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PUTATIVE ESTROGEN AND GLUCOCORTICOID RECEPTORS IN THE LIMBIC BRAIN

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SUMMARY

The brain is a target tissue for both estrogens and adrenal steroids. These hormones alter both neuroendocrine activity and behavior. Putative soluble (cytosol) and cell nuclear receptor sites are concentrated in anterior pituitary and in limbic regions of the brain: hypothalamus, preoptic region, and amygdala for estradiol, and hippocampus, septum, and amygdala for corticosterone. Recent experiments will be summarized dealing with the following aspects of our work: (1) the postnatal ontogeny of the estradiol-binding system in the limbic brain of the female rat and the temporal relationship of its development to another estradiol-binding protein, probably identical to the α-fetoprotein, which gradually disappears after birth; (2) effects of estradiol on activities of enzymes in pituitary and in brain regions which contain the putative receptors, which effects may relate to the function of the receptor sites; and (3) properties of the putative brain glucocorticoid receptors with particular emphasis on their binding of corticosterone, dexamethasone, and progesterone. Glucocorticoid receptor sites have been studied both *in vivo* and *in vitro* using cytosols and tissue slices, thus allowing us to study the cellular mechanism of binding and the extent of receptor occupation by the physiological range of endogenous corticosterone.

INTRODUCTION

An increasing body of information indicates that the brain and pituitary gland contain cells which are targets for both gonadal and adrenal steroids. Both classes of steroids exert important regulatory effects on the secretion of anterior pituitary trophic hormones. Prominent among these are the positive feedback action of estrogen in promoting the LH surge and the negative feedback action of potent synthetic glucocorticoids such as dexamethasone in suppressing ACTH secretion. It is now recognized that in addition to their neuroendocrine actions, these steroids also exert important regulatory effects upon neural processes underlying behavior. Such effects include the facilitation by gonadal steroids of sexual behavior and sexually dimorphic behaviors such as intraspecific aggression, and territorial marking (in rodents) and song (in birds). Adrenal steroids have been associated with alterations in mood, in thresholds for recognition and detection of sensory stimuli, in phases of sleep, and in active and passive avoidance behavior. (For recent summaries of the literature, see [1–3].)

Spectacular progress in the elucidation of cellular and molecular mechanisms of steroid hormone action has made available an attractive model in which putative receptor sites in the cytoplasm of target cells bind the hormone and then move into the cell nucleus where the hormone-receptor complex initiates changes in the transcription of specific genomic sites leading eventually to the biosynthesis of proteins which mediate the physiological response of the cell to the hormone [4]. The identification of specific, limited capacity binding sites for radiolabeled steroid hormones in the soluble (cytosol) and cell nuclear compartments of tissues is a relatively simple, direct and elegant means of detecting steroid responsive cells. This technique, when supplemented by autoradiographic studies which provide anatomical descriptions of the hormone-binding cells, has led to the identification in brain and pituitary of putative hormone responsive systems localized in anatomically meaningful patterns. The anatomical distribution has been shown in several instances to correspond to sites where direct implantation of the steroid brings about neuroendocrine and behavioral effects of that hormone (see [1, 2]).

The purpose of this paper is to summarize the overall development and recent progress of studies in our laboratory on the identification and the physiological significance of putative receptor systems for estradiol and for adrenal glucocorticoids in the brain and pituitary gland.

Putative estrogen receptors in the limbic brain: phylogenetic and developmental aspects

The existence of limited capacity, stereospecific binding sites for estrogen in the brains and pituitaries of female rodents has been known since the pioneering tissue uptake studies of Eisenfeld and Axelrod[5] and Kato and Villee[6]. Subsequent work has demonstrated that these sites reside in the cytosol fraction of tissues from ovariectomized female rats treated *in vivo* with [^{3}H]-estradiol (see [1, 2]). The studies of Zigmond in our laboratory were among the first to show in hypothalamus, preoptic area, and amygdala of ovariectomized

female rats the cell nuclear retention of [^{3}H]-estradiol which is stereospecific for estradiol 17β as opposed to estradiol 17α and testosterone [7]. These regional localizations for estrogen binding sites are consistent with autoradiographic data [8–11], which however also reveals lesser numbers of estrogen binding sites in hippocampus and midbrain [11]. From the autoradiographic work it is also evident that the predominant binding of estradiol is neuronal.

In an attempt to generalize from the rodent to the primate, where important work is underway on estrogenic regulation of behavior and neuroendocrine function [12, 13], we conducted experiments on the distribution of binding sites for [^{3}H]-estradiol in the pituitary and brain of female Rhesus monkeys. These studies were carried out in collaboration with Mr. John Gerlach and Dr. Donald Pfaff of this university and Drs. M. Ferin, E. Zimmerman and P. Carmel of Columbia University College of Physicians and Surgeons. They revealed a very similar concentration of all nuclear estrogen binding sites in uterus and anterior pituitary, and in the anterior hypothalamus and amygdala to that observed in the rat [14]. Autoradiographic investigations fully support these findings [14].

We have also been interested for some time in the developmental aspects of the putative estrogen receptor system in the brain, especially in view of reports that the female rat brain becomes sensitive to the induction by estradiol of precocious puberty sometime during the fourth postnatal week of life [15]. Examination of the relative amount of cytosol estrogen binding material in pooled hypothalamus, preoptic area, amygdala and midbrain revealed a pattern of increasing concentration of this material from detectability in the second postnatal week of life into adulthood [16]. Similar results have been reported by Kato[17, 18]. However, our experiments also revealed the existence in neonatal rat brains of another estrogen binding component which dwarfs in magnitude the adult-type binding protein [19]. This protein has the following unique characteristics: (1) it sediments at 4S in a 5–20% sucrose density gradient at low ionic strength; (2) it binds [^{3}H]-estradiol 17β but not estradiol 17α, testosterone, or diethylstilbestrol, the latter of which does bind to the adult binding protein; (3) unlike uterine receptor protein, the neonatal material does not bind to a DNA cellulose column; (4) it is present equally in male and female brains and is not localized to any brain region; and (5) it is present in large amounts in washes of whole brain tissue and has been detected in CSF. It is present after extensive perfusion of the brain to remove blood, an important consideration since we discovered that in the above-mentioned properties this protein resembles the serum estrogen binding protein described by Raynaud *et al.*[20]. It should be noted that even in unperfused brains of neonatal rats, the blood contribution to brain levels of this protein is no more than 13% [19]. We believe, however, that this protein, which is believed to be identical with the so-called α-fetoprotein (see [19]), originates from the blood by passage, perhaps, into the cerebrospinal fluid and resides in the extracellular fluid spaces of the brain where it may play a role in regulating the amount of circulating estrogen which can reach brain cells. It will be recalled that estradiol has deleterious and paradoxical "defeminizing" effects when administered in large quantities to neonatal female rats [21, 22].

The hypothesized protective effect of this neonatal estrogen binding protein is subject to further experimental testing. However, it is interesting to note that in the human fetus levels of α-fetoprotein are elevated and reach their peak during the presumed critical phase of sexual differentiation of the reproductive tract and brain, believed to occur during the second trimester of gestation [23].

We may have observed one aspect of the protective action of this estrogen binding α-fetoprotein in the brain during a study in neonatal rats of the *in vivo* accumulation by cell nuclei of physiological doses of [^{3}H]-estradiol [16]. In this study cell nuclear accumulation in pooled tissue from hypothalamus, preoptic area, amygdala, and midbrain of female rats was extremely low and not different from cerebral cortex until the fourth postnatal week of life [16], at which time levels of the estrogen binding α-fetoprotein are at or below the limits of detection in brain [19] and serum [20]. An attractive explanation for these findings is that the α-fetoprotein sequesters [^{3}H]-estradiol and reduces the amount available for binding.

Recent studies in our laboratory have revealed that there are detectable cell nuclear binding sites earlier than the fourth postnatal week of life. In these experiments, much larger doses of [^{3}H]-estradiol, 50 times or greater than those used in the study just described [16], do accumulate in significant amounts in cell nuclei of pituitary, hypothalamus, preoptic area, and amygdala at least as early as postnatal day 10 (McEwen, Chaptal and Plapinger, unpublished observations). Studies of the early neonatal period before day 10 are currently in progress in view of the report by Sheridan *et al.*[24] that estrogen binding sites are detectable by autoradiography in these brain regions as early as postnatal day 2. It will be important to examine the role of such sites as these in the neonatal defeminizing action of large doses of estrogen [21, 22]. The role of such sites in the physiological action of androgen in masculinizing the male brain also deserves consideration in view of recent evidence for conversion of testosterone to estradiol by neonatal brain tissue [25, 26].

Effects of gonadal steroids on biochemical parameters of brain and pituitary

That large doses of estrogen are able to alter chemical events in neonatal brain tissue in spite of

high levels of the neonatal estrogen binding protein is supported by recent work of Giulian in this laboratory [27], who showed that administration of 100 μg of estradiol benzoate or diethylstilbestral on day 1 results in elevated cerebral serotonin (5HT) levels on days 8–14 in brains of both sexes. These studies also demonstrated that administration of either testosterone propionate or 5-dihydrotestosterone propionate on day 1 reduced 5HT levels in female brains on days 10 and 14. These effects tend to mimic a normal sex difference in 5HT on days 10–14 in which females have higher 5HT levels than males [27, 28]. Ovariectomy of females on day 1 reduced 5HT levels in females on day 12, suggesting that ovarian secretion is at least in part responsible for the sex difference. While castration of males on day 1 produced in our hands a non-significant elevation of 5HT levels on day 12 [27], testicular secretion may also play a role in these sex differences [28]. Recent evidence indicates that alterations in at least one brain enzyme activity, monoamine oxidase (monoamine: O_2 oxidoreductase, EC 1.4.3.4) accompany these changes in 5HT levels but are opposite in direction to them [29]; Giulian and Luine, unpublished observations).

In the light of these provocative findings on neonatal brains and because of our interest in putative gonadal steroid receptors in immature and adult nervous tissue, Dr. Luine has undertaken to study gonadal steroid alterations in brain enzyme activities in order to obtain biochemical markers for hormone action which may also be useful for the understanding of sexual differentiation of the brain, as well as the role of the putative receptors [30–32]. Two classes of enzymes were selected for investigation: (1) enzymes of intermediary metabolism particularly those which generate reducing equivalents for reductive biosynthesis (*e.g.* glucose-6-phosphate dehydrogenase, isocitrate dehydrogenase); and (2) enzymes more directly associated with neuronal function (monoamine oxidase; choline-O-acetyltransferase).

The efficacy of this approach is perhaps best illustrated by studies of pituitary glucose-6-phosphate dehydrogenase activity. This enzyme is known to be elevated by estrogen treatment in uterine tissue [30] and it also is elevated by estrogen treatment in the pituitary gland [30]. The specificity of various steroids in elevating G6PDH activity in the pituitary of the ovariectomized female rat parallels very closely the binding specificity of the putative estrogen receptor sites: estradiol 17β and diethylstilbestrol are effective; estradiol 17α and testosterone are ineffective. The estrogen antagonist MER 25, which competes for the putative estrogen receptor sites [31, 32], prevents the estradiol induced increase in pituitary G6PDH activity [31].

Control experiments rule out several alternative explanations for the estrogen effect of G6PDH: estrogen addition to the enzyme assay mixture in concentrations from 10^{-8} to 10^{-6} M does not alter activity; food deprivation of ovariectomized rats, which induces weight loss mimicking the weight loss induced by estradiol, does not alter pituitary G6PDH activity [30, 32]. It would therefore seem that estradiol interaction with stereospecific receptor sites is the most likely mechanism for initiating the increase in pituitary G6PDH activity. It remains to be established by more rigorous techniques such as quantitative immunoprecipitation that an actual enzyme induction is involved.

Using this approach, Luine has found that estrogen treatment of ovariectomized female rats elevates activities of a number of enzymes in brain regions which contain putative estrogen receptor sites, namely, hypothalamus, preoptic area, and amygdala [30–32]. To obtain this information, it was necessary to devise a dissection procedure for fresh brain tissue which removed those portions of each of these regions in which estrogen binding sites are highly concentrated [11, 30] and even then maximal elevations of enzyme activities are only in the order of 30%, although they are highly reproducible. Table

Table 1. Estrogen effects on enzyme activities in responsive neuroendocrine tissues

Tissue	Enzyme* 1	2	3	4	5	6	7
Pituitary	+109	+75	ns	ns	+54	—	—
Basomedial hypothalamus	+25	ns	+34	+42	ns	−29	ns
Corticomedial amygdala	ns	ns	+31	+32	ns	−49	+23
Medial preoptic area	ns	ns	ns	ns	ns	ns	+32

* Per cent change from control.

Only statistically significant data are presented; ns—not significant; −1—enzyme not assayed.

A list of enzymes is presented below. Doses of estradiol benzoate are: for enzymes 1–6—30 μg/220 g body wt. for 7 days; for enzyme 7—30 μg/220 g body wt. for 3 days.

Data from [31] and [32]. Enzymes are listed by number: (1) G6PDH—D-glucose-6-phosphate; NADP oxidoreducture EC 1.1.1.49; (2) GPGDH—6-phospho-D-gluconate; NAD(P) oxidoreducture EC 1.1.1.43; (3) MDH—1-malate: NADP oxidoreducture (decarboxylating) EC 1.1.1.40; (4) ICDH—threo-Ds-isocotrate: NADP oxidoreducture (decarboxylating) EC 1.1.1.42; (5) LDH—1-lactate: NAD oxidoreducture e.c. 1.1.1.27; (6) MAO—monoamine: O_2 oxidoreductase EC 1.4.3.4; (7) ChAc—acetyl-CoA: choline O-acetyltransferase EC 2.3.1.6.

1 summarizes by brain region the significant changes, and it is interesting that brain regions such as hippocampus and cerebral cortex which do not contain large amounts of putative estrogen receptors are not responsive to estrogen with respect to any of the enzymes which have been studied. It should be noted that one enzyme, monoamine oxidase, is reduced in activity by estrogen treatment in ovariectomized female rats. The estrogen effects in females parallel those described for pituitary G6PDH activity in the following ways: (1) the effects occur with estradiol 17β but not with testosterone; (2) in at least two cases, monoamine oxidase and cholineacetylase, the anti-estrogen MER 25 prevents estrogen dependent alterations in enzyme activity; and (3) control experiments—adding estradiol to the enzyme assay mixture and food deprivation to induce body weight reduction—rule out alternative explanations for the estrogen effects (see above). Moreover, hyophysectomized female rats also respond to estradiol to induce the same kinds of changes in enzyme activities, with one important exception. Monoamine oxidase activity in hypothalamus is no longer reduced by estrogen treatment in hypophysectomized female rats even though MAO activity in amygdala is still reduced. These observations raise the possibility that pituitary hormones mediate the estrogen effect on hypothalamic MAO, although alternatives, such as damage induced in estrogen responsive hypothalmic cells by the surgical procedure of hypophysectomy, are not ruled out [30, 32].

The profound effects of sexual differentiation of the brain on biochemical parameters of responsiveness to gonadal steroids has been revealed by studies of estradiol and testosterone effects on many of the same enzymes in male brains which were investigated in female brains. As shown in Table 2, the estradiol treatment of castrated male rats elevates fewer enzymes in hypothalamus, preoptic region, and amygdala than were elevated in female brains [31, 32]. The fact that estrogen works at all is consistent with the observation that the male rat brain contains putative estrogen receptor sites in the same regional pattern as is found in the female brain. Somewhat more dramatic is the observation that while female brains do not respond to testosterone treatment with respect to the enzyme activities measured, male brains do respond to testosterone treatment and in many cases the response is greater than the corresponding change resulting from estradiol treatment (Table 2). Thus, for example choline acetylase activity in the preoptic region is elevated by 70% after 7 days of testosterone treatment. Monoamine oxidase activity in the medial preoptic region is also elevated by testosterone, and in no region studied does testosterone decrease MAO activity. The general impression is that the brain of the castrated male rat is decidedly more responsive to testosterone than is the brain of the ovariectomized female rat, while the reverse may be true with respect to the responsiveness to estradiol. Similar observations have been made for the sensitivity of the pituitary gland to testosterone and estradiol dependent increases in enzyme activities (Table 2). Further studies are required to firm up the attractive conclusion that these gonadal steroid effects are mediated by putative estrogen and androgen receptor sites in brain and pituitary and to test the hypothesis that the effects may represent a hormonal induction of enzyme formation.

Whatever the cellular mechanism of these effects, it is important to make several comments about the possible role of testosterone metabolism in the androgen-mediated alterations in enzyme activities.

Table 2. Comparison of estradiol and testosterone effects on enzyme activities in male and female pituitary and brain regions

Tissue	Hormone	Sex	Enzyme* 1	2	3	4	5	6	7
Pituitary	E†	F	+200	+29	ns	ns	+140	—	—
	E	M	+175	+28	ns	ns	+85	—	—
	T†	F	ns	ns	ns	ns	ns	—	—
	T	M	+55	+29	+20	+33	+40	—	—
Basomedial hypothalamus	E	F	+26	—	+31	+26	—	−29	ns
	E	M	+33	—	ns	ns	—	ns	ns
	T	F	ns	—	ns	ns	—	ns	ns
	T	M	+26	—	ns	+25	—	ns	ns
Corticomedial amygdala	E	F	—	—	+29	+32	—	−49	+23
	E	M	—	—	ns	+47	—	ns	ns
	T	F	—	—	ns	ns	—	ns	ns
	T	M	—	—	ns	ns	—	ns	ns
Medial preoptic area	E	F	ns	ns	ns	ns	ns	ns	+32
	E	M	—	—	—	—	—	ns	ns
	T	F	—	—	—	—	—	ns	ns
	T	M	—	—	—	—	—	+28	+70

* See legend for Table 1 for identification of enzymes.

Data are presented as per cent change from control. Only significant data are presented; ns—not significant, enzyme not assayed.

† Doses: estradiol benzoate—30 μm/220 g body wt./7 days (enzymes 1–6); 30 μg/220 g body wt./3 days (enzyme 7); testosterone propionate—28 μg/220 g body wt./7 days (enzymes 1–6); 28 μg/220 g body wt./3 days (enzyme 7).

Conversion of testosterone to 5α dihydrotestosterone (5α DHT) and to estradiol have both been described to occur in hypothalamus and limbic brain structures [33, 34]. The aromatization of testosterone [25, 26, 35] is, however, not an attractive explanation for those testosterone effects so far uncovered where estradiol is less effective in inducing the same response (see Table 2). Attempts to interfere with testosterone effects by anti-estrogens such as MER-25 remains an attractive means of further exploring this possibility. Likewise, treatment of castrated males with 5α DHT, which cannot itself be aromatized, is another means of extending these studies and probing the importance of the other major pathway of androgen metabolism in brain tissue.

Putative glucocorticoid receptors in neural and pituitary tissues

The demonstration of putative estradiol receptors in specific brain regions and in anterior pituitary prompted us to search for binding sites for another important group of steroids, the glucocorticoids. We were motivated to do so because of neuroendocrine and behavioral evidence noted in the introduction which points to important neural effects of these hormones. We are also interested in the possible similarity or uniqueness of the neuroanatomical distribution of putative receptor sites for different steroid hormones. It should be noted that autoradiographic and biochemical studies of the uptake and binding of [^{3}H]-testosterone pointed to a pattern of distribution for this steroid very similar to that observed for [^{3}H]-estradiol [1, 36, 37].

We asked ourselves whether the distribution of [^{3}H]-corticosterone would be different. Indeed it was different: as reported first in 1968, uptake and limited capacity binding of this hormone, the major glucocorticoid in the rat, is highest in the hippocampus and septum of adrenalectomized rats [38–40]. Cell fractionation experiments demonstrated the existence of both soluble (cytosol) and cell nuclear binding sites with considerable specificity for corticosterone not only in hippocampus but also in amygdala [1, 41]. Similar studies on another species, the Rhesus monkey, reveal a predominance of hippocampal cell nuclear binding sites for [^{3}H]-corticosterone [14]. Such sites were also found for both species in lesser concentrations in cerebral cortex, hypothalamus, midbrain and in the pituitary gland [1, 41–43]. Autoradiography has confirmed the high concentration of neuronal binding sites for [^{3}H]-corticosterone in hippocampus, septum, and amygdala and has affirmed that the cell nucleus is the site of highest hormone concentration [44]. At this point it should be stressed that, like [^{3}H]-estradiol, [^{3}H]-corticosterone attaches to its binding sites without any prior metabolic transformation [1, 7, 41]. It should also be pointed out that cell nuclear bound [^{3}H]-corticosterone can be recovered after salt extraction of the pellets of isolated nuclei as a high molecular weight complex with protein [45].

The soluble binding proteins for corticosterone in brain tissue, which were identified by Grosser and collaborators [46, 47] and in our laboratory [42], deserve special mention. They are distinctly different from the serum corticoid binding protein, transcortin or CBG, on the basis of the following criteria: (1) they are found in brain and pituitary tissue after extensive perfusion with Dextran–saline solution has removed detectable blood contamination and are found to be regionally distributed in brain like the cell nuclear binding of [^{3}H]-corticosterone [42]; (2) they bind not only [^{3}H]-corticosterone but also, unlike CBG, bind synthetic corticoids like [^{3}H]-dexamethasone and [^{3}H]-triamcinolone [42, 43, 48, 49]; (3) when labelled *in vivo*, they are quantitatively precipitated by protamine sulfate, while CBG is not [42]; (4) they migrate more slowly than CBG at low ionic strength in polyacrylamide gel electrophoresis [42]; and (5) unlike CBG, they have sulfhydryl groups which are essential for hormone binding [48].

Interaction of these proteins with progesterone has provided an important experimental tool for determining the relationship of cytosol to cell nuclear binding of glucocorticoids [48]. We found that [^{3}H]-progesterone binds to the cytosol binding proteins and that [^{3}H]-corticosterone binding can be inhibited by unlabelled progesterone. Also [^{3}H]-progesterone does not bind to cell nuclei of hippocampus in a limited capacity manner, thus suggesting that the cytosol [^{3}H]-progesterone complex does not transfer its radioactivity to the cell nucleus. However, unlabelled progesterone does inhibit the cell nuclear binding of [^{3}H]-corticosterone in hippocampus. Since it can only do so by occupying cytosol binding sites, we have concluded that the cytosol binding protein is an obligatory way-station in the passage of corticosterone to the cell nucleus [48]. This conclusion is in general agreement with the concept of the translocation of a cytosol hormone receptor complex to the cell nuclear compartment [4].

The potency of the synthetic glucocorticoid, dexamethasone, in suppressing ACTH secretion prompted Dr. de Kloet to investigate the uptake and binding of this steroid in pituitary and neural tissue of adrenalectomized rats. It came as somewhat of a surprise that [^{3}H]-dexamethasone injected intravenously concentrates especially in cell nuclei of the anterior pituitary gland and is found in much lower concentration in cell nuclei of hippocampus and hypothalamus, with very little difference between these two structures [43]. Studies of cell nuclear binding *in vitro* using a tissue slice preparation previously developed [48] also showed highest cell nuclear binding of [^{3}H]-dexamethasone in pituitary and very little difference in cell nuclear concentrations of this hormone in hippocampus and hypothalamus [43]. Careful comparison of the cytosol binding of [^{3}H]-corticosterone and [^{3}H]-dexamethasone in

pituitary, hippocampus, and hypothalamus revealed that binding activity toward dexamethasone decayed more rapidly than that toward corticosterone as a function of time after tissue homogenization. The decay was particularly pronounced in pituitary: 90′ after cell rupture, no specific binding of [^{3}H]-dexamethasone could be detected, while binding of [^{3}H]-corticosterone was substantial [43]. These results suggest that there may be more than one population of glucocorticoid binding sites in pituitary and brain tissue. One possible interpretation of such populations is that they represent the contribution of different cell types within the tissue. In the brain this might mean different binding proteins in neurons and glial cells: neuronal localization of [^{3}H]-corticosterone in hippocampus, septum, and amygdala is clearly established by autoradiography (see above); and there is now evidence that glial cells from rat brain in tissue culture contain glucocorticoid binding sites and respond to dexamethasone in low concentrations and corticosterone in somewhat higher concentrations leading to increases in the activity of glycerol phosphate dehydrogenase [50, 51]. It should also be pointed out that the preference of [^{3}H]-dexamethasone for binding sites in the anterior pituitary is consistent with a growing body of information pointing to the pituitary as the primary site of action for this steroid in suppressing ACTH secretion [43]. It remains to be established to what degree corticosterone interaction with these sites may be involved in the physiological effects of this natural glucocorticoid on ACTH secretion which are revealed, for example, by the compensatory increase in ACTH secretion following adrenalectomy.

Putative corticosterone receptor sites in hippocampus appear to operate within the physiological range of corticosterone concentrations observed in basal and stress conditions. As shown by Stevens *et al.*[52] cytosol binding sites are occupied more fully at the diurnal peak of corticosterone secretion than at the diurnal trough. We have estimated that at corticosterone leads in the morning of 4 μg% as many as 40% of the cell nuclear sites in hippocampus may be occupied, while at stress levels of corticosterone as much as 75% of the cell nuclear capacity may be filled [53]. The removal of adrenal secretion by adrenalectomy is associated with other changes in the binding sites for corticosterone. Within approximately 2–6 h of bilateral adrenalectomy, available cytosol and cell nuclear binding sites in hippocampus increase and level off in concentration until the 11th or 12th h [53]. Thereafter, we have detected a further increase over approximately 48 or 72 h to a new plateau of binding activity which we interpret to mean either: (a) that further endogenous steroid has escaped from the binding sites; or (b) that the binding cells have produced more binding protein [53]. We are presently unable to distinguish between these two possibilities, although the former appears less likely since blood levels of corticosterone decrease below limits of detectability by acid fluorescence within one or two hours after adrenalectomy and since our radioisotope studies of the time course of [^{3}H]-corticosterone retention indicate that once the peak of binding occurs in cell nuclei at 1 h, levels of radioactivity decline rapidly and lag behind declining blood levels by only 1–2 h [39, 41, 48].

CONCLUSION

The studies summarized in this paper are part of a growing body of knowledge that the brain as well as the pituitary gland contain cells which are responsive to steroid hormones and which are involved in regulating behavior as well as neuroendocrine function. Our own work has helped to establish more firmly the view that neural tissue, in spite of its anatomical and functional complexity and in spite of the relative paucity of putative hormone receptor sites, is amenable to biochemical investigations of the mechanism of steroid hormone action.

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