal to that seen in animals adrenalectomized for 72 h. It is difficult to determine whether the increased binding of [^{3}H]-corticosterone (d.p.m./mg protein) in adrenalectomized animals between the 12 h and 24 h group represents continued "washout" of endogenous steroid or indicates a synthesis of new receptor sites. Corticosterone blood levels in this experiment were minimal by 12 h. After adrenalectomy, similar increases of [^{3}H]-corticosterone binding has been reported in the hippocampus [14] for up to 7 days post-adrenalectomy. These studies were extended to much longer time periods after adrenalectomy when an increase was seen in the amount of [^{3}H]-corticosterone bound which reaches a peak at 30 days (Fig. 4). If the amount of corticosterone bound to receptor protein is involved in some negative feedback system, the increased binding capacity observed may be an attempt by the central nervous system to increase the amount of steroid-receptor complex. Since this cannot be accomplished by increased release of corticosterone due to the adrenalectomy, an alternate possibility would be to synthesize more receptor protein. This would suggest that the binding of corticosterone to receptor protein is involved in the measurements of the amount of endogenous hormones circulating in the blood. The subsequent decline in [^{3}H]-corticosterone binding to receptors during the next 30 days could reflect the return of the brain regulatory mechanism to normal receptor turnover rates in response to the continued absence of corticosterone. It is of interest that the amount of [^{3}H]-corticosterone bound at 180 days after adrenalectomy is nearly the same as that seen at 12 h after adrenalectomy. This decrease at 180 days may be due to adrenalectomy and ageing.

The amount of [^{3}H]-corticosterone bound to receptor protein decreases as the rat ages (Fig. 5). This may reflect the gradual senescence of the adaptive mechanism(s) which includes the endocrine regulatory system. Roth has reported a decrease in the amount of glucocorticoid bound in rat brain cytosols of old rats when two month and 25 month old animals were compared [15].

The old rat (22–32 months) has been reported to have a decreased sensitivity of its adrenocortical control mechanism to feedback inhibition when compared to young animals (4–6 months) [16]. The decreased amount of glucocorticoid bound per mg of protein reported in this paper and also by Roth [15], may be used to explain the decreased sensitivity of the adrenocortical control mechanism.

The changes in the amount of [^{3}H]-corticosterone bound to receptor proteins in brain cytosols in young animals [17] can be correlated with a number of important physiological changes which are occurring in the developing rat. In Fig. 6 some of the developmental stages in rat brain maturation are indicated [10]. However, more important are the correlations that can be made with the development of the adrenal-pituitary axis. The newborn rat is only slightly responsive to stress during the first few days after birth [18, 19]. This corresponds to the time when a small number of receptor sites are available for [^{3}H]-corticosterone binding. Thus there is a period in which the animal is non-responsive to stress [19] which corresponds to the period of lowest capacity for [^{3}H]-corticosterone binding (day 7). The amount of brain receptor protein available for binding corticosterone begins to increase on day 10 and continues to increase to day 19. This is the time that the stress response is developing and this response reaches near normal (adult) levels by day 21 [19].

Also, studies by Campbell and Ramaley have shown that a diurnal variation in circulating corticosterone is not present until day 17 in Sprague-Dawley rats and is dependent on the development of a retino-hypothalamic pathway to the supra-chiasmatic nucleus [20]. At this time the capacity of the receptor protein to bind corticosterone is nearly fully developed (Fig. 6). The experimental observations presented here together with those from the literature cited, strongly suggest that the soluble corticosterone receptor molecule in the rat central nervous system is intimately involved in the pituitary-adrenal regulatory mechanism.

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