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ADRENAL STEROIDS AS MODULATORS OF NERVE CELL FUNCTION

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Summary—Adrenal steroids modulate the function of nerve cells. Some, but not all actions of these steroids take place after binding to intracellular receptor systems and translocation of the steroid–receptor complex into the cell nucleus. Studies on the rat brain revealed heterogeneity of receptors. One population of receptor sites is present in abundance in extrahypothalamic limbic brain regions, e.g. neurons of the hippocampus, septum and amygdala. This neuronal receptor system displays a stringent binding specificity towards corticosterone, which is the naturally occurring glucocorticoid of the rat.

Focussing the studies on the corticosterone receptor system in hippocampal neurons has provided further insight in the understanding of some of the actions of the steroid. Certain hippocampus-associated behaviors and indices of neurotransmission (serotonin) were disturbed after removal of the adrenals, but selectively restored after replacement with a low dose of corticosterone. The specificity, localization and dose-dependency of the corticosterone action on behavior and neurotransmission corresponds to the properties of its receptor system.

The responsiveness to corticosterone is altered after changes in number of receptor sites. Chronic stress or high doses of exogenous corticosterone cause a long-term reduction. Other factors involved in regulation of receptor number are the neurotransmitter serotonin and neuropeptides related to ACTH and vasopressin. These substances restore changes in number of hippocampal corticosterone receptor sites due to aging, endocrine or neural deficiencies. Our results show that the number of corticosterone receptors is a sensitive index for brain functioning. Thus, the receptor system mediates some of the modulatory actions of corticosterone on nerve cell function and it may adjust its capacity under the influence of neural and endocrine factors.

INTRODUCTION

Adrenal steroids have a potent influence on brain functioning. Glucocorticoids affect growth and differentiation of nerve cells [1], and have an important action on neuronal processes involved in the expression of mood [2], sleep [3] and adaptation of the organism to the environment [4]. Excess glucocorticoids promote psychopathology [2] and aspects of brain aging [5]. Mineralocorticoids regulate the electrolyte balance, salt appetite [6] and under certain conditions are involved in adapative processes as well [7].

Glucocorticoids are secreted from the adrenal in response to ACTH of the anterior pituitary, which in turn is released in response to CRF's from hypothalamic (CRH or vasopressin) origin. Glucocorticoids feedback on brain and pituitary to block the release and synthesis of ACTH and CRF's [8, 9]. The activity of this pituitary-adrenal system at rest is on the one hand a function of the net result of all inhibitory and stimulatory influences converging on the hypothalamic CRF cells and the magnitude of the corticoid feedback signal. The feedback by glucocorticoids easily can be overridden by excitatory impulses, that are non-specifically termed stress [10]: pain, blood loss, loud noise, changes in environment, anxiety or frustration. In fact every internal or external evoked disturbance in homeostasis activates the pituitary-adrenal system.

It has been suggested that the essential property of all environmental stressors is the ability to elicit arousal that results in the neuroendocrine response and a behavioral response aimed to fight, flight or to cope with the threat. Glucocorticoids that are released in response to the stress serve the restoration of depleted energy resources and behavioral adaptation. Glucocorticoids also protect the organism against stressful stimuli. In the brain these steroids regulate in higher brain centres neuronal processes, that are involved in interpretation of sensoric stimuli [11] and expression of the behavioral and neuroendocrine response. In this way glucocorticoids serve the organism in coping with the threat (Fig. 1).

Corticoids exert the action on the brain by modulation of electrolyte balance, of energy and neurotransmitter metabolism and of electrical signals [12, 13]. The steroids have a slow action on neuronal functioning and may induce long-lasting changes in cell metabolism. This contrasts with the autonomic responses to stress, that produce rapid adaptive changes in many organs including the brain. Steroid hormones also have direct actions on the cell membrane [14], but these actions are less well understood and will not be discussed. Neurons, that respond to steroid hormones in general have intracellular receptor sites. Such sites have been identified for gluco- and mineralocorticoids in the brain. The localization and properties of these receptors have provided the criteria to study the biochemical,

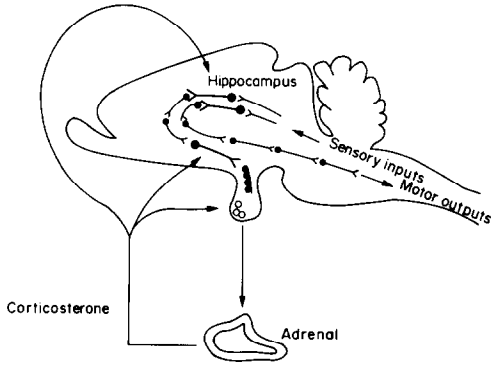

Fig. 1. Feedback action of corticosterone on brain and pituitary. Corticosterone blocks hypothalamic CRH release and pituitary ACTH release. In higher brain centres (hippocampus, septum) corticosterone exerts a modulatory influence on the neuroendocrine and behavioral response to changes in the environment.

neuroendocrinological and behavioral aspects of corticoid action: In the present report these aspects will be discussed mainly for corticosterone, which is the rat's adrenal glucocorticoid.

A RECEPTOR SYSTEM IN LIMBIC NEURONS SPECIFIC FOR CORTICOSTERONE

Autoradiography clearly showed that corticosterone retaining neurons are mainly localized in the hippocampus (pyramidal neurons and granular neurons of the dentate gyrus), dorsolateral septum and regions of the amygdala and cerebral cortex [15–18]. The steroid was found concentrated in the cell nuclei. This observation is not restricted to the rat, but has also been made in many other species suggesting that such limbic neurons are common target cells for corticosteroids, irrespective of whether the principal adrenal glucocorticoid is corticosterone or cortisol [19]. The neuroanatomical localization is very different from that of steroids related to reproductive functions. Estrogens and androgens are accumulated and retained by neurons in the preoptic area, lateral septum and parts of the hypothalamus [20]. Retention of corticosterone in neurons of the hypothalamus is low, even in the neurons of the paraventricular nucleus, recently shown to be the site of synthesis of CRH [18, 21]. Some accumulation is found in the anterior pituitary and in glial cells [22].

A remarkable finding was that the potent synthetic glucocorticoid dexamethasone displayed a completely different neuroanatomical pattern of distribution in the brain. Retention of this steroid by limbic neurons is low [16, 22–24]. The most pronounced retention occurs in endothelial cells around blood vessels, epithelial cells lining the ventricles and the choroid plexus [23]. In contrast, dexamethasone is found retained in particular in the ACTH producing cells of the anterior pituitary [22, 25].

The only other steroid that showed a comparable anatomical retention pattern with corticosterone is aldosterone [26–28], albeit that the uptake of this steroid occurred more rapidly, but never exceeded half the amount of corticosterone, when administered in equimolar doses. In contrast, the retention of the other mineralocorticoid, deoxycorticosterone, is very low [24]. In all these studies it is essential that the adrenals are removed. The endogenous corticosterone circulates in sufficient amounts to prevent retention by receptor sites of the tracer amount of [^{3}H]corticosterone of [^{3}H]aldosterone.

Cell fractionation studies of brain regions containing the corticosterone retaining neurons confirmed the autoradiographical data [22, 24, 29]. These studies indicated high levels of cell nuclear retention of corticosterone in limbic brain regions. Pretreatment with excess of aldosterone or deoxycorticosterone, but not of dexamethasone blocked the uptake of cortocosterone [28], suggesting that the former two steroids can compete for the corticosterone receptor sites. In cytosol, these sites displayed highest affinity for corticosterone (Table 1) [28, 30].

The binding studies have helped to understand the heterogeneity of adrenal steroid receptor sites in the brain. One population is occupied by the "pure" glucocorticoid RU 26988 [28, 31]. The sites represent classical glucocorticoid receptors as they also are found in glucocorticoid target cells in the periphery including the ACTH cells of the anterior pituitary. In the brain their presumptive localization is in glial cells [9, 32]. Dexamethasone is more potent than corticosterone in affecting glial enzymes such as glycerol-6-phosphate dehydrogenase [33] or ornithine decarboxylase [34, 35].

The sites that remain unoccupied by RU 26988 have highest affinity for corticosterone [28, 39], among steroid structures related to adrenal steroids (Table 1). Moreover, corticosterone shows the most extensive retention in neuronal cell nuclei and a number of behavioral and neurotransmitter responses are selectively affected by this steroid (see below). The mineralocorticoid aldosterone also displays appreciable affinity to the corticosterone receptor and appears also translocated efficiently to the cell nucleus. Yet, aldosterone acts as antagonist to corticosterone (see below). Possibly, there is a separate set of specific mineralocorticoid receptor sites, in view of specific effects of the steroid on brain function [13, 28, 31, 36]. Moreover, the curvilinear Scatchard displayed by aldosterone indicates heterogeneity of binding sites. Small amounts of corticosterone can linearize this Scatchard plot, leaving about 10% of the sites available for high affinity aldosterone binding [28].

Specific effects of corticosterone on brain chemistry and function

We are beginning to recognize a number of actions of corticosterone on the brain that fulfil criteria on localization, binding specificity and capacity as prescribed by the corticosterone receptors. The strategy

Table 1. Relative binding affinity (RBA) of steroids related to the pregnane structure for binding sites labelled with [^{3}H]corticosterone in the presence of 100-fold excess of unlabelled steroid

Steroid	RBA*
Corticosterone	100
11-Deoxycorticosterone	33,5
Cortisol	28,5
Progesterone	20
Aldosterone	11,0
Dexamethasone	6,6
Triamcinolone acetonide	2,5

The concentration of unlabelled steroid (cL-IC_{50}) and competing steroid (cC-IC_{50}) required to decrease the binding measured in the absence of competitor by 50% were determined. The ratio (cL-IC_{50}/cC-IC_{50} × 100) represents the RBA.

used in this type of experiment is to define disturbances after removal of the adrenals, that should be restored with corticosterone replacement in doses not exceeding the physiological plasma level (Table 2).

Specific effects of corticosterone are found on [^{3}H]uridine uptake in cell RNA [37] and on [^{3}H]leucine incorporation in a 54 K protein species [38] as well as on Protein I [39], which is a constituent of neurotransmitter vesicles and on the neurotransmitter serotonin. Corticosterone acts on many aspects of serotonin metabolism. The steroid increases the activity of the rate-limiting biosynthetic enzyme, tryptophan hydroxylase [40]; the uptake of the precursor tryptophan [41]; the release of serotonin from synaptosomes *in vitro* [42] and reduces the number of receptor sites for serotonin in parts of the hippocampus (*Biegon, personal communication*). A convenient method for estimation of the serotonin synthesis rate is the determination of the rate of accumulation of serotonin and decline of 5-hydroxyindole acetic acid after administration of pargyline, which is a MAO blocker [43–45].

Serotonin neurons project from the midbrain raphe nuclei to the hippocampus [40]. Using the above mentioned method it was shown that corticosterone increased the serotonin synthesis rate simultaneously in the raphe nucleus (cell body region) as well as in the hippocampus (terminal) region [44–46]. Interestingly, the raphe neuron is devoid of corticosterone receptor sites [47]. These receptor sites are localized in the post-synaptically localized pyramidal neurons (see previous section). Thus, it was concluded that corticosterone induces a change in cellular metabolism to which the raphe neuron responds with increased serotonin synthesis and release. The nature of the chemical process altered in the pyramidal neurons is not known. It could concern an excitatory efferent projection of the hippocampus [48], since lesioning the efferent pathways of the hippocampus prevents the corticosterone induced increase in serotonin synthesis [49].

Table 2. Specific effect of corticosterone on hippocampus chemistry

Biochemical parameter	Effect	Ref.
Serotonin content	+	77
Serotonin synthesis rate	+	44,47
GABA reuptake	−	78
Norepinephrine receptor coupled adenylate cyclase	−	79
B_{max} β-adrenergic receptor sites	−	80
[^{3}H]uridine incorporation	+	37
[^{3}H]leucine incorporation	+	38
Protein I	+	39

+ Increase and − = decrease in biochemical parameter following replacement of adrenalectomized rats with corticosterone.

Corticosterone is the only agonist. All other steroids including aldosterone and dexamethasone are ineffective. These steroids act as antagonist when given prior to corticosterone [44, 47, 50]. Specificity of the corticosterone action, thus, corresponds to the specificity of the corticosterone receptor and reinforces the notion that corticosterone–receptor interaction in hippocampal neurons is required for maximal expression of serotonergic neurotransmission.

The next question was addressed to what brain functions are regulated by corticosterone via its receptor system. Such functions should be associated with the receptor containing cells in the limbic structures (hippocampus) and ultimately be expressed in an altered behavioral and neuroendocrine response. The hippocampus is involved in the interpretation of environmental stimuli and the underlying neuronal processes are modulated by corticosterone. For instance, removal of adrenals increases detection of stimuli, but decreases the ability to interprete and perform the appropriate response [6]. These typical expressions of hippocampal dysfunction are normalized by glucocorticoid replacement in primates as well as in rodents.

Certain behaviors of rats such as exploration of a novel environment, spatial orientation of the animal, and extinction of learned behaviors are associated with hippocampus. Thus exploratory activity [51] was reduced and extinction behaviors deficient after removal of the adrenals [52]. Replacement with a low dose of corticosterone, but not of other steroids restored these behaviors. Pretreatment with progesterone, aldosterone and even dexamethasone revealed antagonistic properties of these steroids [51, 52, 55]. Such subtle and specific actions of corticosterone are not easy to define in neuroendocrine regulation. Most studies concerned with feedback actions show that stress-induced ACTH release is blocked by synthetic and naturally occurring glucocorticoids via the classical glucocorticoid receptors in the pituitary [56]. Yet, there are studies on fast-feedback at the CRH neuron and on steroid control of circadian rhythmicity in pituitary-adrenal activity following hippocampal implants [57], that suggest specificity for the naturally occurring glucocorticoid.

In conclusion, the corticosterone effect on certain behaviors and serotonergic neurotransmission corresponds to the properties of the corticosterone receptor system in the hippocampus. These indices require corticosterone for full expression.

Regulation of receptor capacity for corticosterone in hippocampus

Receptor capacity for corticosterone in the hippocampus shows large changes during life span. On post-natal day 1, the lowest number of receptor sites is measured. Receptor number reaches adult level around four weeks of age [58,59] and declines at senescence [60–62]. In naive mature animals of the same age there is a large variability in receptor capacity [63]. Classification of animals on the basis of acquisition of a conditioned avoidance response showed that "good learners" have the highest number of corticosterone receptors [63]. Individual differences as well as the changes during life span may be genetically determined. However, in recent years there also have been factors defined that are involved in long-term regulation of receptor number. These factors are the proper hormone corticosterone, neurotransmitters, neuropeptides and changes induced by hippocampal lesion. There were no changes observed in apparent affinity but only changes in receptor capacity (Table 3).

High levels of corticosterone either produced by administration of exogenous hormone or evoked endogenously due to chronic stress or pathologic hyperactivity of the adrenals, caused a long-term reduction in receptor sites [64–68]. This is an important observation since downregulation may imply that subsensitivity to corticosterone develops, which may lead to reduced resistance to stress. The development of subsensitivity to corticosterone remains to be firmly established. Upregulation of receptor number is observed after surgical removal [69, 70] or chemical lesioning of part of the receptor containing neurons [71]; the number of receptor sites in the remaining cells displays a compensatory increase in order to make up for the loss of receptor containing cells. For instance, unilateral removal of the dorsal hippocampus causes after a week a considerable increase in number of receptor sites in the contralateral lobe [70]. A similar compensatory increase occurred in hippocampus pyramidal neurons that survived kainic acid lesion [71]. Such kainic acid lesioned rats displayed an increased responsiveness to corticosterone, that in part can be explained by a supersensitive receptor system after increase in receptor number.

Table 3. Regulators of corticosterone receptor capacity in hippocampus

	Down	Up	Ref.
Chronic stress	×		64,67,68
Exogenous corticosterone	×		65,66
Serotonin	×		72
Diabetes insipidus animals	×		73
+ vasopressin related peptides		×	74
Hypophysectomized animals		×	65,66
+ ACTH related peptides	×		65,66
Aged animals	×		60,61
+ ACTH related peptide		×	62
Hippocampus lesions		×	69,70,71

Table 4. Effect of ORG 2766 on hippocampal corticosterone receptor system of senescent rats

Experimental group	B_{max}	K_D
Old	177	4.1
Old + ORG 2766	227	4.1
Young	214	4.0

Binding constants from Scatchard analyses. B_{max} is expressed in fmol [^{3}H]corticosterone/μg cytosol protein. K_D in nM.

A long-term increase in receptor number also is observed after partial lesioning of serotonergic terminals in the hippocampus by infusion of the neurotoxic drug, 5,6-dihydroxytryptamine [72]. This finding further extends the interrelationship between corticosterone and serotonin. Corticosterone, as mentioned in the previous section, increases serotonin turnover, and reduces number of receptor sites; the reverse is observed after adrenalectomy. We now have shown, that when the serotonin system is hypoactive after the lesion, there is upregulation of corticosterone receptor number. Future studies should demonstrate that the effect of the increase in number of corticosterone receptor sites results in a larger steroid signal, that eventually restores serotonin turnover.

The role of neuropeptides was derived from studies with animal models characterized by a hereditary lack or a defect in utilization of a particular neuropeptide [73, 74]. An example is the animals of the Brattleboro strain homozygous for diabetes insipidus. Such rats have a hereditary lack in the synthesis of the antidiuretic hormone, arginine-vasopressin; which becomes manifest in the inability to retain water. Diabetes insipidus rats also have a severe memory disturbance. Receptor capacity for corticosterone in hippocampus and glucocorticoid receptors in anterior pituitary, but not in other brain regions, was about 35% less than homozygous normal Brattleboro rats [73, 74]. Chronic treatment with arginine-vasopressin normalized the water balance, improved memory and restored receptor capacity towards normal. The same effect on brain function and receptor capacity was observed with desglycinamide-arginine-vasopressin, which is a peptide devoid of antidiuretic activity.

That the number of cortocosterone receptor sites may serve as a sensitive index for brain functioning became also apparent from studies with senescent rats treated with a behaviorally active peptide, the $ACTH_{4-9}$ analog ORG 2766. This peptide improves deficits in learning, displayed by aged rats [75] and has been found to improve mood of elderly people [75]. Moreover, chronic treatment with the peptide delays age-associated changes in hippocampus morphology [5] and speeds regeneration of injured peripheral nerves [76]. In a recent study we have shown that the number of receptor sites for corticosterone is reduced in the hippocampus of aged rats. Treatment with ORG 2766 infused with a subcuta-

neously implanted minipump (rate of release 0.5 μg/0.5 μl/h) restored the number of receptor sites to the level observed in young animals (Table 4).

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