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Glucocorticoid-sensitive hippocampal neurons are involved in terminating the adrenocortical stress response

(corticosterone/steroid receptor/aging/negative feedback)

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ABSTRACT **The hippocampus is the principal target site in the brain for adrenocortical steroids, as it has the highest concentration of receptor sites for glucocorticoids. The aged rat has a specific deficit in hippocampal glucocorticoid receptors, owing in large part to a loss of corticoid-sensitive neurons. This deficit may be the cause for the failure of aged rats to terminate corticosterone secretion at the end of stress, because extensive lesion and electrical stimulation studies have shown that the hippocampus exerts an inhibitory influence over adrenocortical activity and participates in glucocorticoid feedback. We have studied whether it is the loss of hippocampal neurons or of hippocampal glucocorticoid receptors in the aged rat that contributes most to this syndrome of corticosterone hypersecretion. To do this, we used two model systems for producing reversible glucocorticoid receptor depletion in the hippocampus, and we found that depletion of receptors without inducing cell loss results in corticosterone hypersecretion. Furthermore, correction of the receptor deficit results in normalization of corticosterone secretion. These results focus attention on the hippocampus as an important glucocorticoid sensor in relation to the stress response. They also provide important new physiological correlates for the remarkable plasticity of the hippocampal glucocorticoid receptor system, which is under independent control by corticosterone and by vasopressin.**

In the mammalian brain, the hippocampus contains the highest concentration of glucocorticoid receptors and retains the highest concentration of [^{3}H]corticosterone after *in vivo* injection (1). This phenomenon was initially reported in 1968 (2), and considerable speculation still remains regarding the physiological and behavioral significance of the hippocampal uptake system (1, 3). However, the hippocampus has been persistently implicated as an inhibitory influence on the hypothalamic–pituitary–adrenocortical (HPA) axis. Destruction of the hippocampus, for example, leads to hypersecretion of glucocorticoids under basal and stressed conditions (4–9). Furthermore, stimulation of most parts of the structure inhibits stress-induced HPA activation (10–13). At least some of this inhibitory influence of the hippocampus represents mediation of feedback inhibition by glucocorticoids. For example, hippocampectomized subjects are less sensitive to the suppressive effects of exogenous glucocorticoids on HPA secretion (6). Moreover, corticotropin (ACTH) is increased after hippocampectomy and the difference in corticotropin levels between lesioned and sham-lesioned animals is abolished by adrenalectomy, suggesting that the relative increase in corticotropin due to the lesion resulted from lesion-induced disinhibition from corticoid-feedback suppression (5). This evidence that circulating glucocorticoids exert some of their feedback effects via the hippocampus suggests that such actions are mediated by the hippocampal glucocorticoid receptor. Thus, one can postulate that decreased numbers of hippocampal glucocorticoid receptors might result in HPA hypersecretion. We present evidence for this hypothesis in the present study.

The aged male rat is relatively insensitive to the suppressive effects of exogenous glucocorticoids on the HPA axis (14). Furthermore, such subjects hypersecrete corticosterone, the principal glucocorticoid in the rat; specifically, aged male rats show a delay in terminating corticosterone secretion at the end of stress (15). The aged rat hippocampus has a loss of neurons (16–18) and, as a result, shows a decrease of corticosterone receptors (19). The lesion data cited above suggest that the age-related destruction of hippocampal neurons might account for the corticosterone hypersecretion at the end of stress. However, a loss of corticosterone receptors without cell loss might also lead to corticosterone hypersecretion. In this paper, we examine the dissociation between receptor loss and cell loss, and we show that the loss of corticosterone receptors is sufficient to produce the corticosterone hypersecretion. We have studied three rat models: aged rats, vasopressin-deficient Brattleboro rats, and chronically stressed rats. All show selective losses of hippocampal corticosterone receptors. The latter two models involve reversible receptor depletion without loss of hippocampal neurons. We show that decreased concentrations of hippocampal corticosterone receptors are associated with corticosterone hypersecretion following the end of stress.

MATERIALS AND METHODS

Subjects. Aged male rats (24- to 26-month-old Fischer 344 rats, obtained from the pathogen-free colony of the National Institute on Aging) shown to have a loss of hippocampal corticosterone receptors (19) were compared with 3- to 5-month old control rats.

Male Brattleboro rats (a strain derived from the Long–Evans strain and congenitally deficient in vasopressin) have a selective depletion of hippocampal corticosterone receptors that can be normalized by treatment with Des-glycinimide arginine vasopressin (dGVP), a centrally acting vasopressin analogue with no peripheral diuretic or corticotropin-releasing effects (18, 20). Subjects were injected s.c. daily for 7 days with 2 μg of dGVP dissolved in a slow-release zinc phosphate solution (18). Control subjects were injected with the zinc phosphate solution alone.

In the third model, Long–Evans male rats were subjected to daily intermittent stress for 7 days, a treatment that has been shown to preferentially and transiently down-regulate corticosterone receptors in the hippocampus (21). Subjects were exposed to sham adrenalectomy, vibration stress (1 hr), vasopressin (500 milliunits/kg, s.c.), cold (4°C for 4 hr),

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Abbreviations: HPA, hypothalamic–pituitary–adrenocortical; dGVP, Des-glycinimide arginine vasopressin.

ether (1 min), histamine (1 mg, i.m.), and immobilization (1 hr). Subjects were subjected to three stressors daily; all received the same stressors in the same sequence.

All subjects were given access to food and water *ad lib* and were maintained on a 14:10 light/dark cycle (lights on, 0500–1900 hr).

Cytosolic Binding Assays. Assays for cytosolic corticosterone receptors were conducted as described (21). Briefly, rats were decapitated 12 hr after adrenalectomy. Hippocampus was removed, homogenized in Tris·HCl, pH 7.4/1 mM EDTA/10 mM sodium molybdate/10% (wt/vol) glycerol/1 mM dithiothreitol, and spun at 1°C for 30 min at 105,000 × *g*. Extracts were incubated with [^{3}H]dexamethasone (5–40 nM) and for nonspecific binding with a 500-fold excess of unlabeled corticosterone. Macromolecular bound steroid was separated from free steroid with LH-20 columns, and the eluate was counted in Liquiscint. Cytosol protein concentrations were determined. B_{max} (fmol of cytosol protein per mg) and K_d (dissociation constant, mol/liter × 10^{-9}) were derived by Scatchard analysis.

Immobilization Stress and Measurement of Corticosterone. Corticosterone titers were measured in rats under basal, stress, and post-stress conditions. Rats were immobilized 1 hr into the light cycle in a restraint tube and then returned to their home cages to recover quietly. All blood samples were taken from the tail vein within 2 min of disturbing the subjects. Only a single recovery period blood sample was taken per subject. Corticosterone titers were determined by radioimmunoassay (22); intra- and interassay coefficients of variation were 0.08 and 0.10, respectively (n = 25).

Hippocampal Ablation. Rats were anesthetized and infused stereotaxically with the excitotoxin kainic acid. Infusions were made bilaterally with 2 μg of kainic acid into the hippocampus at each of the following coordinates (lambda = bregma): site A: AP, 4.1; ML, 2.1; DV, 2.8. Site B: AP, 3.0; ML, 3.4; DV, 3.2. Site C: AP, 1.8; ML, 3.8; DV, 5.0. Kainic acid was prepared in ascorbic-acidified saline, and injected with a Hamilton syringe in a 1-μl vol over 1 min, followed by a 1-min wait for the kainic acid to diffuse. Subjects were allowed 1 week to recover and then were given the immobilization stress test. After testing, subjects were perfused with 10% formalin, and brains were removed and stored in formalin for 5 days. Coronal sections (32-μm) of the hippocampus were cut and stained with cresyl violet. Damage to the hippocampus was assessed at ×40 magnification, and data (see below) are from subjects sustaining at least 50% destruction in all hippocampal cell fields over the length of the structure.

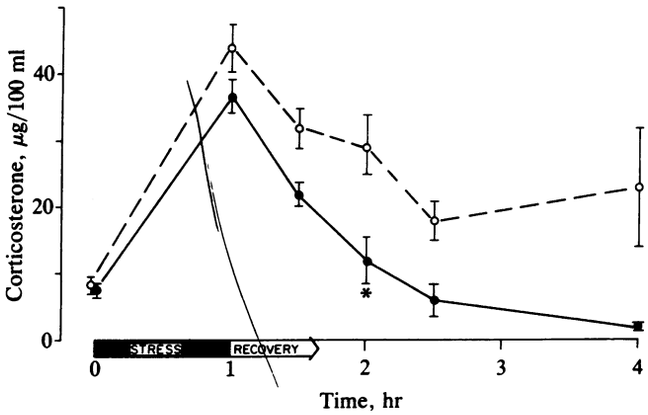

FIG. 1. Corticosterone titers in young and aged rats during 1 hr of immobilization stress, followed by 3 hr of post-stress recovery. * indicates time when titers are no longer significantly elevated above baseline (determined by two-tailed paired t test). In the case of young subjects, this was after 1 hr of the recovery period; for aged subjects, such recovery did not occur. Sample size for young: n = 42 for times 0 and 1; n = 19 for time 1.5; n = 11 for time 2; n = 6 for time 2.5; n = 5 for time 4. Sample size for old: n = 48 for times 0 and 1; n = 22 for time 1.5; n = 11 for time 2; n = 10 for time 2.5; n = 5 for time 4. ●, Young; ○, old.

RESULTS

We examined circulating corticosterone titers in young and aged subjects under basal conditions, at the end of 1 hr of immobilization stress, and at various times during quiet recovery from such stress. Corticosterone titers in the aged rats resembled those of young controls under both basal and stressed conditions (Fig. 1). However, at the end of stress, corticosterone titers in the young subjects declined to basal levels within 90 min, whereas aged subjects still displayed significantly elevated titers after 3 hr.

The aged hippocampus presents at least two deficits that may be relevant to this corticosterone hypersecretion. Aged rats have a 30% decrease in cytosolic corticosterone receptors in the hippocampus, but no change in receptor affinity (Table 1). Accompanying this receptor loss is a decline in the number of pyramidal neurons in the hippocampus (16–18). This neuronal loss at least partially explains the receptor loss, as it is steroid-concentrating neurons that disappear with age (18).

Because of the extensive literature relating hippocampal damage to corticosterone hypersecretion (4–9), we next in-

Table 1. Maximal cytosolic binding capacity for [^{3}H]dexamethasone in the hippocampus (fmol bound per mg of protein)

Subject	B_{max}	K_d	r	% control
A. Aged model				
Young (8)	153 ± 26	2.5 ± 0.7	0.95	1.00
Old (8)	108 ± 14*	2.1 ± 0.6	0.93	0.70
B. Stress down-regulated model				
Control (17)	136 ± 14	1.0 ± 0.1	0.94	1.00
Stressed (9)	90 ± 13*	1.0 ± 0.2	0.91	0.66
Stressed and 1 week recovery (8)	140 ± 15	1.0 ± 0.1	0.92	1.02
C. Brattleboro model				
Long-Evans control (4)	125 ± 8	1.3 ± 0.2	0.97	1.00
Brattleboro (4)	95 ± 11*	1.2 ± 0.2	0.88	0.76
dGVP-treated Brattleboro (4)	120 ± 6	1.1 ± 0.1	0.93	0.96
dGVP-treated Brattleboro, 6 weeks after end of treatment (4)	85 ± 5†	1.8 ± 0.6	0.82	0.68

* and †, significantly lower than young Fischer controls (in aged model), unstressed controls (in down-regulated model), or Long–Evans controls (in Brattleboro model) at $P < 0.05$ and $P < 0.01$, respectively. Newman–Keuls test was used after one-way analysis of variance in down-regulated and Brattleboro models; two-tailed paired t test was used in aged model. Data from the first three experimental groups in part C and from the third group in part B have been previously published (refs. 18 and 21, respectively). Numbers in parentheses represent number of rats tested.

Table 2. Effects of total hippocampal damage on corticosterone titers under basal, stressed, and post-stress conditions

Time, min	Control (n = 11)	Experimental (n = 12)
0 (basal)	4.5 ± 1.0	6.3 ± 1.2
60 (stressed)	18.5 ± 2.3	37.6 ± 6.2
120 (post-stress)	5.3 ± 1.1	22.3 ± 6.2

Corticosterone titers are in μg per 100 ml (mean ± SEM). Subjects were bled 1 hr into the light cycle, exposed to 1 hr of immobilization stress, bled at the end of that period, and then were returned to home cages for 1 hr recovery. At the end of that period (120 min after initial blood sample), subjects were bled again. Corticosterone titers of experimental subjects were significantly elevated above baseline at the 120-min mark ($P < 0.02$, two-tailed dependent t test), whereas control subjects had returned to basal values by that time. Experimental subjects had received multisite infusions of kainic acid, producing at least 50% destruction of all hippocampal cell fields throughout its length.

vestigated whether the impaired termination of corticosterone secretion at the end of stress in aged subjects might be related to the loss of hippocampal neurons. Table 2 demonstrates that complete ablation of the hippocampus with multisite infusions of kainic acid led to a similar syndrome of corticosterone hypersecretion. Rats had significant elevations during stress and during the post-stress period. We next investigated whether it was the loss of neurons after such damage that led to this hypersecretion or whether it was the loss of the corticosterone receptors in these neurons that was critical.

We studied two animal models in which a selective loss of corticosterone receptors in the hippocampus occurred, independent of any loss of hippocampal neurons. Furthermore, in both of these models, the corticosterone receptor losses were reversible. We determined whether loss of corticosterone receptors in the hippocampus was associated with corticosterone hypersecretion, much as seen after overt destruction of hippocampal neurons.

Table 1 summarizes the hippocampal corticosterone receptor profiles in these animals. The Brattleboro rat has a 24% decrease in the number of cytosolic corticosterone receptors in the hippocampus but no change in receptor affinity (Table 1). The hippocampal deficit involves only decreases in the numbers of corticosterone receptors per hippocampal neuron and no change in the number of corticosterone concentrating neurons in the hippocampus or in the total number of hippocampal neurons (18). This receptor deficit is apparently related to the congenital absence of central vasopressinergic projections, because treatment with dGVP normalized the receptor deficit. This effect was not permanent: 6 weeks after the end of dGVP treatment, receptor number had declined to pretreatment levels (Table 1).

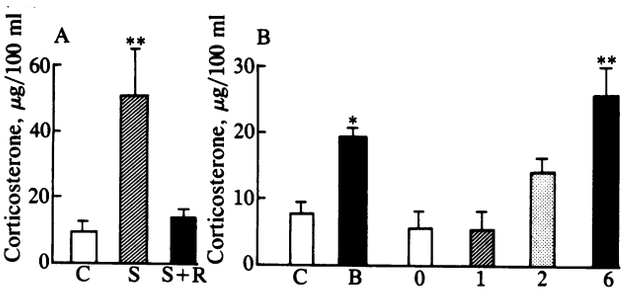

FIG. 2. Corticosterone titers of subjects taken 1 hr into the recovery period after 1 hr of immobilization stress. (A) Subjects were Long–Evans control subjects (C), subjects exposed to 1 week of daily stressors (S), and stressed subjects allowed 1 week to recover from the stress regimen (S+R). (B) Subjects were Long–Evans controls (C), untreated Brattleboro rats (B), and Brattleboro rats treated for 1 week with dGVP and tested either 0, 1, 2, or 6 weeks after the suspension of dGVP treatment. See Table 1 for sample sizes of each experiment. * and **, Significantly elevated above basal corticosterone titers at $P < 0.05$ and $P < 0.02$, respectively (paired t tests).

Exposure of rats to 1 week of chronic stress, which provoked sustained corticosterone secretion, decreased the number of corticosterone receptors by 34% in the hippocampus; no change in receptor affinity occurred (Table 1). This loss of receptors involves no loss of hippocampal neurons but merely numbers of receptors per neuron (unpublished observations). One week after the end of chronic stress, the receptor loss had recovered to control values (Table 1).

Thus, both syndromes have in common a depletion of hippocampal corticosterone receptors separate from any neuronal loss in the structure. Consistently, subjects with depletions of hippocampal corticosterone receptors showed impairment in their recovery from stress. In particular, they continued to secrete significant amounts of corticosterone at times when the control rats had terminated secretion and had basal serum levels. In the case of stress down-regulated rats (Fig. 2A), corticosterone titers 60-min into the recovery period were significantly elevated above those of controls, indicating that they experienced a relative delay in their recovery from the effects of stress. (Basal and stressed titers were comparable with controls.) However, 1 week after daily stressors had been terminated, an interval that allowed for recovery of receptor concentrations back to control levels (Table 1), post-stress corticosterone titers had declined to control levels (Fig. 2A).

In the case of the Brattleboro rat (Fig. 2B), post-stress titers were significantly elevated above controls. After normalization of receptor number with dGVP treatment, recovery from stress was also normalized. Over the 6-week period after the end of dGVP treatment, when receptor levels decayed to pretreatment values, post-stress titers gradually became elevated again.

DISCUSSION

Stimulatory influences on the HPA axis are diverse, involving direct effects on the pituitary and/or hypothalamus, as well as indirect ones via projections from various brain sites to the hypothalamus (22–25). Similarly, several forms of inhibitory regulation by glucocorticoids are recognized—i.e., rate-sensitive fast feedback and level-sensitive delayed feedback (26). Such inhibitory influences are thought to be mediated by the pituitary, hypothalamus, and certain extrahypothalamic sites, including the hippocampus, amygdala, septum, and reticular formation (1, 3, 27). These varied inhibitory and stimulatory influences interact in complex manners to determine basal, stress, and post-stress aspects of corticosterone secretion. For example, increases in circulating titers of glucocorticoids will at least partially inhibit subsequent stress-induced increases in corticosterone secretion. However, individual stressors differ in their capacity to override this inhibitory negative-feedback signal (27). Within this complex framework, the hippocampus has been shown to have an inhibitory influence on HPA activation under a variety of circumstances. For example, destruction of the hippocampus or its efferents leads to increases in corticotropin and corticosterone under basal conditions (4–7) and during stress (5, 6, 8, 9). Furthermore, stimulation of most parts of the hippocampus limits the extent of HPA activation to an array of stressors (10–13). Our present data suggest that the hippocampus also plays a role in terminating the HPA stress response. [It appears legitimate to interpret this hypersecretion at the end of stress as evidence of an inability to terminate the stress response, rather than a prolonged perception of a stressor. For example, we have shown (15) that the aged rat is slow in adapting to mild cold stress, continuing to hypersecrete corticosterone at a time when young controls have titers resembling basal levels. Yet, aged subjects are

less sensitive to cold than young subjects, as measured by cognitive and sensorimotor functions (see discussion in ref. 15).] Aged rats with neuronal loss in the hippocampus are delayed in terminating their stress response. As more direct evidence, we show in this paper that ablation of the hippocampus with kainic acid produces a similar hypersecretion of corticosterone during the post-stress period. (In agreement with the studies cited above, such hippocampal ablation also produces corticosterone hypersecretion during the stressor itself.)

Our findings suggest that it is the loss of corticosterone receptors in such hippocampal damage that is critical to the altered efficacy with which the hippocampus terminates the stress response. We have examined corticosterone secretion in chronically stressed rats and in Brattleboro rats and we found that there are consistent secretory abnormalities associated with depletions of corticosterone receptors. Both have a corticosterone receptor deficit at a common anatomical locus, the hippocampus. In chronically stressed rats, a small decline also occurs in the amygdala, with no change elsewhere in the brain or pituitary (21); Brattleboro rats have a decline in the pituitary, but nowhere else in the brain (20). In neither case is the hippocampal receptor depletion due to a loss of neurons (ref. 18; unpublished observations) but rather to a reversible modulation of receptor levels by corticoids, on the one hand, and by vasopressin on the other. [Corticosteroids and vasopressin appear to represent independent systems of control of hippocampal corticosterone receptors, each capable of influencing hippocampal function. Autoradiographic analysis demonstrates differing hippocampal cell fields sensitive to each of the substances. Furthermore, dGVP does not modulate down- or up-regulation of corticosterone-receptor number following corticosterone administration or adrenalectomy (unpublished work).] In both of these models, the common deficit of depletion of hippocampal corticosterone receptors was accompanied by elevated corticosterone titers during the post-stress recovery period. Furthermore, in the two cases in which the receptor deficit was reversible, normal receptor concentrations were paralleled by normal corticosterone titers during the post-stress recovery period.

These functional deficits all represent syndromes of hypersecretion of the steroid. Clearance rate of corticosterone is unchanged in aged rats (15) and cannot account for the elevated post-stress titers. We have not tested corticosterone clearance rates in the chronically stressed rats, but it is highly unlikely that a decreased rate, if it occurred, would account for the profoundly elevated titers shown in Fig. 2. Finally, the untreated Brattleboro rat has an elevated corticosterone clearance rate (28), implying that the abnormality of corticosterone hypersecretion in these subjects is even more pronounced than presented here.

The apparent role of the hippocampal glucocorticoid receptor in this hypersecretion phenomenon suggests that this represents an impaired sensitivity to negative-feedback regulation by glucocorticoids of the HPA axis. In support of this, aged rats and Brattleboro rats show impaired sensitivity to negative-feedback regulation (ref. 14; unpublished data); specifically, they are less sensitive to the suppressive effects of exogenous glucocorticoids on subsequent stress-induced corticosterone secretion. (Parallel studies with chronically stressed subjects have not been conducted.) Furthermore, subjects with hippocampal lesions have similar subsensitivities to feedback regulation (6). Finally, the corticotropin hypersecretion following hippocampal lesions appears to represent disinhibition from negative-feedback suppression, because adrenalectomy subsequent to the lesion produces equally high basal corticotropin titers in both lesioned and control subjects (5). This is not due to a ceiling effect on maximal corticotropin titer, as subjects elevate corticotropin titer in response to ether stress (5).

These findings suggest that termination of the stress response involves the feedback influence of high circulating titers of corticosterone. As part of this, we postulate that glucocorticoids might alter function within the hippocampus to eventuate in either the direct inhibition of release of CRF-like neurohormones or the sensitization of the hypothalamus to direct corticoid negative-feedback inhibition. Our observations suggest that a decrease in the number of glucocorticoid receptors in the hippocampus, independent of any loss of neurons, attenuates the effect of circulating glucocorticoids on the structure and, thus, the inhibitory influences of the hippocampus on the HPA axis. Although our results strongly implicate the hippocampus, they do not rule out the possible involvement of an additional and, as yet, unexamined structure in the brain.

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