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The Neuroendocrinology of Stress and Aging: The Glucocorticoid Cascade Hypothesis*

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AS RECENTLY as 1900, tuberculosis, influenza, and pneumonia were the leading causes of death in our country (1). For the most part, however, these infectious diseases, as well as those of poor hygiene or undernutrition, no longer plague us. Instead, we succumb most frequently to heart disease and cancer, diseases of slow degeneration (1). Most of all, unlike so many in the generations before us, we are in a position to age. Regardless of what else occurs, we age, we become more constrained by the discrepancy between what we were and what we have become, and each step becomes harder. The goal in the study of aging is not to halt the process, because we can no more be cured of aging than of birth. The goal, instead, is to slow and soften the sharpest edges of the biological unraveling that constitutes aging.

Over the past 5 yr, we have examined some of the sharpest edges of the pathology of aging. We have studied the capacity of aged organisms to respond appropriately to stress and the capacity of stress to cumulatively damage aging tissue. The idea of a relationship between stress and aging has permeated the gerontology literature in two forms. First, senescence has been thought of as a time of decreased adaptiveness to stress (2). This idea has been supported frequently, as many aged physiological systems function normally under basal conditions, yet do not adequately respond to a challenge. For example, aged and young humans have similar basal body temperatures, but the former are relatively impaired in thermoregulatory capacities when heat- or cold-challenged (3). A second theme in gerontology concerning stress is that chronic stress can accelerate the aging process. Selye and Tuchweber (2) for example, postulated a finite "adaptational energy" in an organism, with prolonged stress prematurely depleting such reserves, thus accelerating the onset of senescence. This idea was derivative of earlier idea (*cf.* Ref. 4) that the "rate of living" could be a pacemaker of aging. Experimentally, varied approaches have supported the notion that at least some biomarkers of age can be accelerated by stress (5, 6).

The above hypotheses led us to examine the adrenocortical axis, the endocrine axis which is among the most central to the stress response. Our findings support both of these concepts. We find that the aged male rat is impaired in terminating the secretion of adrenocortical stress hormones, glucocorticoids, at the end of stress. This hormonal excess may be due to degenerative changes in a region of the brain which normally inhibits glucocorticoid release; the degeneration, in turn, is caused by cumulative exposure to glucocorticoids. Together, these effects form a feed-forward cascade with potentially serious pathophysiological consequences in the aged subject.

The adrenal cortex secretes glucocorticoids in response to a variety of stressors. This is the final step in a neuroendocrine cascade that begins with a perception of a stressor by the brain and the triggering of hypothalamic release of CRF and of other ACTH secretagogs. In turn, these stimulate release of ACTH from the anterior pituitary, and this hormone subsequently stimulates glucocorticoid release from the adrenal gland (7–9). Glucocorticoids, in turn, interact with the brain and pituitary to regulate the entire axis by inhibiting subsequent release of CRF and ACTH. Thus, the axis forms a closed-loop feedback system (7–9). Glucocorticoids cause tremendous shifts in carbohydrate metabolism throughout the body that increase circulating energy substrates at the cost of stored energy; they also increase cardiovascular tone, alter cognition, and inhibit growth, the immune and inflammatory responses, and reproduction (7, 8, 10). These changes are central to successful adaptation to acute physical stress, as they increase readily available energy and supportive metabolism and defer energetically costly anabolism until less stressful times. The notorious fragility of organisms with adrenocortical in-

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sufficiency in adapting to stress testifies to the importance of glucocorticoids. However, as these are predominantly catabolic responses to acute emergency, excessive exposure to the steroid (as seen during prolonged stress or in pathological, Cushingoid states) imposes a cost in the form of myopathy, steroid diabetes, hypertension, immunosuppression, infertility, and inhibition of growth (8, 10). Thus, both an absence of and an overabundance of glucocorticoids during stress have profound, if contrasting, pathophysiological consequences, and an inability to appropriately terminate glucocorticoid secretion at the end of a stressor can ultimately be as damaging as the inability to appropriately initiate secretion at the onset of a stressor. Our initial studies determined whether aged rats can appropriately regulate glucocorticoid secretion during and after stress.

Terminating the Stress Response: The Problem of Feedback Inhibition

After a variety of stressors, aged male rats show no impairments in their adrenocortical stress response. Figure 1 shows the secretion of the species-typical glucocorticoid, corticosterone (B) in response to 1 h of immobilization stress in young and aged rats. A similar lack of an age effect is seen in B secretion in response to other stimulators of B secretion, such as ether or cold exposure, cage transfer, laparotomy, or histamine injection (11–14). An appropriate reserve capacity for B secretion is also present in the aged adrenal, as old rats adequately secrete B in response to a new stressor after a period of chronic stress (11). Additional features of the axis remain functional, including the typical circadian rhythm of B secretion and a normal clearance rate of the steroid from the blood of unstressed animals (11). In the female rat,

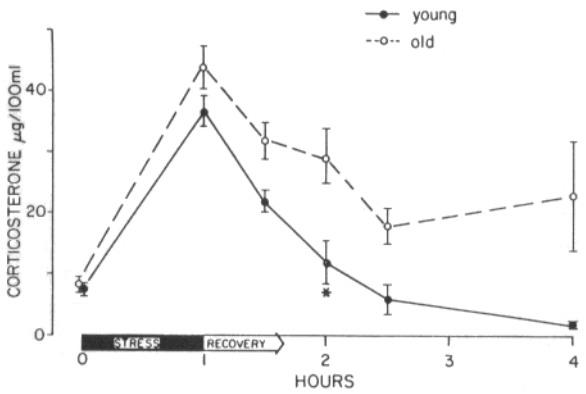

FIG. 1. B titers in young (3–5 months) and aged (24–28 months) Fischer 344 rats during 1 h of immobilization stress, followed by 4 h of poststress recovery. *Asterisk* indicates time when titers are no longer significantly elevated above baseline (determined by two-tailed paired *t* test). In the case of young subjects, this was after 1 h of the recovery period; for aged subjects, such recovery did not occur within the monitored time period. [Reproduced with permission from R. Sapolsky *et al.*: *Proc Natl Acad Sci USA* 81:6174, 1984 (20).]

stress-induced B concentrations have been reported to decline with age (15, 16), and it initially appeared that this represented a diminished adaptive capacity in these animals. However, levels of the B-binding globulin are also likely to decrease with age (due to a decrease in concentrations of estrogen, which increases levels of the globulin) and thus, concentrations of unbound B—the biologically active pool of the steroid—are unlikely to be changed (17, 18).

While the aged rat seems capable of appropriately initiating a B stress response, it is dramatically impaired in its capacity to terminate it (11). In Fig. 1, subjects were monitored during the recovery period after immobilization. B concentrations in young rats return to basal range within 60 min after the end of stress. In contrast, concentrations of aged rats remain elevated for as much as 24 h post stress (11, 19, 20). Factoring the clearance rate of B out of the data in Fig. 1 shows the elevated poststress concentrations of B in the aged rats to be due to continued secretion of the hormone (11).

This case of hyperadrenocorticism is but one in a larger syndrome of B hypersecretion. In addition to the delay in terminating B secretion at the end of stress, aged rats show delays in adapting to mild sustained stress, such as moderate cold exposure (11). Furthermore, basal concentrations of B have often been reported to rise progressively (11, 14, 20–23). Given the unchanged B clearance rate with age (11), and the increased body size and blood volume of aged rats, this represents a substantial increase in adrenocortical output during senescence.

Throughout this paper, we will propose that this problem of B hypersecretion is due to degenerative changes within the aging brain, specifically in the hippocampal region of the limbic system. Should the brain be the genesis of the hypersecretion, one would expect B to be only the last in a cascade of hormones that are hypersecreted during stress; in fact, this is observed to be the case. Basal concentrations of ACTH also rise with age (along with β-endorphin) (14, 24, 24a). ACTH concentrations increase approximately 4-fold with age, considerably more than the approximate doubling of B concentrations. Accompanying this is a decreased adrenal sensitivity to ACTH (13, 14, 25, 26). This diminished responsiveness can be viewed as an only partially successful adrenal compensation for the more substantially amplified ACTH signal in the circulation. The moderate rise in basal B concentrations with age is the result of these coupled changes. Higher in the axis, similar changes also seem to occur. While CRF concentrations have not yet been measured in aged rats, the aged pituitary shows the same dampened sensitivity to CRF as does the aged adrenal to ACTH (27). This suggests that hypersecretion occurs throughout the aged adrenocortical axis.

These cases of hypersecretion are likely to arise from a progressive loss of sensitivity of the axis to negative feedback regulation (28–31). Elevated concentrations of circulating glucocorticoids normally inhibit both subsequent basal and stress-induced concentrations of glucocorticoids. However, B and the synthetic glucocorticoid dexamethasone (DEX) are relatively ineffective in suppressing endogenous B secretion in old rats (28–31). Feedback inhibition within the axis is diverse, and both the rapid, rate-sensitive, and the delayed, level-sensitive forms of B inhibition of adrenocortical secretion are diminished in old rats (31). The varied cases of elevated B concentrations discussed earlier appear due to this loss of sensitivity to feedback inhibition.

The Problem of Hippocampal Neuron Loss

Hormones influence target tissues by interacting with macromolecular receptor proteins which, in turn, stimulate second messenger cascades or directly alter genomic events. Glucocorticoids are bound by macromolecular receptors which, upon forming complexes with the steroid, interact with genomic material. [At present, it remains controversial whether such receptors occur in the cytoplasm (32, 33); however, we will henceforth refer to the unoccupied form as "cytosolic."] The demonstration of a decreased sensitivity with age of some unknown regulatory region of the brain or pituitary to the inhibitory feedback signal of glucocorticoids led us to postulate that a loss of glucocorticoid-binding receptors might underlie this desensitization.

There are at least two receptor types in the brain which are capable of recognizing glucocorticoids. One type (called "GC receptor") is recognized by antibodies to the liver glucocorticoid receptor and is found in many regions of the brain (32). It is labeled *in vivo* by [^{3}H] DEX (34). The other type (called "corticosterone" or "B receptor") is similar to the so-called "mineralcorticoid receptor" of the kidney (36) and it recognizes B with a high affinity (35). Both B and GC receptors are labeled *in vitro* by [^{3}H]DEX and by [^{3}H]B (36).

Our initial studies demonstrated that glucocorticoid-binding receptors are lost with age in the hippocampus. The hippocampus is the principal uptake site in the brain for tracer doses of [^{3}H]B (37, 38), and this uptake is due in large part to binding to B receptors (36). Only at higher doses of B or with DEX is there evidence of labeling of the lower affinity GC receptor in the hippocampus (34, 39). We and others found that the aged hippocampus sustains a loss of approximately 50% of glucocorticoid binding sites (22, 40, 41). This deficit occurs, at least in part, in the population of B receptors, since the loss was first demonstrated by *in vivo* administration of [^{3}H]B, which selectively labels B receptors (34, 36, 39). We have not yet conducted a similar study which would preferentially label GC receptors in the aged hippocampus; thus, it is not clear whether a loss occurs in that population. In the rest of this article, when discussing *in vitro* experiments utilizing [^{3}H]DEX, we will refer to the "GC + B" receptor, whereas in experiments in which [^{3}H]B was administered *in vivo*, selectively labeling B receptors, we will refer only to B receptors.

The loss of hippocampal B receptors appears to be anatomically specific, as receptor levels are unchanged in other target sites for B, such as the pituitary, hypothalamus, cortex, and midbrain. (It should be noted that this does not rule out the possibility of losses in small subregions of these loci.) There is also a small and relatively inconsistent receptor loss in the amygdala which can be detected by biochemical, but not by autoradiographic, techniques. The loss of GC + B receptors in the hippocampus is due entirely to a loss of cytosolic receptors affinity of binding, and the capacity of the receptor, once having formed a complex with the steroid, to bind tightly to the cell nucleus does not change with age (40).

Since glia also contain glucocorticoid receptors (42), we next investigated whether the receptor decreases are predominately of neuronal or nonneuronal origin. We determined this by comparing age-related *in vivo* uptake of [^{3}H]DEX *vs.* [^{3}H]B. A short time after administration, the former selectively labels GC + B receptors found in glia (43–45). Furthermore, DEX induces a glial-specific enzyme and fails to induce a neuron-specific protein (42, 46). In labeling glial glucocorticoid receptors, we found that no age-related decrease occurs and, in fact, a trend toward increased [^{3}H]DEX uptake is observed (40). This is likely to reflect the glial hypertrophy typical of senescence (47–49). These studies suggest that this age-related receptor loss may be restricted exclusively to the neuronal receptor population.

We next determined the anatomical specificity of this loss. The hippocampus is a large, heterogeneous structure, with multiple neuron types, anatomically distinct cell fields, and differing functions ascribed to different portions of the structure (50). Quantitative autoradiographic techniques with [^{3}H]B showed the receptor loss (in this case, the B receptor) to be anatomically discrete, in that some portions of the hippocampus show no age-related losses (*e.g.* subiculum, dentate gyrus, and the CA4 cell field) while others show profound depletion (*e.g.* pyramidal cell layer of CA3) (51, 52).

The CA3 cell field contains considerable concentrations of both B and GC receptors (36) and, as discussed, it is not yet clear whether there is a decline in GC receptors to accompany the demonstrated loss of B receptors.

Finally, we determined whether the B receptor loss in these regions is due to decreased average numbers of receptors per neuron or to loss of the neurons themselves. Using high resolution autoradiography of [^{3}H]B binding coupled with cell counting techniques, we found that the receptor loss is at least partially due to death of the target neurons. This was observed in the CA3 cell field, where previous quantitative autoradiographic studies had revealed extensive receptor losses. Importantly, no neuron loss occurs in the CA4 cell field, an area with no overall decrease in [^{3}H]B binding (51, 52). Previous work had shown hippocampal neuron loss with age (53, 54); our studies demonstrated that it is [^{3}H]B concentrating neurons which are lost, with surviving neurons having a smaller complement of receptors.

Is There a Relationship between the B Hypersecretion and the Receptor Loss?

The studies described in the previous section demonstrated that the aging hippocampus loses cytosolic B and possibly GC receptors in some of its cell fields, and that a loss of neurons richest in B receptors accounts for this decline. We next investigated whether there is a relationship between the two age-related deficits uncovered at this point—the problem of B hypersecretion (with the underlying problem of loss of sensitivity to negative feedback inhibition), and the loss of GC + B receptors in the hippocampus (with the underlying problem of loss of the neurons themselves).

We assumed the possibility of a causal, rather than merely correlative, relationship between these dysfunctions because of the frequency with which they appear together. Elevated basal glucocorticoid concentrations, delayed recovery from stress, and insensitivity to glucocorticoid negative-feedback consistently appear in association with decreased hippocampal binding of glucocorticoids and/or damage to that structure. We briefly review these correlations.

As detailed, such a cluster of traits is found in the aged rat. The Brattleboro rat, a strain cogenitally deficient in vasopressin (VP) (see below) shows a similar pattern, in that there is a loss of GC + B receptors which is most evident, within the central nervous system, in the hippocampus (55), as well as a hypersecretion of B after the end of stress (20). Pharmacological manipulations that normalize the number of such GC + B receptors in the hippocampus of the Brattleboro rat are accompanied by normalization of the B secretion (20). Streptozotocin-induced diabetes mellitus in the rat results in both an insensitivity to glucocorticoid negative-feedback inhibition, as well as a loss of cytosolic GC + B binding throughout the limbic system (56–59). Similarly, chronic stress leads to preferential down-regulation of GC + B receptors in the hippocampus (Ref. 60; discussed below) as well as hypersecretion of the steroid and negative-feedback insensitivity (20, 61).

These consistent correlations are also observed developmentally. The neonatal rat has a pronounced paucity of limbic GC + B receptors (61–63), and adult-like concentrations of receptors develop only gradually during the first few weeks of life. Chronic B exposure selectively decreases hippocampal GC + B receptor concentrations in day 35 rats; this reversal of the developmental progression of this system has been shown to produce a syndrome of B hypersecretion that accompanies such receptor loss (63). Furthermore, stimulation of the neonatal rat (specifically, daily handling) produces persistent increases in hippocampal GC + B receptor concentrations (64) as well as an enhanced ability of rats to terminate B secretion after the end of stress (65, 66).

These correlations are also observed phylogenetically, as New World monkeys have cortisol concentrations 1 order of magnitude higher than those in Old World monkeys and, in addition, are 1 order of magnitude less sensitive to the suppressive effects of glucocorticoids. Such species do not have a paucity of glucocorticoid receptors, but rather are reported to have receptors with an affinity for cortisol considerably lower than in Old World monkeys [(67); it should be noted that this does not appear to be the sole unique feature of the hypothalamic-pituitary-adrenal axis in New World primates (*cf.* Ref. 68)]. A similar pattern is shown for guinea pigs, as compared to related species. The former have a 3-fold increase in cortisol concentrations, are DEX resistant (requiring a higher dose for suppression and a faster escape from such suppression). The species is found to have receptors with a 20-fold decrease in affinity for the steroid (69).

A number of insults that damage the hippocampus are associated with glucocorticoid hypersecretion. As will be detailed below, experimental destruction of the structure is associated with instances of hypersecretion and resistance to feedback inhibition. Furthermore, Alzheimer's disease (AD), the primary foci of which includes damage to the hippocampus, nucleus basalis of Meynart, and cortex, is associated with DEX resistance in approximately 50% of cases (see below). In addition, chronic alcohol exposure, which reduces hippocampal neuron number in both adults and fetuses, is associated with hyperactivity of the hypothalamic-pituitary-adrenal axis (70–73).

Finally, diverse studies of large numbers of different social species, inculding mouse (74–78), rat (79, 80), wolf (81), and primates (82–91), demonstrate that elevated basal glucocorticoid secretion, adrenal enlargement, and DEX resistance are associated with social subordinance in a stable dominance hierarchy; such subordinance is

also associated with down-regulation of B receptors in the brain (92).

This extensive and catholic array of studies suggested a relationship between damage to the hippocampus and/or to its glucocorticoid receptors, and syndromes of glucocorticoid hypersecretion. We thus began to study the complex patterns of causality between these two classes of defects. We initially investigated whether the hippocampal damage typical of senescence could eventuate in the associated syndrome of hypersecretion.

The Hippocampus and Feedback Inhibition

Stimulatory influences upon the adrenocortical axis are complex; some stressors act directly upon the hypothalamus and pituitary to release CRF, related secretagogs, and ACTH, while others influence these structures via neural projections (9). Negative-feedback regulation of the axis by glucocorticoids is also diverse, involving both rapid rate-sensitive and delayed level-sensitive forms of regulation (9). Studies with hypothalamic explants or pituitary cell lines indicate that most feedback inhibition by glucocorticoids occurs at these target sites. However, suprahypothalamic structures also mediate small but significant portions of the inhibitory glucocorticoid signal. Thus, the inhibitory effects of glucocorticoids are attenuated when the afferent connections to the hypothalamus are severed (93). We hypothesized that the hippocampus is a mediating locus of glucocorticoid feedback inhibition at the end of stress, and that in the aged rat, the observed hippocampal degeneration is responsible for the loss of sensitivity of the axis to feedback inhibition.

There was much reason to suspect a hippocampal involvement as, of all suprahypothalamic loci, the structure has been most consistently implicated as an inhibitory influence upon the adrenocortical axis. This appears to include regulation of basal ACTH and glucocorticoid secretion, as total hippocampal lesion, lesion of only the dorsal hippocampus, or fornix transection results in basal hypersecretion of these hormones (94–98) [with some conflicting suggestions of a circadian alteration in the strength of this inhibitory hippocampal regulation (94–99)]. Furthermore, the structure appears capable of inhibiting stress-induced activation of the adrenocortical axis, as destruction of either the entire, or just the dorsal portion of the hippocampus produces glucocorticoid hypersecretion after a number of different stressors (95, 96, 100, 101). In addition, electrical stimulation of the structure (particularly the CA3, subicular, or dentate gyrus cell fields) inhibits an adrenocortical stress response (102–105). Finally, such hippocampally induced inhibition of the axis appears to be a manifestation of negative-feedback inhibition by circulating glucocorticoids. As evidence, destruction of the entire hippocampus, the dorsal component, or the fornical outflow from the structure attenuates the suppressive effects of DEX upon the stress response (91, 106, 107). In addition, ACTH secretion is increased after hippocampectomy, and the difference in concentrations between lesioned and sham-lesioned animals is abolished by adrenalectomy (95), suggesting that the relative increase in ACTH due to the lesion resulted from disinhibition from corticoid feedback suppression.

As a body, these studies heavily implicated the hippocampus as a potentially inhibitory influence upon the adrenocortical axis. This conclusion should be accompanied by a number of caveats, however. The structure should not be considered homogeneous; this is clearly the case from an anatomical perspective, and this literature supports the notion of heterogeneity of function. Thus, the dorsal hippocampus appears to have more of an inhibitory influence upon the axis than does the ventral portion (96). Furthermore, within any given lamella, stimulation of different cell fields produces differential effects; CA1, for example, appears to stimulate adrenocortical secretion, in contrast to all other cell fields (105). The hippocampus must also be thought of as playing, at best, only a minor role in regulating the axis, as judged by the size of the effects reported in these studies. In addition, there appears to be redundancy in such regulation, as there is the potential for recovery of normal adrenocortical function with time after hippocampal damage (98). Finally, the inhibitory role of the structure is not apparent at all times during the circadian cycle (94–99). Thus, with regard to inhibition of adrenocortical function, the hippocampus is neither structurally monolithic nor functionally of primary importance.

Despite these caveats, we felt that the data implicating the hippocampus as potentially inhibiting the adrenocortical axis was sufficiently robust to determine the adrenocortical consequences of the hippocampal damage typical of senescence. First, we found that complete hippocampal lesion eventuates in B hypersecretion at the end of stress, as in the aged rat [(20) although it should be noted that, unlike the aged rat, hippocampectomy also produced B hypersecretion during stress; see also Ref. 108]. We next determined whether this is due to the loss of the neurons after lesioning, or whether loss of the GC + B receptors *per se* contained within those neurons could produce the hypersecretion. As negative-feedback regulation can be conceptualized, a glucocorticoid signal is detected by hippocampal neurons; the first step in the detection process is occupation of hippocampal glucocorticoid receptors by the steroid. The neurons then transduce this endocrine signal into an inhibitory neural signal to the hypothalamus. Thus, destruction of the hippocampus itself damages both detection and trans-

duction, whereas lesioning of the fornical projection from the hippocampus to the hypothalamus results in a hippocampus capable of detecting the endocrine signal but incapable of subsequently inhibiting the hypothalamus. In both cases, feedback insensitivity and hypersecretion ensue. Would impairing detection while leaving communication to the hypothalamus intact (by depleting the hippocampus of GC + B receptors without damaging the neurons themselves) also result in B hypersecretion? To answer this, we developed two rat models in which we selectively and reversibly depleted the hippocampus of GC + B receptors without altering neuron number. Consistently, we observed that a loss of receptors is coupled with the B hypersecretion syndrome at the end of stress.

In the first model, we administered high dosages of B to rats, which decreased GC + B receptor number (20, 60). Such down-regulation of receptors by sustained exposure to elevated levels of ligand is a well-known compensatory feature of endocrine and neural systems. Within the brain, hippocampal GC + B receptors are most sensitive to such regulation (60, 109), and with a proper protocol of B administration, we could reduce hippocampal GC + B receptors in a reversible and fairly discrete fashion. Although we did not distinguish between GC and B receptors in most studies of down-regulation, one instance where we did use [^{3}H]B *in vivo* to selectively label B receptors indicated that this population is decreased in CA1 and CA2 (109). Figure 2 (*left*) demonstrates that such receptor-depleted rats hypersecrete B after the end of stress; this agreed with a previous report showing that rats treated chronically with stress become less sensitive to feedback inhibition by glucocorticoids (109a). Importantly, Fig. 2 also shows that a week after the cessation of B administration, when GC + B receptor concentrations in the hippocampus return to normal, the capacity to turn off the B stress response promptly also normalizes (20).

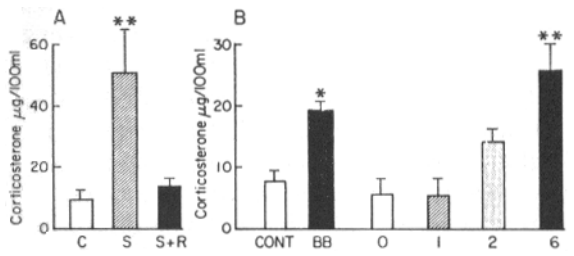

FIG. 2. B titers of rats taken 1 h into the recovery period after 1 h of immobilization stress. Panel A, Rats were Long-Evans control subjects (C), rats exposed to 1 week of daily stressors (S), and stressed rats allowed 1 week to recover from the stress regimen (S + R). Panel B, Rats were Long-Evans controls (CONT), untreated Brattleboro rats (BB), and Brattleboro rats treated for 1 week with the VP analog desglycinimide VP and tested either 0, 1, 2, or 6 weeks after the suspension of treatment with the peptide. * and ** indicate significantly elevated above basal B titers at 0.05, 0.02 levels, respectively (paired *t* tests). [Reproduced with permission from R. Sapolsky *et al.*: *Proc Natl Acad Sci USA* 81:6174, 1984 (20).]

As our second model, we studied the Brattleboro strain of rat, which is congenitally deficient in the peptide VP. VP serves both as an antidiuretic hormone in the pituitary, a modulator of ACTH release, and as a neurotransmitter or neuromodulator in the brain. Neural VP can apparently regulate hippocampal GC + B receptors, as Brattleboro rats are deficient in such receptors; a loss has not been reported anywhere else in the brain and is corrected by administration of VP or a centrally acting VP analog (55). We found that the Brattleboro rat, deficient in hippocampal GC + B receptors, hypersecretes B at the end of stress (Fig. 2, *right*). Furthermore, normalization of the receptor deficit with a VP analog normalizes B secretion. Finally, after suspension of VP-analog therapy, receptor levels decline over 6 weeks to pretreatment levels, and B hypersecretion reemerges in parallel (20).

Thus, the aged hippocampus, with its loss of neurons and of their B and possibly GC receptors, is doubly impaired in its regulation of adrenocortical secretion. The receptor depletion desensitizes the structure to the presence of circulating B and, in effect, causes circulating concentrations of the steroid to be underestimated. The problem is further compounded because not only is there a loss of the receptors, but also of the neurons that contained them; consequently, neural communication through and out of the hippocampus is impeded. The problem of feedback desensitization and B hypersecretion thus appears due to the degenerative loss of neurons and receptors in the aging hippocampus. As noted above, the B and GC receptors differ in their affinities for B. Under conditions of basal circulating B concentrations, approximately 90% of hippocampal B receptors are occupied, whereas perhaps 10% of the lower affinity GC receptors are occupied at that time (36). During stress, occupancy of the B receptors changes only minimally, whereas occupancy of GC receptors changes considerably (36). It has been theorized that hippocampal B receptors mediate signals concerning tonic changes in basal B concentrations, whereas hippocampal GC receptors are responsive to stress signals (36). The age-related depletion of B receptors in the hippocampus may thus be most related to the elevated basal B concentrations observed in the aged rat; that B is hypersecreted in the aftermath of stress makes it of considerable importance to determine whether there is also an age-related loss of hippocampal GC receptors.

It should be mentioned that the construct developed in this section, namely that hippocampal damage impairs the capacity of the aged rat to terminate B secretion after the end of stress, differs from (but does not contradict) the more traditional views of B feedback regulation.

Essentially all of the numerous reports concerning such regulation have examined the effects of B feedback on either basal or stimulated (*i.e.* stressed) adrenocortical secretion (*cf.* Ref. 9). In general it appears that sustained elevation of B concentrations over a period of days inhibits both basal and stimulated secretion. Over the course of hours, B feedback is more effective at inhibiting stimulated rather than basal secretion. In both of these time domains, the extent of inhibition is proportional to the steroid dose. Finally, over a course of minutes, B feedback, in proportion to the rate of rise of concentration of the steroid, can inhibit stimulated adrenocortical secretion (9). To our knowledge, before reports concerning aged rats (11, 19, 20), little attention had been paid to regulation of secretion during the poststress period. As noted, we found that complete hippocampal destruction leads to B hypersecretion both during and after stress (20). This represents a more severe neurological lesion than in aged rats [who have only a moderate loss of hippocampal neurons (52–54)], as well as a more severe endocrine defect [as aged rats do not appear to hypersecrete B during stress (11–14)]. When a more subtle defect is induced in the hippocampus (*i.e.* depletion of GC + B receptors without destruction of neurons), B hypersecretion is only observed during the poststress period (20). This suggests a particularly important role for the hippocampus in terminating poststress B secretion, and that hippocampal damage as a normal part of aging is insufficient to produce elevated B concentrations during stress.

Regulation of Receptor Number per Neuron

At this point, we sought to understand the cause of the degenerative changes in the senescent hippocampus by searching for experimental manipulations which would mimic its features. A number of models initially seemed to fulfill these criteria.

The Brattleboro rat and the aged rat appeared to have a number of features in common. The former completely lacked neural VP, while the latter had decreased levels of the peptide (110, 111). Both had the similar B hypersecretion syndrome described, as well as similar cognitive impairments (55, 110, 111). Finally, both had a selective and extensive loss of glucocorticoid-binding receptors in the hippocampus (40, 55). The demonstration that replacement of the absent VP in the Brattleboro rat normalized the receptor depletion (55) [as well as the endocrine and cognitive dysfunctions typical of the strain (55)] suggested that declining hippocampal VP concentrations may underlie the similar problems of senescence. Thus, aged rats were administered a VP analog which normalized the receptor loss in the Brattleboro rat. Unfortunately, the peptide fails to correct the aged receptor deficit (51). Just as in the aged rat, quantitative autoradiography after [^{3}H]B administration *in vivo* revealed the Brattleboro hippocampus B receptor deficit to be the most dramatic in the CA1 cell field and to spare CA4, dentate gyrus, and subiculum. However, high resolution autoradiography revealed the critical difference: Brattleboro rats have a profound and VP-reversible loss of receptors per hippocampal neuron, but, unlike the aged rat, have not lost any of the neurons themselves (51). Thus, depletion of VP is not the likely cause of the senescence-induced degeneration in the hippocampal B receptor system, and our subsequent characterization of the VP regulation of these receptors showed it to be rather specialized and limited (112).

A second possible model concerned ACTH, which has been reported to regulate GC + B receptor number in the brain (41, 113). Administration of an ACTH analog has been reported to potentiate GC + B receptor number in the aged hippocampus (41). This suggested that an absence of ACTH may underlie the senescent receptor deficit. However, there are at least two inconsistencies with this hypothesis. First, as discussed, ACTH concentrations rise with age (14, 24). Next, the directions of the ACTH effect on GC + B receptors in the two reports were contradictory (41, 113).

In a third model, we investigated reports that disruption of specific neurochemical inputs into the hippocampus could alter its number of GC + B receptors. We disrupted dopamine, norepinephrine, and serotonin projections but, in contrast to other reports (114, 115), found no reliable changes in GC + B receptor number after such lesions. Furthermore, in examining catecholamine and indolamine content in the aged hippocampus, we found no evidence that significant depletions of any of these neurotransmitters occur with aging (Renner, K., R. M. Sapolsky, and V. Luine, unpublished data).

As a final model, we examined whether the down-regulation of hippocampal GC + B receptor concentrations after elevated B concentration is an appropriate model for the senescent hippocampus. We first demonstrated that either a week of sustained stress, or a week of B administration sufficient to mimic the stress-induced concentrations of circulating B, down-regulates GC + B receptor number. As in the aged rat, the loss is most profound in the hippocampus, less reliably so in the amygdala, and not at all in the pituitary or in other brain regions. As in the aged rat, the receptors that remain are unchanged in their affinity for the steroid ligand (60). However, this down-regulation appears due to either decelerated receptor synthesis or accelerated degradation, as there is only a change in the number of receptors per neuron, with no change in the number of neurons themselves (116). Finally, as noted, the receptor loss spontaneously normalizes within a week of the end

of B treatment (60). Therefore, this is not the likely mechanism for the degeneration of the senescent hippocampus.

Thus, hippocampal GC + B receptor number can be regulated by VP, short term exposure to stress, or elevated B concentrations. In contrast with the receptor loss in the aged rat, such alterations of receptor number are transient, presumably involve changes in receptor-processing rates, and do not involve changes in neuron number.

Glucocorticoid Neurotoxicity in the Hippocampus

Despite our demonstration that 1 week of stress or of exposure to high titers of B produced only transient receptor loss, we speculated that more prolonged B exposure could produce permanent degenerative changes in the hippocampus similar to those observed in the aged rat. This was based on two observations in the literature. First, pharmacological concentrations of glucocorticoids produce hippocampal degeneration (117); the seemingly anomalous anatomical preference for the hippocampus may be explained by the demonstration that the structure had the highest concentration of B receptors in the brain (37). Second, in a series of important and difficult studies, Landfield and colleagues (23, 49, 53, 118) produced evidence that cumulative exposure to basal B concentrations over the lifespan might mediate hippocampal neuron death. After characterizing the senescent features of the hippocampus, including the decreased neuron density and compensatory glial clustering and reactivity, they demonstrated that the extent to which basal B concentrations are elevated with age in the rat predicts the severity of the neuropathological changes in the hippocampus. Finally, they demonstrated that removal of B at midage by adrenalectomy prevents the emergence of these markers of hippocampal senescence.

As a result of these observations, we examined whether truly prolonged elevation of B concentrations produces a "senescent" hippocampus. We administered B to rats at a dosage producing the concentrations seen after major stress continuously for 3 months (representing approximately 12% of the lifespan). After this time, hippocampal GC + B receptor number is down-regulated approximately 50%, about the same extent as after only 1 week of B administration (116). This is not surprising, as we had previously shown that to be the maximal extent of down-regulating achievable in the structure (60). However, in contrast to rats which were exposed to 1 week of B and in which hippocampal receptor concentrations normalized within 1 week of the cessation of treatment, the receptor depletion in 3 month B-treated rats was far more persistent, if not permanent; 4 months after the end of treatment, no recovery of receptor numbers is observed (Fig. 3). The depletion appears due to loss of the host neurons themselves. Total cell number is decreased, and the decline is entirely attributable to loss of B-concentrating cells. As with the aged hippocampus, surviving cells bind less B. Furthermore, area determinations of cell bodies showed that the cells lost are of the same size class as the neurons lost in the senescent hippocampus. Importantly, just as in the aged structure, this loss is accompanied by a significant increase in small cells which, by morphological and cytological criteria, resemble the glia which proliferate and infiltrate in response to neuronal damage. Finally, the cell loss in both experimental and aged rats is most profound in the CA3 cell field. Thus, this model produced features identical to that of the aged hippocampus: persistent GC + B receptor loss most probably due to loss of the host neurons, a preferential vulnerability of the CA3 cell field, and glial hyperplasia accompanying this neuronal damage (116).

These findings, when combined with the Landfield studies (23, 49, 53, 118), suggest that cumulative exposure to basal concentrations of B lead to the degenerative loss of neurons and B receptors in the senescent hippocampus, and that chronic stress, with its resultant increase in B concentrations, accelerates this process. This was, in many regards, a puzzling finding: neuron loss due not to toxins, exogenous insults, slow viruses, or autoimmune attack, but rather to cumulative exposure to normal concentrations of a hormone that is essential for life.

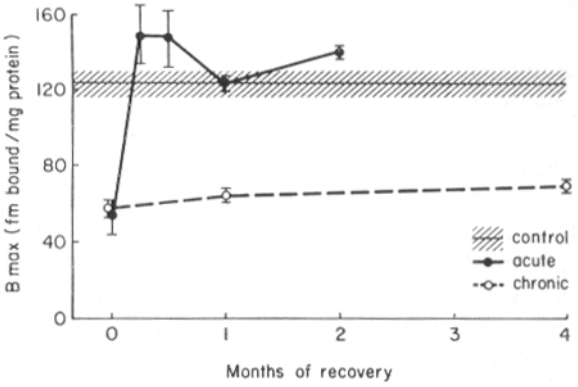

FIG. 3. Maximal binding capacity (in femtomoles [^{3}H]DEX bound per mg cytosolic protein) of hippocampi of control, acute, and chronic subjects. Acute subjects were injected sc daily with 5 mg B for 2 weeks, chronic subjects for 3 months. Rats were then allowed to recover from such treatment; four subjects from each group were culled at each time point during the recovery period for receptor assay. Both acute and chronic rats had significantly diminished binding capacity, relative to controls, at the beginning of the recovery period (0.01 level of significance, Scheffe test after two-way analysis of variance). Acute rats recovered from this quickly, such that receptor levels were comparable to those of controls within a week. Chronic rats, however, showed no evidence of recovery during the 4-month followup. [Reproduced with permission from R. Sapolsky *et al.*: *J Neurosci* 5:1221, (116).]

However, there are precedents for degeneration induced by sustained exposure to an endogenous ligand. The female rodent loses the capacity to ovulate with age, and such reproductive failure arises from loss of the triggering surge of LH before ovulation. Degenerative changes in the senescent hypothalamus (in particular, the arcuate nucleus) appear to underlie the failure of the LH surge, and an extensive and elegant body of studies showed that cumulative exposure to basal concentrations of the ovarian steroid, estrogen, accelerated the hypothalamic degeneration (119). Furthermore, stress has been shown to damage the retina, decreasing the numbers of photoreceptor and bipolar neurons, and this toxicity could be prevented by adrenalectomy (120, 121).

Thus, we turned our attention to the cellular mechanisms of glucocorticoid neurotoxicity in the hippocampus and found evidence for at least one model of action. Potentially, B could be directly and intrinsically toxic to these neurons; *i.e.* in the absence of any challenges or insults, neurons continuously exposed to B would die faster, relative to B-free controls. No evidence for such an action has been demonstrated. As an alternative or additional mechanism, B might be insufficiently toxic to kill neurons directly but might, in some manner, compromise their capacity to survive subsequent extrinsic challenges. Such a model predicted that a variety of toxins and insults which damaged the hippocampus would be more lethal in stressed or B-treated rats and less so in adrenalectomized subjects. We and others have found evidence for such glucocorticoid modulation of hippocampal neuronal vulnerability (122–127). Two neurotoxins, kainic acid and 3-acetylpyridine, and hypoxia-ischemia, all of which preferentially damage the hippocampus, are all more neurotoxic in rats with physiologically elevated B levels. Conversely, adrenalectomy protects against these insults. The effect is large, with the number of dead neurons varying by more than 1 order of magnitude depending on the B milieu. These findings are strikingly reminiscent of those of O'Steen and Donnelly (120), who reported that the damaging effects of photic stimulation upon the retina are potentiated by acute stress, and that this synergy is attenuated by adrenalectomy.

A considerable amount of work has been done recently examining this capacity of glucocorticoids to potentiate damaging insults to the hippocampus, and a number of features of this modulation are now understood:

1. Glucocorticoids impair the ability of hippocampal neurons to survive the insults, rather than to alter the quality of the insults themselves. This view is most broadly strengthened by the sheer variety of mechanisms by which these insults damage the hippocampus. For example, kainic acid is an excitotoxin which is an analog of the excitatory amino acid glutamate and appears to exert some of its damage by influencing glutaminergic synapses (128, 129). In contrast, 3-acetylpyridine is an antimetabolite which disrupts the electron transport chain (130), while hypoxia-ischemia is proposed to damage the hippocampus via ATP depletion, inappropriate calcium and/or chloride fluxes, and interaction with the glutaminergic system (131–134). Yet all of these insults are more potent in the presence of elevated concentrations of glucocorticoids. Furthermore, glucocorticoids do not increase the diffusion or binding of kainic acid within the hippocampus (122), which also supports the idea that the steroids are influencing the capacity of neurons to withstand the insults, rather than influencing the insults themselves. Finally, the potentiation of damage by glucocorticoids occurs either exclusively or most dramatically in the hippocampus, suggesting that these neurons are atypically vulnerable to glucocorticoids (122–124).

2. Glucocorticoids themselves are the damaging agents within the hippocampus. The steroids have a vast number of metabolic effects throughout the body, and one might readily speculate that the potentiation of hippocampal damage by the steroids arises secondarily to glucocorticoid actions elsewhere. The rather unique vulnerability of the hippocampus to the damaging actions of glucocorticoids, and the high concentrations of glucocorticoid receptors within the structure suggest, instead, that the steroids exert a direct effect in compromising neuronal viability. This is strongly supported by our recent observation that glucocorticoids enhance kainic acid and 3-acetylpyridine-induced neuron death in primary cultures of dispersed fetal rat hippocampal neurons (Sapolsky, R. M., and W. Vale, submitted). This conclusion is at odds with one facet of the work of Landfield and colleagues who have suggested that at least some of the damaging actions of glucocorticoids in the hippocampus arise secondarily via glucocorticoid-induced inhibition of ACTH secretion (*i.e.* that chronic diminution of exposure to ACTH is damaging to the hippocampus). In support of this view, they demonstrated that some of the protective effects of adrenalectomy upon the aging hippocampus could be mimicked with administration of an ACTH analog (118). Potentially, both sustained exposure to glucocorticoids and sustained deprivation of ACTH could each be damaging; however, it should be noted that in the aging rat, ACTH concentrations are elevated (13), contrary to the prediction of the data of Landfield and colleagues.

3. Some of the glucocorticoid actions in damaging hippocampal neurons are mediated by the GC receptor. As evidence, the capacity of glucocorticoid to enhance insult-induced damage *in vitro* is attenuated by coincubation of cultures with the GC receptor antagonist, RU 38486 (Sapolsky, R. M., and W. Vale, submitted). Whether some of the endangering actions of the steroid

also arise from interactions with the B receptor in the hippocampus (*cf* Ref. 135) is currently being examined.

4. Both a history of exposure of the hippocampus to elevated glucocorticoid concentrations before an insult, as well as the acute presence of elevated concentrations in the aftermath can potentiate damage. This was shown by limiting glucocorticoid administration to adrenalectomized rats to either a week before or a week after the insult, but not both; significant potentiation of damage still occurs (125).

5. Glucocorticoids endanger the hippocampus in a rapid and persistent manner. A pair of studies examined the time-window of hippocampal neuronal vulnerability to the compromising actions of glucocorticoids; these showed that as little as 24 h of exposure to elevated concentrations of the steroid bracketing the administration of kainic acid or 3-acetylpyridine can potentiate damage (123, 125). This suggests a fairly rapid steroid action, eliminating a number of possible mechanisms of toxicity. For example, glucocorticoids can nonenzymatically form adducts with proteins in ways that can theoretically impair protein function or lead to destructive cross-linked protein aggregates; such a mechanism has been proposed for glucocorticoid-induced retinal damage (136). The speed with which glucocorticoids impair hippocampal neuronal viability suggests that the formation of such adducts is unlikely to underly this phenomenon.

The glucocorticoid effect upon neurons appears to be relatively persistent. As evidence, exposure of adrenalectomized rats to high glucocorticoid concentrations from 7–4 days before kainic acid administration potentiates damage (125).

6. Some of the damaging actions of glucocorticoid arise from their disruption of hippocampal neuronal energy metabolism. Neurons are notoriously vulnerable to depletion of energy. They consume energy at a high rate, have only limited abilities to store glycogen, and utilize only a few energy substrates (134). In addition, the three insults the potencies of which are modulated by glucocorticoids either impair the capacity of neurons to generate energy (hypoxia-ischemia, 3-acetylpyridine), or place pathological demands upon the neuron for energy (kainic acid). It appears that glucocorticoids potentiate their damage, at least in part, by exacerbating the state of energy depletion that they induce. Glucocorticoids inhibit glucose uptake in peripheral tissues such as adipocytes, skin, and thymocytes (137). While measures of whole brain glucose content have not indicated a similar steroid action throughout the entire organ (138), the hormone significantly inhibits glucose uptake and utilization in the hippocampus, as determined by both measurement of labeled glucose in dissected individual brain regions (139) and by 2-deoxy-glucose studies (M. Kadekaro, personal communication). The mechanisms of action of these insults upon energetically vulnerable neurons, and the ability of GCs to themselves disrupt neuronal energy metabolism suggested that supplementing rats with additional brain fuels might counteract the synergy between glucocorticoids and these insults. We have now observed this to be the case (126); supplementation of rats with glucose, mannose, the ketone β-hydroxybutyrate, or (to a much lesser extent) fructose attenuates the synergy between glucocorticoids and either kainic acid or 3-acetylpyridine. Why energy depletion damages neurons is, in effect, the central and most challenging question in cellular neuropathology.

These studies demonstrate that physiological elevations of B can impair the ability of hippocampal neurons to survive extrinsic challenges. This is clearly relevant to acute insults. In the aftermath of cerebral ischemia or seizure, exogenous administration of glucocorticoids can enhance hippocampal damage. Even more importantly, after such insults, adrenalectomy reduces damage, suggesting that what is viewed as "normative" hippocampal injury after these insults is, in fact, normative damage exacerbated by coincident secretion of glucocorticoids [thus, as we have suggested (124), pharmacological attenuation of glucocorticoid secretion in the aftermath of these insults may prove protective of the hippocampus]. These studies suggest that hippocampal neuron loss during aging might arise from a decline after each external insult, the size of the decline being modulated by the B milieu at that time. As noted, whether the hormones are also directly toxic (*i.e.* whether, superimposed on the punctate loss is a continuous decrement) is unknown. Finally, it is unknown whether such declines are exacerbated by intrinsic senescence of these neurons or are entirely a function of external hit frequency (*i.e.* whether, with the same extrinsic insult in the same B milieu, the punctate decline is greater in a population of aged neurons).

In a thoughtful review, Munck (137) viewed the metabolic actions of glucocorticoids as designed to transfer energy during stress from storage sites in fat, skin, thymocytes (and apparently some brain regions) to muscle—the tissue most likely to have an increased demand for energy during a somatic stressor and the capacity of which to utilize glucose is not inhibited by glucocorticoids (137). It appears both maladaptive and puzzling that cerebral ischemia or seizure can provoke glucocorticoid secretion as robustly as do numerous somatic stressors (140), as energy utilization is then curtailed in critically vulnerable neurons. The hippocampus has been long-recognized by neuropathologists as being inordinately vulnerable to numerous insults (134), and various mechanisms have been offered as explanations for this vulnerability, including the sparse microvasculature in the region, or aspects of hippocampal electrophysiology

(134). The studies just outlined suggest that, as an additional factor, enhanced hippocampal vulnerability might arise from the catabolic actions of glucocorticoids and the extremely high concentrations of their receptors in the hippocampus.

An Integrated Model with Possible Pathophysiological Implications

The separate features of this system—the cumulative B effects in the hippocampus, and the hippocampal regulation of B secretion—combine to form a feed-forward cascade of degeneration with age (Fig. 4). Periods of stress, of excessive B secretion, down-regulate the number of B receptors per hippocampal neuron, and once the period of B hypersecretion terminates, the receptor loss can be self-correcting. At some point, however, the down-regulation of receptors is sufficient to dampen hippocampal feedback inhibition of the adrenocortical axis, and B hypersecretion emerges. This precipitates further down-regulation of receptors and, further hypersecretion until permanent loss of the hippocampal neurons themselves occur, and irreversible commitment to the cascade begins. However, a number of questions still remain unanswered. First, is there a linear relationship between down-regulation of the B receptors in the hippocampus and the loss of feedback sensitivity (*i.e.* does hypersecretion begin with the most minimal of down-regulation)? There appears to be a fair linearity in the relationship, as both the B hypersecretion and the hippocampal GC + B receptor decline emerge progressively with age (11, 21–23). At what juncture does neuron death begin, and to what extent must excessive B secretion and extrinsic metabolic challenges temporally coincide to damage neurons? Finally, in addition to disrupting feedback inhibition, does the finite down-regulatory loss of B receptors also temporarily protect the neurons from the more toxic B effects and thus temper the emergence of the cascade?

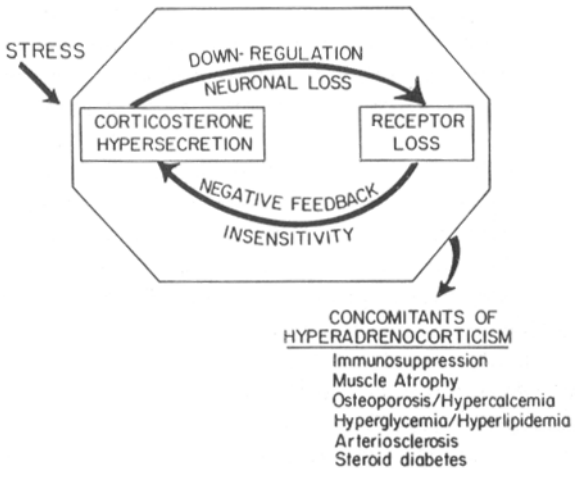

FIG. 4. Schematic representation of glucocorticoid cascade hypothesis.

This model casts light on why the adrenocortical axis in aged male rats responds to stress inefficiently, how this can emerge from a normal lifespan of occasional stress and metabolic challenges to the brain, and how excessive stress and challenges accelerate the process. The degenerative cascade can, potentially, have varied pathophysiological consequences. As described earlier, hyperadrenocorticism has a pathophysiological price, inducing catabolic degeneration in varied organ systems (8, 10). Figure 4 lists pathologies that share two features: occurrence after excessive glucocorticoid exposure, and a dramatically increased spontaneous occurrence with age (8, 10). [It should be noted that in the human, depression should be added to this list, as its incidence increases with age; glucocorticoid administration, as well as Cushing's disease, is similarly associated with an increase in depressive symptoms (141, 142)]. As an additional pathological consideration, the hippocampus plays an important role in cognition (143). Hippocampal damage or decreased GC + B receptor number in the hippocampus are both associated with learning impairments (21, 143), as is aging (118), and Landfield and colleagues (118) have shown that aged rats, after having been adrenalectomized at midage, are not only spared degenerative changes typical of the senescent hippocampus, but also display improved cognition. Could the B hypersecretion syndrome that emerges with age play a role in some of the other pathologies of aging? We have tested this idea in one study. Stress, at least in part through glucocorticoid secretion, promotes the establishment of tumors and accelerates tumor growth (144–146). The long-known immunosuppressive effects of glucocorticoids, as well as their effects on tumorigenesis factors and metabolism, might play a role in this (7, 147–150). If aged rats, because of the regulatory dysfunctions of this cascade, secrete more B per stressor than do young subjects, will chronic stress be more tumorigenic in aged subjects? After injecting rats with cells transformed with Fujinami sarcoma virus, we found that aged rats were vastly more vulnerable to stress-induced acceleration of the growth of these tumor-forming cells. Furthermore, replication of the aged pattern of B hypersecretion at the end of stress in young rats similarly increased their vulnerability to tumor growth (151). While indirect, this study presents first evidence that this senescent cascade of glucocorticoid hypersecretion may be a predisposing factor in some of the pathologies that are concomitants of aging.

Appropriateness of this Model to the Primate and Human

As noted, all of the studies described have utilized rats. Is the model outlined in Fig. 4 relevant to the human

and/or primate brain? The general features of the model are phylogenetically conserved from rodents to primates. For example, the endocrine axis involving CRF (and related secretagogs), ACTH, and glucocorticoids (in the case of primates, cortisol) is identical in the two groups (152). Furthermore, as for the rodent, the primate hippocampus is the principal neural target for glucocorticoids (153). Moreover, excessive glucocorticoid exposure can have many of the same pathophysiological consequences in both groups (10). However, do the more specific features of this model also apply to the primate?

The Effect of Glucocorticoids upon the Primate Hippocampus

Can glucocorticoids damage the hippocampus, or any glucocorticoid-sensitive tissue of the primate brain? Not surprisingly, only very tentative and almost anecdotal data can be considered. Before the emergence of modern therapeutic methods for managing Cushing's syndrome, such patients were likely to sustain prolonged exposure to elevated glucocorticoid concentrations. A rather old literature examining the postmortem status of the brains of such individuals reports a low but consistent incidence of neural atrophy and lesions in the limbic brain, frontal lobe, and hypothalamus (discussed in Ref. 153a). Hydrocephalus was reported to accompany many such cases. In these reports, the cerebral damage was invariably considered as a possible cause, rather than consequence, of the endocrine abnormalities of the syndrome. In a more recent but equally sparse literature, torture victims have been reported to have high incidences of cerebral atrophy, ventricular enlargement, and dementia (154, 155). Some of these neurological correlates of torture appear to be transient, however. In an ongoing study, the brains of vervet monkeys who had died during a period of sustained social stress have been examined. Animals had been recently captured and housed in pairs, and in a number of cases, the socially subordinate member of the pair died, with associated renal failure and peptic ulcers. As compared with age- and sex-matched controls, such stressed animals showed a significant loss of neurons, along with incidences of pyknosis, neuronophagia, and glial infiltration throughout the hippocampus and cortex (Uno, H., and R. M. Sapolsky, in preparation).

The most useful observations concerning the capacity of glucocorticoids to damage the primate hippocampus has emerged from examination of the fetal brain. In these studies, DEX was administered to 132 gestation day rhesus monkeys, and their fetuses were removed at 135 days. In such fetuses, total numbers of neurons were dramatically reduced in the CA2 and CA3 regions of the hippocampus, as well as in motor and visual cortex. Less pronounced declines occurred in the CA4 and CA1 hippocampal cell fields and in sensory and frontal cortex. The 135 gestation day hippocampus is cytoarchitecturally mature and differentiated in this species; this implies that the decrement in neuron number can be ascribed to reduction by the steroid of preexisting neurons rather than to arrest of subsequent neurogenesis (Ref. 156; and H. Uno, personal communication). Thus, there is some suggestion that glucocorticoids can damage the hippocampus, and that the CA3 region is particularly vulnerable to this effect. This relationship remains to be tested, of course, in the adult primate brain.

Can stress and/or sustained glucocorticoid exposure down-regulate hippocampal cortisol receptors in the primate? There are no relevant data yet concerning down-regulation of primate hippocampal cortisol receptors, much less consideration of whether the structure is preferentially sensitive to such regulation.

The Effect of the Primate Hippocampus upon Glucocorticoid Secretion

To consider the other side of the feed-forward cascade outlined in Fig. 4, does the primate hippocampus mediate glucocorticoid negative feedback? What little data there are support this conclusion. First, there is a correlation between hippocampal damage and glucocorticoid hypersecretion (either basally and/or after DEX administration) in a number of human disorders, including AD and chronic alcoholism (discussed below). Furthermore, in perhaps the only such report involving the human hippocampus, stimulation of the structure results in inhibition of adrenocortical secretion (157). Finally, we have recently obtained preliminary evidence that fornix transection in the macaque produces cortisol hypersecretion throughout the circadian cycle as well as DEX resistance (Sapolsky, R. M., S. Zola-Morgan, and L. Squire, unpublished observations).

Normative Human Aging

Thus, the primate hippocampus appears to have a similar inhibitory influence upon the adrenocortical axis as in the rodent. Furthermore, the structure appears to lose neurons with age (with the loss most pronounced, as in the rat, in the CA3 region and far less so in the CA1 cell field) (H. Uno, personal communication). Finally, this pattern of neuron loss can arise from exposure to elevated glucocorticoid concentrations, at least in the fetus. Thus, a number of features of Fig. 4 appear, tentatively, to be relevant to the primate. Do these features produce a syndrome of glucocorticoid hypersecretion as a normative aspect of human aging? Quite clearly, the answer is no. Neither the secretion of cortisol nor of 17-hydroxycorticosteroids increases with age in the human (158–161), and circadian rhythmicity of the

secretion is demonstrable (161–164). Pituitary ACTH content is unchanged with age, as is adrenal responsiveness to stress (158, 159). Finally, most (159, 164, 165) [although not all (29)] studies report no age-related changes in the responsiveness of the adrenocortical axis to either metyrapone or DEX.

Neuropathology and Psychopathology in the Aged Human

Thus, while most of the regulatory features of Fig. 4 appear to be potentially operable in the primate brain, the syndrome of glucocorticoid hypersecretion is not a normative part of human aging. However, these features do emerge as a function of age when senescence is coupled with a pathological state. We will specifically discuss this interaction between age and both AD and affective disorders.

AD is among the most common causes of dementia and produces a profound disruption of cognition. Its neurocytological hallmarks are its neurofibrillary tangles and neutitic plaques of amyloid. Of considerable importance, such cytological degeneration is most marked in the hippocampus and neocortex (166). Considerable attention has focused on the cholinergic components of the disease, in that cholinergic perikarya are lost in the nucleus basalis of Meynart which projects to both the hippocampus and neocortex; accompanying this loss is a decline in choline acetyltransferase activity in those latter sites (167–169). The neurochemical abnormalities of the disease are not limited to the cholinergic system, however, and include declines in somatostatin and CRF concentrations within the cortex (170–173).

A hallmark of AD is the glucocorticoid hypersecretion of its sufferers. This includes elevations of basal cortisol concentrations as well as DEX resistance (174–178). As proposed by Carroll *et al.* (179), the DEX suppression test involved administering 1 mg DEX orally at 2300 h, followed by sampling of serum cortisol concentrations at 1600 h and 2300 h the next day. Nonsuppressors are defined as those who fail to suppress below 5 μg/100 ml at either time point, since some individuals may fail to suppress altogether whereas others may show premature escape from otherwise normal suppression (180). Using this criterion, researchers have reported an approximately 50% rate of DEX resistance in AD patients, and this appears unrelated to coincident affective disorders (see below) (174, 175, 178). Importantly, recent work demonstrates that in the AD patient, DEX resistance becomes more prevalent with age (181).

In viewing these data, we speculate that the glucocorticoid hypersecretion arises from the hippocampal damage typical of AD. In most younger patients, DEX responsiveness is intact (181) which suggests that the primary hippocampal damage attributable to AD is, as yet, below threshold for disrupting adrenocortical function. In older patients, the hippocampal impairment attributable to AD and presumably to aging, each alone typically insufficient to disrupt adrenocortical function, combine and produce a far higher prevalence of DEX resistance (182).

A similar picture may characterize affective disorders such as endogenous depression. The illness has been linked to abnormalities in adrenocortical function (183) and to resistance to DEX (179, 184). Other related indices of abnormal activity of this pathway in depression include elevated CRF levels in the CSF (185) and elevated ACTH levels (186) [which, under some circumstances, may be dissociated from elevated glucocorticoid concentrations (187)]. In addition, depressed patients have been found to have phase shifts in diurnal patterning of adrenocortical function, reflecting an earlier nadir in cortisol levels; this pattern can be dissociated from DEX resistance (188). Carroll and others (179, 184) have found that approximately half of patients with major depression are DEX resistant. Many reasons have been proposed for this partial correlation, including the possibility that there are subtypes of depression with different degrees of adrenocortical dysfunction, different patient populations, or that the conditions which lead to DEX resistance are not operative all the time in depressed patients. In fact, all three notions may be valid.

With regard to subtype of depression, higher incidences of nonsuppression have been identified in familial pure depressive disease, compared with sporadic depressive and depression spectrum disease (188). Furthermore, in keeping with the original work of Sachar *et al.* (183) which identified abnormal cortisol secretion in psychotic depression, this subtype is also recognized as having a high incidence of DEX resistance (189–191). Another recent study indicates that symptoms of melancholia together with either symptoms of agitation or delusion are associated with elevated cortisol concentrations and a high incidence of DEX resistance (R. Brown, Payne-Whitney Clinic, personal communication). With regard to inherent differences in patient populations, it has recently become clear that with a wide variety of depression subtypes, DEX resistance becomes more prevalent with age (192–195c). This was shown quite dramatically in one study by Georgotas *et al.* (193), in which fully 83% of depressives over the age of 60 were DEX-resistant.

Finally, the sensitivity of the adrenocortical axis to DEX waxes and wanes as a function of stress, which could explain some of the patterns of responsiveness to DEX in depressives. Remission of symptoms of depression is associated with normal DEX responsiveness (184), and scoring of patients for lifetime occurrence of DEX resistance, rather than relying only upon one test,

produces a higher percentage of nonsuppressors among bipolar depressed subjects, familial pure depressive disease, and sporadic depressive disease (188). It is therefore conceivable that individuals prone to depression are more prone to psychological stress and perhaps more susceptible as well to the consequences of physical or psychological stress.

These data suggest a number of tentative conclusions regarding depression. First, there appear to be subtypes of depression more strongly associated with glucocorticoid hypersecretion than others. Next, stressors, either preceding or coincident with the depressive episode, might well increase the prevalence of DEX resistance. In support of this, acute stress among physicians associated with preparing and delivering a lecture leads to DEX resistance in half of the subjects (Ref. 196; see also Ref. 196a). Such resistance disappears within 1 week. In the same study, depressed and schizophrenic patients showed the same incidence of DEX resistance (40–50%) as the stressed physicians, although healthy nonstressed controls were all normal suppressors. Thus, stress of unknown duration appears to be an important factor in determining DEX resistance. As discussed, stress in the rat eventuates in preferential down-regulation of glucocorticoid receptors in the hippocampus, and that such transient down-regulation is associated with glucocorticoid hypersecretion and resistance to negative feedback regulation (20, 60, 119). Given the demonstrated role of the primate hippocampus in similar mediation of glucocorticoid feedback regulation, we speculate tentatively that the DEX resistance observed in some depressive disorders [which are, in fact, recognized as often being precipitated by stress (197)] can arise from transient stress-induced down-regulation of glucocorticoid receptors in the hippocampus. As with AD, the prevalence of DEX resistance among depressives becomes more pronounced with senescence and we interpret this, once again, as representing an interaction between the normative impairments of the aged hippocampus (which are typically below threshold for disrupting adrenocortical function) and impairments attributable to the pathological state.

These data concerning humans and primates generate a number of conclusions. It appears that the broadest features of the model presented in Fig. 4 may be operable in the primate, in that the hippocampus plays a role in adrenocortical feedback regulation, and that glucocorticoids can potentially damage that structure. The mechanisms for such damage are unknown and it is, of course, of enormous importance to determine whether glucocorticoids can sensitize the human hippocampus to the damaging effects of acute neuropathological insults such as stroke or seizure (198). Despite the similarities between the rodent and primate, it is nevertheless clear that the thresholds for eliciting the features of Fig. 4 are higher in primate; in other words, this dysfunctional cascade does not emerge as a normative part of human aging. However, when compounded with a pathological state such as AD or depression, the syndrome of glucocorticoid hypersecretion does emerge with age. That there is such "a direct correlation between age and cortisol in interaction with a third variable" (194) suggests that the secretory dysfunctions of Fig. 4 are normally subclinical in the aged human.

Conclusions

Our studies have generated information about normative features of the adrenocortical axis, including B regulation of GC + B receptors, and hippocampal GC + B receptor modulation of the stress response. In addition, they provide insights into how these regulatory features, when combined, can produce some of the characteristic endocrine abnormalities of the aged rat. The initial reversibility of the receptor loss ensures that the abnormalities emerge only slowly; the B-induced neuron death that eventually occurs makes the abnormalities ultimately irreversible; and the exacerbation of the process by chronic stress implies a strong environmental or experiential component in this aging process. Finally, they show the synergism between B and toxins, which suggests that glucocorticoids may not only influence the rate of some aspects of aging, but may also influence the capacity to withstand acute neuropathological insults. Finally, a rather preliminary literature suggests that these studies are relevant, if not to normal human aging, then to some pathological concomitants of human aging. It is our hope that the research described here will aid in understanding and tempering the excesses of aging which each of us must inevitably face.

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