# Regulation of 5-HT Receptors and the Hypothalamic-Pituitary-Adrenal Axis

## Implications for the Neurobiology of Suicide[a]

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**ABSTRACT: Disturbances in the serotonin (5-HT) system is the neurobiological abnormality most consistently associated with suicide. Hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis is also described in suicide victims. The HPA axis is the classical neuroendocrine system that responds to stress and whose final product, corticosteroids, targets components of the limbic system, particularly the hippocampus. We will review results from animal studies that point to the possibility that many of the 5-HT receptor changes observed in suicide brains may be a result of, or may be worsened by, the HPA overactivity that may be present in some suicide victims. The results of these studies can be summarized as follows: (1) chronic unpredictable stress produces high corticosteroid levels in rats; (2) chronic stress also results in changes in specific 5-HT receptors (increases in cortical 5-HT2A and decreases in hipocampal 5-HT1A and 5-HT1B); (3) chronic antidepressant administration prevents many of the 5-HT receptor changes observed after stress; and (4) chronic antidepressant administration reverses the overactivity of the HPA axis. If indeed 5-HT receptors have a partial role in controlling affective states, then their modulation by corticosteroids provides a potential mechanism by which these hormones may regulate mood. These data may also provide a biological understanding of how stressful events may increase the risk for suicide in vulnerable individuals and may help us elucidate the neurobiological underpinnings of treatment resistance.**

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## INTRODUCTION

Research studies in suicide attempters and suicide victims (two overlapping but not identical populations) have implicated disturbances in the serotonin (5-HT) system as the neurobiological "alteration" most consistently associated with suicide.[1,2] As we will see below, several studies have also shown that dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis can be found in suicide victims.[3–5] Although each of these systems have been studied independently, their interaction in the brain, as it relates to the pathophysiology of suicide, has received relatively little attention.

That disturbances in these two systems may share a common pathophysiological mechanism is not surprising, inasmuch as we know from animal studies that they interact extensively and that they are related in a variety of ways.[6] The hippocampus, in particular, is an anatomical region in which components of the HPA and the 5-HT systems have a rich representation. This region is part of the limbic system, an area implicated in the regulation of several vegetative functions (arousal, sleep, appetite, and hedonic capacity) as well as in the control of cognitive function and of mood. Therefore, the hippocampus provides an ideal anatomical substrate to study the HPA axis, the serotonin system, and their potential role in suicide.

It is clear that neurobiological abnormalities can be found in suicide victims, irrespective of diagnosis. However, not all suicides have a common underlying psychiatric condition. An important question is whether the biological abnormalities that have been found in suicide victims are characteristic of a subpopulation or if there is a neurobiological precursor common to all suicides. For example, although disturbances in the 5-HT and HPA systems have been identified in suicide victims, they have also been implicated in affective disorders.[7] This is particularly relevant, because, depending on the population, 40 to 60% of suicide victims have a history of affective illness.[8,9] Given this neurobiological link, an understanding of the relationship between these two circuits can give us clues to the pathophysiology of both suicide and affective illness.

## STRESS AND THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

The HPA is the classic neuroendocrine system that responds to stress. Perception of stress by an organism results in a series of events whose final result is the secretion of glucocorticoids (cortisol in humans, corticosterone in rats) from the adrenal cortex.[10] Activation and termination of the adrenocortical stress response is critical for adaptation and survival. Inhibition of

stress responsiveness is partly achieved by the binding of circulating glucocorticoids to specific cytoplasmic receptors in the hypothalamus, where they inhibit corticotropin (CRH) and consequently pituitary adrenocorticotropin (ACTH) secretion. Additional modulation of the system is apparently achieved in limbic structures, specially the hippocampus, a structure that is linked to the hypothalamus through neuronal connections that converge on the paraventricular nucleus of the hypothalamus (PVN), where the stress responsive CRH and vasopressin (AVP) neurons reside.[10] There are several lines of evidence that highlight the importance of the hippocampus for HPA feedback mechanisms. For example, hippocampal lesions in the adult rat result in increased circulating corticosterone, and increased CRH/AVP mRNA, suggesting a lost of a site critical to glucocorticoid-negative HPA feedback on basal expression of these secretagogues.[11,12] Further study of corticoid receptor molecules within this structure have provided evidence of their relevance to HPA feedback mechanisms. It is also well established that the hippocampus is a central component of limbic circuitry and is fundamental in controlling aspects of cognitive and behavioral functions.

Chronic stress can lead to specific alterations and changes in the activity of the HPA axis. For example, in rats, chronic foot shock leads to an aberrant pattern of pituitary responsiveness to glucocorticoids and an impairment in feedback mechanisms.[6,10] This impaired feedback following chronic stress is associated with a decrease in glucocorticoid receptor (GR) gene expression in the hippocampus.[13] Interestingly, some of the HPA changes seen after chronic stress in rodents can be prevented by the administration of antidepressant medications,[14] suggesting that correction of the HPA axis disturbances is associated with the therapeutic action of these compounds.

The feedback abnormalities in chronically stressed animals resemble those seen in patients with depressive illness.[15,16] This HPA dysregulation in depression is manifested by cortisol hypersecretion, failure to suppress cortisol secretion after dexamethasone administration and blunted response to CRH administration,[17-19] This last observation has been interpreted as evidence for increased CRH drive, inasmuch as chronic CRH administration is known to cause down-regulation of pituitary CRH receptors.[20]

## STRESS, DEPRESSION, AND SUICIDE

The relationship between stress and suicide is complex. It is important to distinguish between the psychological aspects of stress (with issues such as predictability, control, and coping) and the biological aspects of stress, which include activation of specific neuroendocrine cir-

cuits, such as the HPA axis. Although it is clear that the stress-related brain circuits (in particular the HPA) play a key role in stress responsiveness, the sites critical for stress perception are not as clear. The exact relationship between the psychological aspects of stress, the biological aspects of stress, and suicide is unknown. However, we do know the following: (1) Several clinical and epidemiological studies in suicide victims have identified psychosocial stressors (*e.g.*, unemployment, a recent stressful life event) as important risk factors in suicide.[21-24] These factors have been identified both in suicide attempters and completers as a group, although clearly this does not imply that psychosocial stress is a key variable in all suicides. (2) As we have mentioned above, subjects with affective illness are a significant subpopulation in suicide victims.[8,9,25] Stressful life events have also been identified as important risk factors prior to the onset of depression.[26] (3) Depressed patients have evidence of dysregulated stress systems at the biological level. Therefore, it is possible that evidence of HPA overactivity may be found in at least the subpopulation of suicide victims with a history of mood disorders, or in those who may have suffered from more severe psychological (and/or biological) stressors.

Clinical neuroendocrinological studies have indeed found evidence of HPA overactivity both in completed suicides and in suicide attempters. For example, Norman[27] found that depressed patients who had undergone a dexamethasone suppression test (DST) and later committed suicide had significantly higher postdexamethasone levels than other (nonsuicidal) depressed patients. In another study, Roy[28] found that violent suicide attempters had higher cortisol levels than those who made nonviolent suicide attempts. Postmortem studies have also found evidence of chronic HPA activation in suicide victims, such as adrenal hyperplasia,[4,29] increased CRH content in the cerebrospinal fluid,[5] and downregulation of CRH receptors in the frontal cortex.[30] We have found increases in POMC (the precursor molecule for ACTH) mRNA and peptide content in the pituitaries of suicide victims,[3] which is an indication of chronic activation of the HPA axis. It is not clear if these postmortem changes are due to an underlying mood disorder in a subset of this population, to the stress surrounding the suicidal act itself, or to a neurobiological abnormality common to all suicides irrespective of diagnosis.

Nevertheless, the presence of a chronically hyperactive HPA axis has important biological consequences to the organism. In this paper, we review animal studies that indicate that the final products of the HPA axis can exert an important modulatory influence in some components of the serotonin system. These data strongly suggest that HPA overactivity is not merely an epiphenomenon of the suicidal state, but that it may be responsible for (or worsen) some of the 5-HT abnormalities found in suicide. This HPA dysregulation, therefore, is an important contributor to some pathophysiological disturbances that may lead to suicidal behavior.

## SEROTONIN 1A RECEPTORS AND THE HPA AXIS

Animal studies have shown that corticosteroids can alter several elements of serotonergic neurotransmission. Removal of circulating corticosteroids by adrenalectomy (ADX) results in anatomically specific decreases in indices of serotonin metabolism, whereas stressful procedures, which raise corticosteroid levels, produce corresponding increases in serotonin turnover.[31,32] Activity of tryptophan hydroxylase, the rate-limiting serotonin biosynthetic enzyme, appears to be sensitive to circulating corticosteroid levels.[33] However, corticosteroids may also act to directly modulate serotonergic neurotransmission by regulating 5-HT receptors. Autoradiographic studies[34] first identified increased 5-HT1 receptor binding in the rat hippocampal formation one week after bilateral ADX. Subsequent investigations have confirmed the sensitivity of 5-HT1 receptors to circulating corticosteroid levels[35] and indicate that specific hippocampal subfields are exquisitely sensitive to adrenal steroids. More recent electrophysiological studies have shown a suppression of 5-HT-induced hyperpolarizations within CA1 pyramidal cells after brief application of steriods,[36] establishing a functional coupling for steroid–serotonin receptor interactions within the hippocampus.

The signal transduction mechanism for corticosteroids involves the translocation of hormonally bound cytosolic receptors to the cell nucleus, where association with specific genomic sites induces alterations in transcriptional efficiencies for particular genes.[37] Pharmacological studies have defined at least two subtypes of corticosteroid receptors that differ in their affinity for corticosterone;[38] the mineralocorticoid receptor (MR), which resembles the peripheral kidney MR receptor and binds corticosterone with high affinity; and the GR, which exhibits a 3 to 5-fold lower affinity for the endogenous ligand. Both autoradiographic and immunohistochemical studies[39,40] indicate that the hippocampus contains particularly high concentrations of both MR and GR compared to other brain regions. *In situ* hybridization studies confirm the intrahippocampal synthesis of these sites and reveal a heterogeneous distribution of both MR and GR mRNAs across hippocampal subfields.[41] It, therefore, seems likely that the high concentration of corticosteroid receptors within the hippocampus underlies the sensitivity of 5-HT receptors to corticosteroid regulation in this region.

The ascending serotonergic innervation of hippocampal neurons arising in midbrain raphe nuclei provides one means by which the serotonergic system may act to regulate limbic function. Thus, the hippocampus represents a key anatomical structure involved in the central control of HPA axis function[10] *and* limbic circuitry.[42] As such, this area provides a unique anatomical environment in which to study the molecular interplay between serotonergic systems and corticosteroids.

The 5-HT1A receptor has been identified both as an inhibitory somatodendritic receptor in raphe serotonergic cells and a post-synaptic receptor in selective serotonergic terminal fields.[43] Its abundance in the limbic system, as well as evidence from animal and clinical studies, strongly suggests that the 5-HT1A receptor plays an important role in the pathophysiology of mood disorders. For example, a number of antidepressant drugs, monoamine oxidase inhibitors, and tricyclics have been found to regulate hippocampal 5-HT1A receptor number and responsiveness,[44–46] suggesting that this receptor subtype may be important in relation to the therapeutic actions of these compounds. In clinical studies, 5-HT1A partial agonists have been shown to be effective in the treatment of anxiety disorders,[47] and major depression,[48] including the melancholic subtype.[49] Interestingly, the 5-HT1A agonist, buspirone, has been reported to augment the antidepressant response in patients who are antidepressant nonresponders.[50]

Although original ADX studies did not differentiate between 5-HT1 receptor subtypes, autoradiographic data using subtype-specific ligands[51] and *in situ* hybridization histochemistry[52] indicated that the predominant postsynaptic hippocampal 5-HT1 receptor is of the 5-HT1A type, suggesting that 5-HT1A receptors may be regulated by adrenal steroids. Reported alterations in 5-HT1A receptor binding in response to ADX[53] is certainly supportive of such a conclusion. This evidence suggests that the mechanisms underlying corticosteroid regulation of 5-HT1A receptors may contribute to steroid-mediated modulation of mood and, as such, may represent an important linkage both in the pathophysiology of suicide and in psychotherapeutic drug action.

Our laboratory underwent a series of experiments designed to investigate regulation of the 5-HT1A receptor in response to alterations in circulating corticosteroids.[6, 54–56] Studies were designed to further our understanding of the mechanisms involved in corticosteroid-mediated modulation of the 5-HT1A receptor at the molecular and subanatomical level under conditions related to physiological and pathophysiological levels of steroids. As corticosteroids act predominantly at the level of neuronal gene expression, we employed *in situ* hybridization techniques in combination with receptor autoradiography to allow simultaneous visualization and quantification of both receptor gene expression and receptor dynamics in discrete anatomical subfields. More specifically we asked (1.) How do steroids regulate 5-HT1A receptors? Is there any evidence for anatomical or receptor specificity? What are the roles of MR and GR in mediating hippocampal 5-HT1A receptor regulation? How do hippocampal 5-HT1A receptors react to chronic stress? and Can antidepressant drug treatment alter 5-HT1A receptor binding and gene expression under conditions of chronic stress?

We found that removal of circulating steroids by adrenalectomy resulted in a significant increase in 5-HT1A receptor binding and mRNA

in rat hippocampus.[54,57] This increase was prevented if a low dose of corticosterone was administered after adrenalectomy. The dose of corticosterone used in this study is probably occupying MR selectively. Dexamethasone replacement after adrenalectomy was only partially effective in reversing the adrenalectomy-induced upregulation of 5-HT1A . Dexamethasone is primarily a GR agonist. These studies indicated that 5-HT1A hippocampal receptors are under tonic inhibition by corticosteroids, and this effect is predominantly mediated by MR. The role of MR in modulating hippocampal 5-HT1A under basal conditions has been confirmed by other laboratories[58,59] using specific pharmacological agents. This regulation appears to be relatively site specific, inasmuch as 5-HT1A mRNA and binding in the dorsal raphe was not altered by removal of circulating steroids.[57] There is also some receptor specificity, because dopamine D1 receptors and 5-HT2C receptors were not affected.[57]

We also examined the effect of persistent HPA activation on hippocampal 5-HT1A receptors by using a chronic unpredictable stress paradigm.[60–62] This paradigm causes persistent elevation of circulating corticosteroids and is therefore probably occupying both MR and GR simultaneously. After two weeks of chronic stress, 5-HT1A receptor mRNA levels and 5-HT1A binding densities were significantly reduced in the hippocampus, relative to control animals.[55,56] If imipramine is administered concomitantly to the chronically stressed animals, it prevents both the downregulation of hippocampal 5-HT1A receptors and the stress-induced increase in plasma corticosteroids. The mechanism by which chronic imipramine administration prevented 5-HT1A downregulation is not clear, but its ability to attenuate the elevations of corticosterone after chronic stress suggests that the beneficial effect of antidepressants on 5-HT1A receptors may be mediated, at least in part, by reducing HPA hyperactivity.[63] Further evidence for this is provided by the observation that, when imipramine is unable to decrease corticosterone levels, it is also unable to prevent 5-HT1A downregulation after chronic stress.[64]

The direction of the effect of chronic stress on 5-HT1A receptors is in agreement with what would be expected based on the adrenalectomy studies (*i.e.*, upregulation in the absence of corticosteroids, downregulation with excess of corticosteroids). However, these results are merely correlative, because stress is a very complex biological response and, in addition to corticosteroid secretion, many other events are occurring, which could potentially be affecting 5-HT1A receptor levels.

In order to elucidate whether the 5-HT1A downregulation observed in this paradigm is a consequence of the increase in plasma corticosterone, or if it is mediated by a central mechanism (*i.e.*, brain circuits activated during stress), we investigated the effect of chronic unpredictable stress in adrenalectomized (ADX) rats with and without corticosterone replacement (cort-replaced).[56] This was done in orther to prevent the persistent

rise of plasma corticosterone during prolonged stress. Six groups of animals were studied: (1.) sham-operated animals that were not stressed (controls), (2.) sham animals that were stressed, (3.) ADX animals, nonstressed, (4.) ADX, stressed, (5.) cort-replaced, nonstressed, and (6.) cort-replaced, stressed. Chronic unpredictable stress was administered for 14 days. All animals were sacrificed 24 hours after the last stress session. Corticosterone was measured by radioimmunoassay. Hippocampal 5-HT1A mRNA was measured by *in situ* hybridization. The results of this experiment are seen in FIGURE 1. We found that plasma corticosterone significantly increased in the sham-stressed animals compared to the other groups. In these sham-stressed animals, chronic stress caused a consistent decrease in 5-HT1A mRNA in all hippocampal subfields, although the decrease achieved statistical significance only in CA3 and the dentate gyrus (DG) ($p < 0.05$ by ANOVA). No 5-HT1A mRNA downregulation was observed in the ADX stress or cortisone -replaced stress groups compared to controls. Therefore, elimination of the corticosterone rise after stress prevented the decrease in hippocampal 5-HT1A. These results indicate that the 5-HT1A down-regulation observed after chronic stress is mostly mediated by increases in plasma corticosterone levels. Interestingly, chronically stressed rats that were ADX and received corticosterone replacement also had lower 5-HT1A levels than controls. Whether this represents a chance event or the fact that there is indeed a small effect of stress independent of corticosteroid levels, remains to be investigated.

The results of these experiments suggest that corticosteroids, by interacting with the 5-HT1A receptor, may play an important role in the relationship among stress,[65] mood changes,[65] and perhaps suicide. Because hypersecretion of endogenous corticosteroids in animal models can decrease 5-HT1A receptor expression, it may be possible that the hypercortisolemia found in depressed patients can exacerbate disturbances in affective states associated with 5-HT1A receptor function. If this is the case, then antidepressant medications, in addition to directly correcting a central monoaminergic disturbance, may act to improve serotonergic function indirectly by decreasing cortisol hypersecretion. We have found some evidence that these mechanisms may be operating in the human brain. In a small number of suicide victims with a history of depression, we found decreases in 5-HT1A mRNA levels in several hippocampal subfields, compared to nonsuicide controls.[56] The decreases in 5-HT1A gene expression ranged from 30% to 45%, depending on the hippocampal subfield, with the greatest decrease found in CA3. There was also a reduction in MR mRNA levels in the same hippocampal regions. This is consistent with a history of exposure to high corticosteroid levels, and therefore consistent with the animal data of corticosteroid-mediated 5-HT1A mRNA downregulation. Serotonin 1A receptor binding has also been found to be decreased in the pars opercularis and temporal lobe of elderly depressive

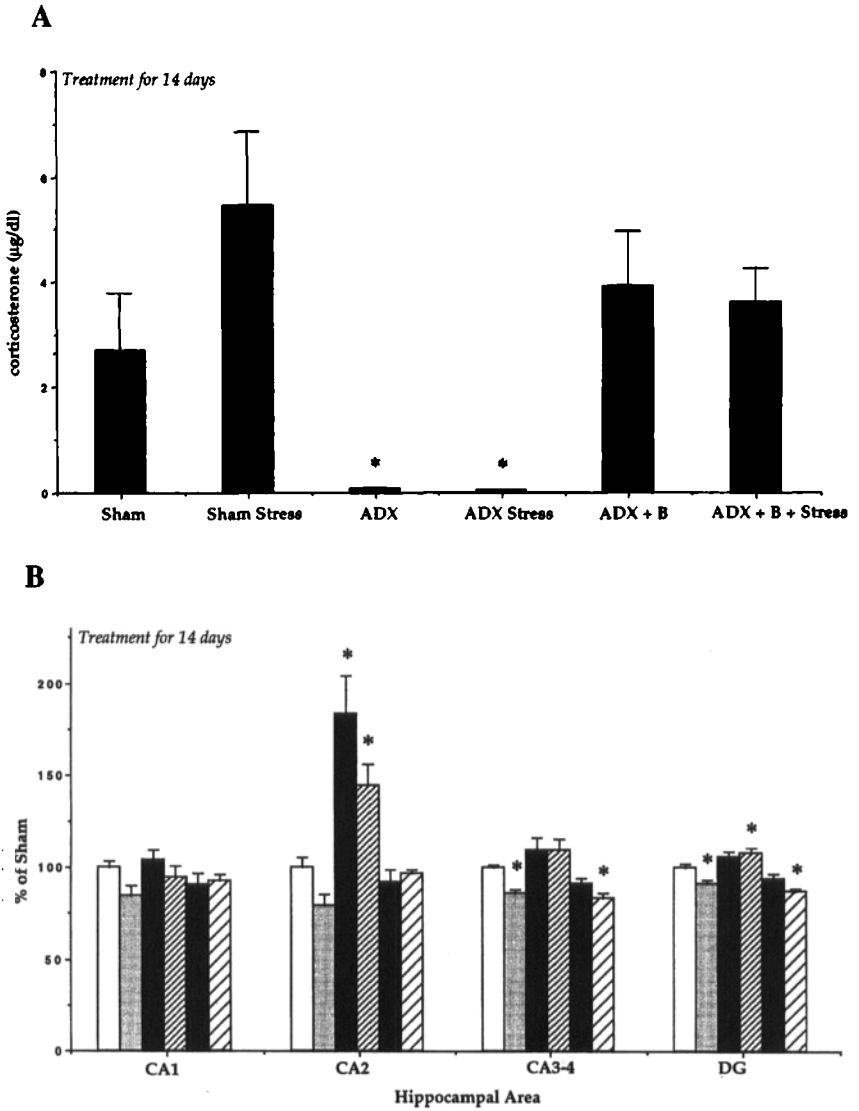


**FIGURE 1.** Effect of chronic unpredictable stress, adrenalectomy (ADX), and corticosterone (B) replacement on plasma corticosterone (**A**) and 5-$HT_{1A}$ mRNA levels (**B**). Intact (sham) animals that underwent 14 days of chronic unpredictable stress showed a decrease in 5-$HT_{1A}$ mRNA compared to the non-stressed group. ADX with and without B replacement abolished the differences between the stressed and nonstressed animals. *Significantly different from sham by ANOVA, $p \leq 0.05$. □, sham; ▩, sham stress; ■, ADX; ▨, ADX stress; ■, ADX + B; ▨, ADX + B + stress.

patients.[66] This last observation is of interest to the potential relationship between hypercortisolemia and 5-HT1A receptors, inasmuch as aging is associated with worse impairment of HPA axis function.[67]

Because ADX and steroid administration are known to cause acute effects on 5-HT content and release, it is possible that corticosteroids are modulating 5-HT1A receptor levels secondarily, through 5-HT synaptic changes. However, hippocampal 5-HT1A receptors, as assessed by ligand binding, have proven to be remarkably resistant to homologous regulation, even after complete loss of serotonin stimulation as a result of a neurotoxic lesion.[68,69] Similarly, significant depletion of hippocampal serotonin appears to produce only relatively small, or no, changes in 5-HT1A receptor mRNA within hippocampal subfields.[70] It is, therefore, most likely that changes in both 5-HT1A mRNA expression and 5-HT1A binding observed in response to stress do not result from stress-induced changes in serotonergic activity but rather reflect a direct effect on hippocampal neurons.

Due to the abundance of MR and GR receptors in this brain area, corticosteroid-induced downregulation of 5-HT1A is likely an effect specific to the hippocampal formation. Some postmortem studies have reported an increase in 5-HT1A binding in the prefrontal cortex of suicide victims.[71,72] Although this is in apparent contradiction with the findings of decreased 5-HT1A in the hippocampus, the results of these studies are not necessarily inconsistent. Regulation of 5-HT1A receptors may be different in different brain regions. For example, hippocampal 5-HT1A receptors, due to their colocalization with MR and GR,[35,36,52] may be more sensitive to circulating steroids, whereas receptors in the prefrontal cortex may be less responsive to steroids and more responsive to changes in local 5-HT levels. As we will see below, these 5-HT1A changes in the prefrontal cortex are in the same direction as those reported for the 5-HT2A receptor in the same anatomical region.[73]

## SEROTONIN 1B RECEPTORS, SEROTONIN 2A RECEPTORS, CORTICOSTEROIDS, AND STRESS

The 5-HT1A receptor is not the only serotonin receptor whose function may be relevant to the pathophysiology of suicide. Recent animal studies have implicated the 5-HT1B receptor in impulsive and aggressive behavior.[74,75] In a resident-aggression test, mutant mice lacking the 5-HT1B receptor show a significant increase in impulsive attacks, compared to wild-type mice.[74,75] In addition, some 5-HT1B agonists, called "serenics," have been shown to decrease aggression in several behavioral paradigms.[76] The 5-HT1B receptor is located both presynaptically (where it is believed to control serotonin release) and postsynaptically.[77] The antiaggressive effects of 5-HT1B are believed to be mediated by the postsynaptic receptor,[78,79] inasmuch as lesioning the central serotonergic system does

not alter the antiaggressive properties of serenics. These animal studies have important implications for the neurobiology of suicide, inasmuch as impulsive behavior in humans (including violent suicide) has been associated with a decrease in central serotonergic activity.[80] Ramboz *et al.*[75] have speculated that low serotonergic activity could result in a decreased activation of the postsynaptic 5-HT1B receptor, which may result in aggressive and/or impulsive behavior.

There is a paucity of studies investigating whether stress and/or corticosteroids can modulate 5-HT1B receptors. Mendelson and McEwen[81] measured 5-HT1B receptor binding in the rat hippocampus after ADX and corticosterone administration. They found that ADX increased 5-HT1B binding in the DG and that high doses of corticosterone reduced the binding below sham levels. Corticosterone also reduced 5-HT1B binding in area 2 of the cortex.[81] Because receptor binding studies using $^{125}$iodocyanopindolol will visualize both the presynaptic and postsynaptic 5-HT1B receptor, it is not known which hippocampal cells and which receptors (pre- vs. postsynaptic) are regulated by corticosterone.

We therefore used *in situ* hybridization to study the regulation of the postsynaptic 5-HT1B receptor in the hippocampus, using the same ADX-cort-replaced animals in which 5-HT1A regulation was studied (FIG. 2). 5-HT1B mRNA is found in the cell bodies of the pyramidal neurons of the

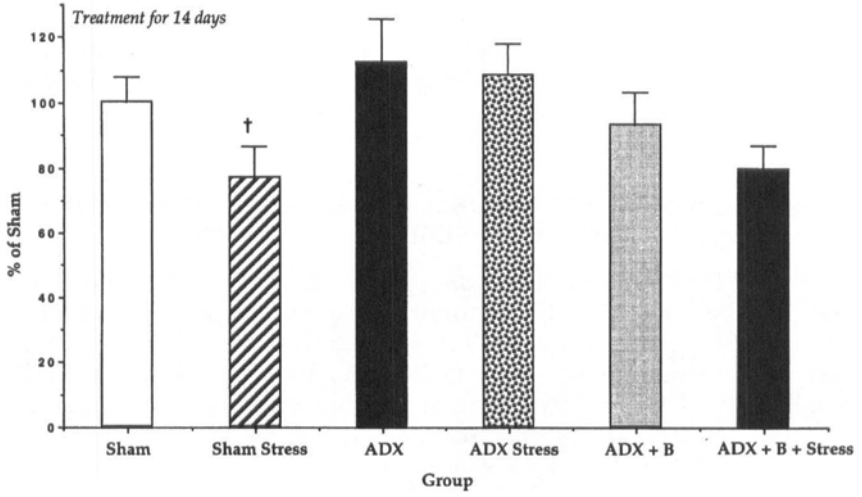


**FIGURE 2.** Two weeks of chronic unpredictable stress resulted in a small, but significant, decrease in 5-HT1B mRNA in CA1 compared to controls. † Significantly different from sham animals by ANOVA, $p \leq 0.05$.

CA1 hippocampal subfield, and these neurons project axons to the dorsal subiculum.[82] Two weeks of chronic unpredictable stress resulted in a small, but significant decrease in 5-HT1B mRNA in CA1 compared to controls (Fig. 2). This decrease was prevented if rats were ADX prior to undergoing the stress schedule, indicating that the decrease in postsynaptic 5-HT1B after stress is mediated, for the most part, by circulating corticosteroids. To the extent that postsynaptic 5-HT1B receptors are involved in modulating impulsive and/or aggressive behavior, these results would suggest that stress, by downregulating 5-HT1B receptors, may be capable of influencing, triggering, or worsening impulsive behavior.

Another serotonin receptor closely associated with the neurobiology of suicide is the 5-HT2A receptor. An increase in 5-HT2A receptor number (a postsynaptic receptor) in the prefrontal cortex of suicide victims has been reported by several investigators.[83–85] Some studies have not replicated these findings, but factors such as prior medication use may account for some of the discrepancies. Interestingly, chronic antidepressant treatment causes opposite changes of those found in suicide, that is, downregulation of cortical 5-HT2A receptors.[86,87]

Some animal studies have shown that the 5-HT2A receptor can be regulated by steroids and by stress. Chronic social stress increases 5-HT2A binding in the parietal cortex of subordinate rats.[88] Administration of ACTH for ten consecutive days decreased 5-HT2A binding in the neocortex of the rat forebrain.[89] This effect is abolished by ADX and mimicked by corticosterone administration for ten days.[89] Dexamethasone treatment for the same amount of time also causes a dose-dependent increase in 5-HT2A binding in the cortex,[90] suggesting that this effect is mediated by the GR. These effects of stress and steroids on cortical 5-HT2A are again in the direction expected if stress and HPA activation are contributing to the 5-HT receptor changes observed in suicide victims. Like the hippocampal 5-HT1A changes, it is not clear if this is a direct effect of corticosteroids on the receptors themselves or if these effects are secondary to steroid-induced changes in serotonin levels. Interestingly, the effect of corticosteroids on 5-HT2A receptor number seems to be specific for the cortex. Neither chronic social stress[88] nor dexamethasone administration[90] altered 5-HT2A binding in the rat hippocampus. Given the abundance of both MR and GR in the hippocampus, and the lack of MR in the cortex, it is plausible that 5-HT receptor regulation in the cortex is mediated through different mechanisms than in the hippocampus. For example, 5-HT2A may be more responsive to antecedent changes in the endogenous ligand (*i.e.*, serotonin) than to direct regulation by corticosteroids, as occurs with hippocampal 5-HT1A receptors.

Some investigators have suggested that a balance between cortical 5-HT2A and hippocampal 5-HT1A receptors is essential for an animal's ability to respond to stress.[88] It is not clear whether the 5-HT2A and 5-HT1A receptor changes observed in hypercorticoid states represent an adaptive

response to stress or a breakdown of this adaptive mechanism. Nevertheless, the fact that antidepressants reverse many of the behavioral and neuroendocrine changes provoked by chronic stress and at the same time revert or oppose the 5-HT2A and 1A receptor changes points to an important role of these two receptors in the control of mood and perhaps in the pathophysiology of suicide.

## SEROTONIN TRANSPORTER AND CORTICOSTEROID REGULATION

The 5-HT receptor changes discussed above describe mostly a postsynaptic effect of corticosteroids on serotonergic function. However, serotonergic transmission is also controlled by presynaptic mechanisms. For example, antidepressants are believed to exert their therapeutic action by blocking the reuptake serotonin from synaptic terminals.[91,92] The reuptake of serotonin is mediated by a specific 5-HT transporter located in the presynaptic membrane. Changes in transporter sites and transporter mRNA levels have been reported to follow chronic antidepressant treatment,[91,93] indicating that regulation of this molecule is intimately linked to the concentration of 5-HT in the synapse. Arango and collaborators[72] have reported a significant decrease in serotonin transporter binding in the prefrontal cortex of suicide victims. This suggests that abnormalities in the serotonin transporter are linked to the serotonergic dysfunction postulated to occur in suicide victims.

In order to investigate whether corticosteroids have an effect in serotonin receptor binding and whether that effect was consistent with that found in suicide victims, we performed two experiments investigating the effect of ADX, dexamethasone administration, and chronic unpredictable stress on serotonin transporter sites in the hippocampus and cortex.

In one experiment (FIG. 3), rats were either ADX (n = 12) or sham-operated (n=11), as described above. ADX animals received 0.9% saline as drinking water. Five ADX animals received once daily injections of dexamethasone (50 ug ip) for 1 week; the remaining ADX animals received daily injections of saline for the same time period. Sham ADX animals received daily injections of saline (n = 6) or dexamethasone (n = 5) in an identical fashion to ADX groups. After one week, all animals were sacrificed by decapitation, brains were removed and total serotonin transporter sites were measured in the hippocampus and cortex by receptor autoradiography, using $^{3H}$paroxetine. We found no significant differences between any of the groups. Therefore, neither removal of circulating steroids nor dexamethasone administration had an impact on serotonin transporter sites in the areas studied.

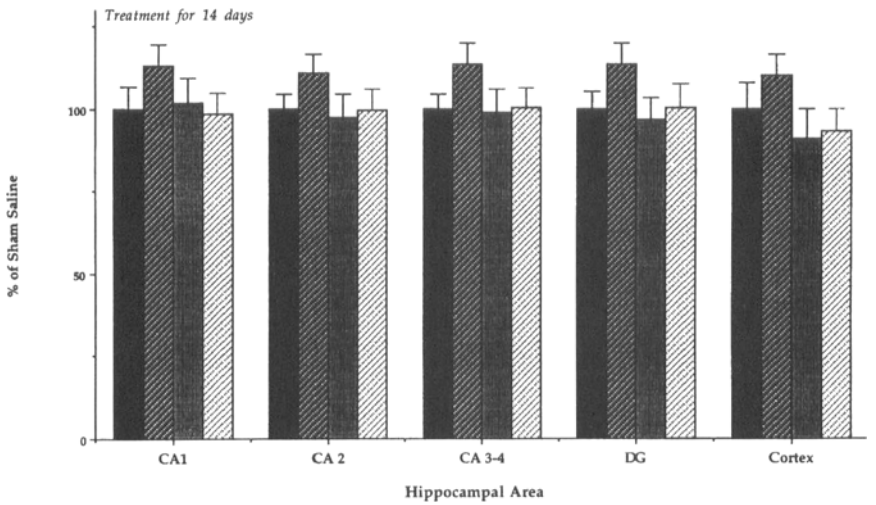


**FIGURE 3.** 5-HT transporter binding sites in the hippocampus. Animals were adrenalectomized (ADX) or sham operated for one week and received daily injections of dexamethasone (Dex) or saline. No differences were detected in total $^{3H}$paroxetine binding between any of the groups. ■, sham saline; ▨, sham Dex; ■, ADX saline; ▨, ADX Dex.

In another experiment we investigated the effect of two weeks of chronic unpredictable stress(ref) and antidepressant administration on $^{3H}$paroxetine binding in the same brain areas (FIG. 4). Rats were divided into four groups (n = 6/group). One group received saline injections daily (controls), another group received daily intraperitoneal (ip) injections of imipramine (10 mg/kg), a third group underwent daily sessions of chronic unpredictable stress and saline injections, and a fourth group received both daily stress and imipramine. Animals in all treatment groups were sacrificed by decapitation on day 15; brains were removed and processed for serotonin transporter measurement as above. As in the ADX study, we did not find any effect of chronic stress on 5-HT transporter sites in the hippocampus or cortex.

These studies suggest that circulating corticosteroids have very little effect in modulating the number of 5-HT transporter sites, at least in the areas studied. This speaks against the possibility that hyperactivity of the HPA axis is responsible for the decrease in transporter sites reported in suicide victims.[72] This of course does not rule out the possibility that steroids may be affecting the function of the serotonin transporter. A recent paper has reported that dexamethasone can increase the reuptake of serotonin in platelets, without affecting the number of transporter sites.[94] The possibility that this may be occurring in the brain deserves further exploration in animal studies.

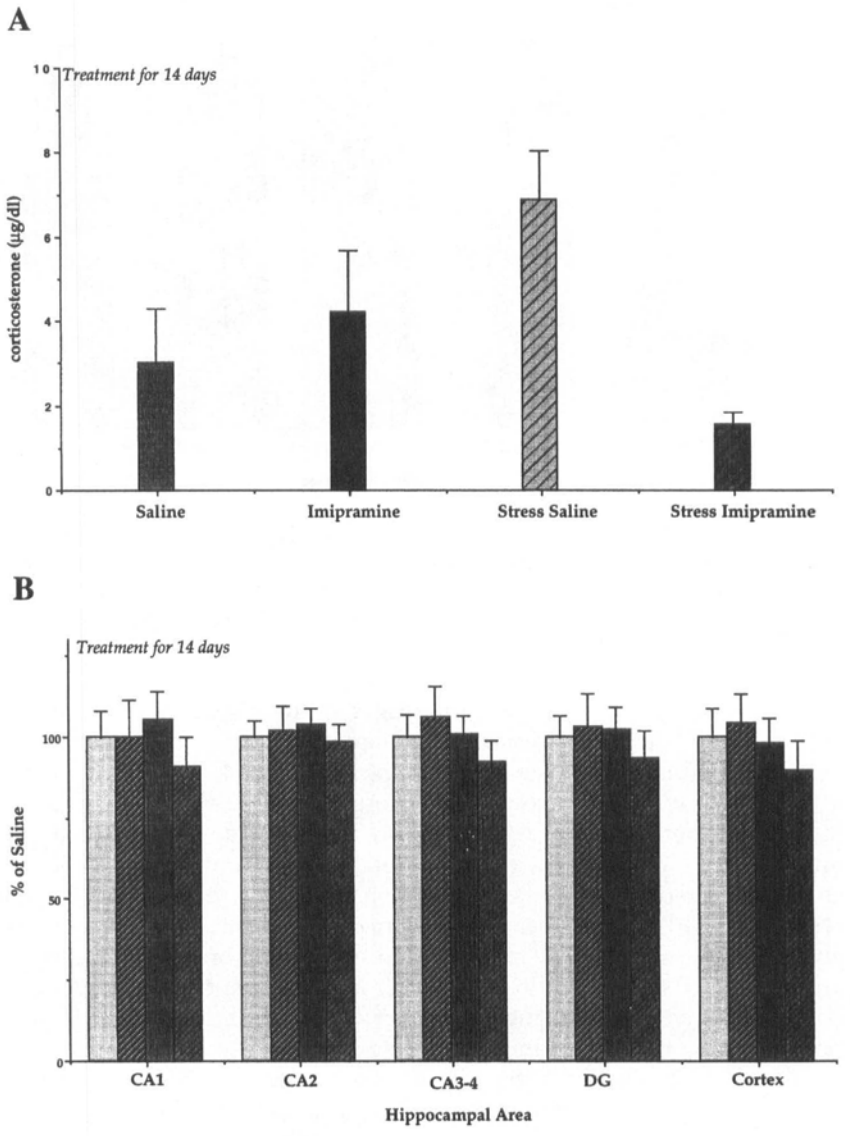


**FIGURE 4.** Two weeks of chronic unpredictable stress or daily imipramine administration (10 mg/kg) had no significant effect in the binding of [$^3$H]paroxetine in rat hippocampus. Panel **A** shows the plasma corticosterone levels. Panel **B** shows the paroxetine binding ($B_{max}$) across all hippocampal subfields and the cortex. ■, saline; ▨, imipramine; ■, stress saline; ▨, stress imipramine.

## EARLY LIFE STRESS AND HPA REGULATION

Up to this point we have reviewed evidence showing that stress has important biological consequences in the brain of the adult animal. The impact of stress, manifested by hyperactivity of the HPA axis and resulting in biochemical changes in the limbic circuitry, may provide a neurobiological underpinning to the correlation that has been described between life stressors, depression and suicide. Another important correlation that emerges in the clinical literature is between psychosocial stressors early in life and suicidal behavior.[21] For example, psychiatric patients with a history of suicide attempts have a higher incidence of parental loss,[95] maternal loss,[96] and parental separation[97] than do nonsuicidal patients. Suicide attempters experience significantly more negative and less positive parental rearing factors than nonattempters.[95] Children and adolescents who attempt suicide have significantly more chaotic family situations than controls.[98] Parental loss may also be a factor in completed suicides.[99]

It is likely that these early life events have important psychological consequences, manifested by an increase in suicide risk, as well as an increased risks for depression and substance abuse. However, in addition to the psychosocial consequences, animal studies suggest that early "stressful" events may also have important neurobiological effects. It is known that stress, and activation of the neuroendocrine stress system, can have important consequences for a developing organism. Studies in rodents show that the developing brain may be more sensitive to changes in circulating corticosteroids than the adult brain,[100–102] and therefore activation of the HPA axis could have a more negative impact on the brain systems that participate in the modulation of mood, cognition, and behavioral control.

Early in life, there is a delicate and critical balance of the activity of the HPA axis to maintain very low glucocorticoid levels. During the first two weeks postnatally, from day 3 to 14, the HPA stress system is characterized by a "silent period," during which the developing infant rat is hyporesponsive to stress (stress hypo-responsive period or SHRP).[103,104] During this time the animal maintains very low levels of circulating corticosterone even under conditions of stimuli that normally elicit corticosterone elevations in adults. In view of the catabolic influence of corticosteroids on growing and differentiating systems, the SHRP serves a highly adaptive function, allowing anabolic events to predominate during this period.

Maternal behavior influences several physiological processes in the developing infant, including the relative stress hyporesponsiveness unique to the SHRP. Levine and co-workers have found that, following 24

hours of maternal deprivation the neonatal rat responds with significant increases in ACTH and corticosterone when exposed to novelty, injection of isotonic or hypertonic saline, and ether vapors.[105,106] These endocrine responses observed in the maternally deprived animals are unique and resemble the stress response seen in developing animals during their weaning period (25 days old)[102] and also the stress response of adult animals subjected to disruption of their hippocampal-hypothalamic connections.[12] Under all of these models, the animal has elevated basal corticosterone levels, and these levels also remain elevated for a prolonged time after the stressor. Thus, deprivation of maternal nurturing activities results in a hyperresponse of the HPA axis to a stressor at a time in the animal's life when the axis is usually quiescent.

Vázquez and collaborators[107] have shown that the HPA response to maternal separation is associated with a decrease of MR mRNA expression in the CA1 region of the developing hippocampus. These animals also show a significant decrease in 5-HT1A mRNA in the same brain region (FIG. 5). It is not clear if these receptor changes in the hippocampus are due to a direct effect of maternal deprivation or are secondary to the initial increase in corticosteroid levels. However, it is apparent from this study that the small but significant decrease in MR mRNA levels may be sufficient to offset the normal age-related increase in MR mRNA levels in this region. Given the importance of hippocampal MR in maintaining or enhancing synaptic responsiveness, a decrease in MR gene expression may have direct repercussions in the modulation of HPA activity. It may also have important consequences for the activity of serotonin in the hippocampus.

There is evidence that the disturbances in HPA activity secondary to maternal separation have long-lasting effects into adulthood. Adult rats that underwent maternal separation have higher hypothalamic CRH mRNA content than unhandled rats.[108] In addition, maternally deprived rats have an increase in both basal and stress-induced ACTH levels, as well as an increase in CRH immunoreactivity in the median eminence.[109] Interestingly, brief daily periods of handling (15 minutes) during the first two weeks of life have opposite effects in the HPA axis of adult animals.[110] Handled animals show, as adults, lower CRH mRNA levels, an increased number of glucocorticoid receptors in the hippocampus and hypothalamus and enhanced negative feedback of the HPA axis.[110] These adult animals also have significantly lower 5-HT2A binding in the frontal cortex compared to unhandled animals, as well as an increase in 5-HT turnover.[111]

It is clear then from these studies that manipulations early in the life of the developing organism influences the activity of the HPA axis and alters the stress response. These alterations are long lasting and are accompanied by alterations in the serotonin system, which may persist into adulthood.

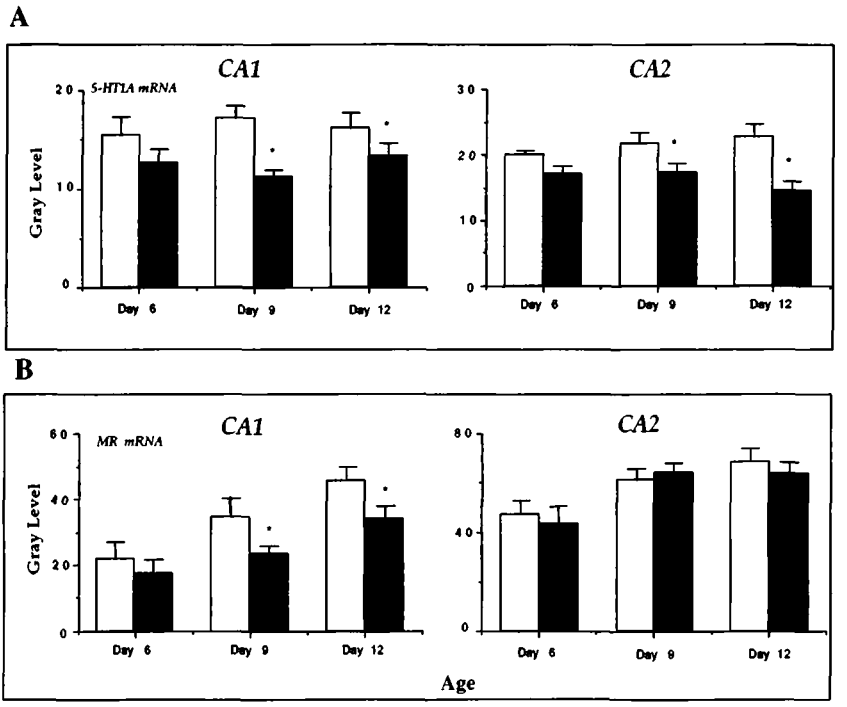


**FIGURE 5.** Twenty-four hours of maternal deprivation reduces 5-HT1A mRNA levels in CA1 and CA2 hippocampal subfields in 9- and 12-day old rats (**A**). A decrease in MR mRNA levels was also observed in CA1, but not in CA2 (**B**). *Significantly different from nondeprived animals, $p \leq 0.05$. □, nondeprived; ■, deprived.

## MONOAMINE REGULATION OF GR AND MR IN THE HIPPOCAMPUS

Although it is clear that corticosteroids can regulate 5-HT receptors, it is also important to remember that regulation can exist in the other direction. Acute administration of 5-HT1A and 5-HT2 agonists causes release of ACTH and corticosteroids, and chronic administration of 5-HT agonists and reuptake inhibitors are capable of upregulating GR and MR in the limbic system.[112] Monoamine tone seems to be essential for the maintenance of normal GR and MR levels in the hippocampus. In order to study the effect of removing this monoamine tone on MR and GR receptors, we gave rats daily injections of methamphetamine (20 mg/kg, sub cutaneously, twice daily for 4 days) and compared them to animals that received saline injections. High doses of methamphetamine are neurotoxic to both dopamine and serotonin neurons.[113,114] Animals were sacri-

ficed after two weeks, and we analyzed GR and MR levels in the hippocampus using *in situ* hybridization. We found that amphetamine administration caused a decrease in both MR and GR mRNA levels in the hippocampus (FIG. 6). That this effect of methamphetamine is mediated

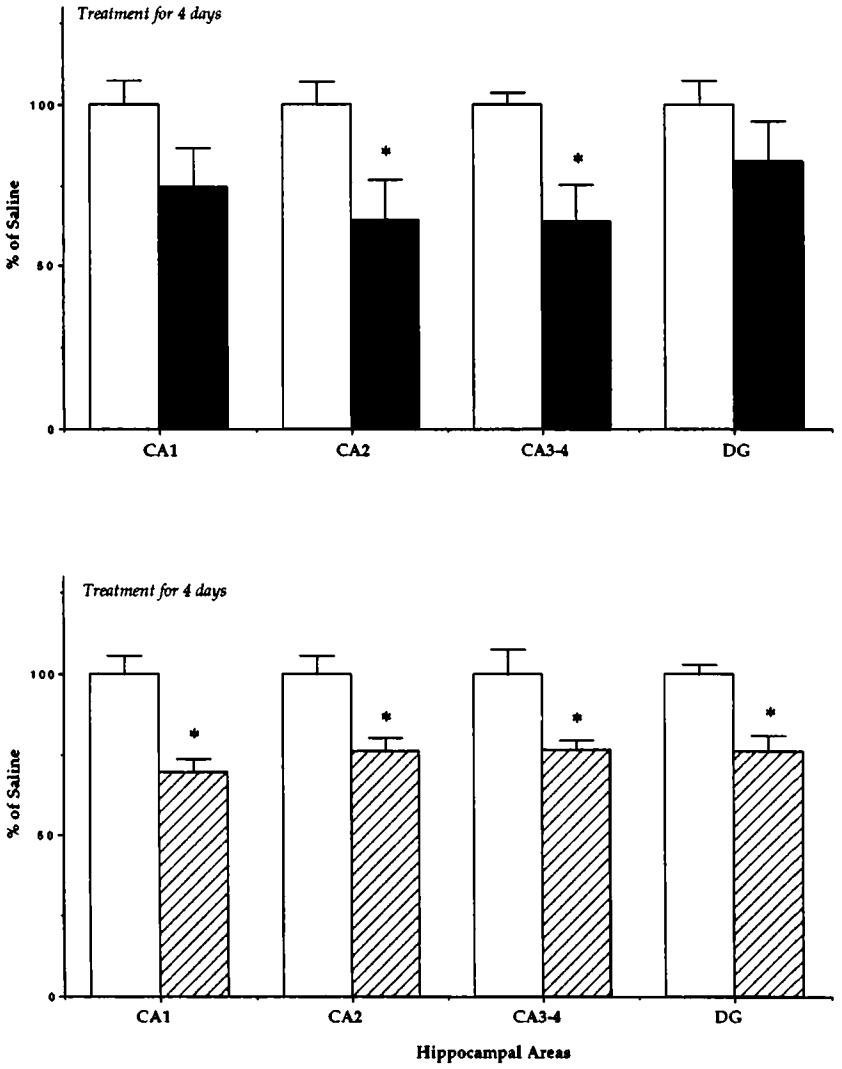


**FIGURE 6.** Four consecutive days of amphetamine administration at neurotoxic doses results in significant decreases in GR (**A**) and MR (**B**) mRNA levels in rat hippocampus. *Significantly different from saline, $p \leq 0.05$. □, saline; ■, methamphetamine.

by 5-HT has been confirmed by the fact that specific destruction of central serotonergic neurons using 5,7-dihydroxytryptamine also decreases hippocampal GR and MR gene expression.[115] These data clearly point out that the relationship between corticosteroids and serotonin in the hippocampus is bidirectional, complex, and tightly controlled.

## CONCLUSIONS AND FUTURE PROSPECTS

Based on the animal studies reviewed above, we can generate a working model of the interplay between the stress axis and the monoamines, and their potential interactions in suicide and depression. Although it is possible that some monoamine receptor changes are due to antecedent changes in their endogenous ligands, we propose that many of the receptor changes observed may be a result of the HPA overactivity present in at least some suicide victims, in particular those with a history of affective disorders. The rationale for this hypothesis derives from the above evidence, which can be summarized as follows.

(1) Suicide victims, in general, and depressed patients, in particular, show evidence of overactivity of the stress axis. (2) Chronic stress and/or high steroid levels in rats results in an alteration of specific 5-HT receptors (*e.g.*, increases in cortical 5-HT2A, decreases in hippocampal 5-HT1A) in terminal projection fields. (3) Most human receptor binding studies show the same changes in the brains of suicide victims (*e.g.*, increases in cortical 5-HT2A) as are found in hypercorticoid states. Our human data suggest a decrease in 5-HT1A mRNA levels in the hippocampi of depressed suicide victims (as seen with chronically stressed animals). (4) Chronic antidepressant administration causes opposite receptor changes to those seen with chronic stress (*e.g.*, downregulation of 5-HT2A cortical receptors, upregulation of hippocampal 5-HT1A receptor). (5) Antidepressant administration reverses the overactivity of the HPA axis. Keeping in mind these observations, it is possible to construct a model of the relationships among stress hormones, monoamine receptors, and mood. We will illustrate these relationships using the 5-HT receptors, but the same model can be applied to the noradrenergic system.

If indeed the 5-HT1A and 5-HT2A (and perhaps 5-HT1B) receptors have at least a partial role in controlling affective states (either directly or secondarily through other systems), then their modulation by corticosteroids provides a potential mechanism by which these hormones may regulate mood. This of course does not exclude the possibility that steroids can be simultaneously acting through other systems, such as the $\alpha$ and $\beta$ adrenoreceptors, thereby synergistically affecting mood and behavior.

If hypersecretion of endogenous corticosteroids can affect these 5-HT receptors, is it possible that the hypercortisolemia present in some depressed patients may be contributing to the affective disturbance? This possibility has been raised by some investigators[116,117] based on the parallelisms between Cushing's disease and depression. Interestingly, patients with major depression who are resistant to antidepressant treatment experienced a dramatic improvement when they received steroid suppression agents.[118] Additionally, patients who respond to antidepressant treatment, but who continue to show cortisol nonsuppression after dexamethasone administration, have a much greater risk of relapsing than patients who show dexamethasone suppression.[119] Therefore, antidepressant agents, in addition to having a direct effect by correcting the central neurotransmitter "disturbance," may also be improving depression indirectly by decreasing cortisol hypersecretion.

Corticosteroid modulation of 5-HT receptors has important implications for the pathophysiology and treatment of both affective disorders and suicide. This may be one of the mechanisms by which stressful events can precipitate depressive episodes in some (genetically) vulnerable individuals and or precipitate suicidal behavior. Another implication is that altered 5-HT levels or metabolism do not necessarily have to be present for 5-HT receptor abnormalities to occur. Based on the animal data, it is apparent that specific 5-HT receptors may be directly regulated in response to alterations of corticosteroid levels. Thus, in depressed patients normal levels of serotonin and its metabolites may not necessarily reflect normal central 5-HT activity.

We know that stress affects corticosteroid release, and that this may be one of the mechanisms through which it affects 5-HT receptor function. We cannot rule out, however, that stress can also affect brain 5-HT receptors through noncorticosteroid-mediated pathways (even perhaps through noradrenergic-mediated circuits). The interplay of these factors may lead to the emergence, or maintenance, of affective symptoms. Similarly, antidepressants can counteract this phenomenon by affecting 5-HT receptor function directly[7,46] and by simultaneously regulating stress-induced corticosteroid secretion.[14,63] The same interaction could of course be occurring with other molecules implicated in the pathophysiology of suicidal behavior, such as the $\alpha$ and $\beta$ adrenoreceptors. Clearly, more animal research is needed exploring the modulation by corticosteroids of other monoaminergic receptors that postmortem studies have linked to the neurobiology of suicide.[83,120]

Inasmuch as most antidepressants are known to reverse many of these receptor changes in rodents, it will be interesting to compare these molecules in suicide victims with and without a history of chronic antidepressant intake. We may expect that some of these predicted biochemical changes may be absent in suicide victims with a history of psychotropic use. Alternatively, it is possible that, in some subjects, antidepressant

administration was unable to reverse some of these biochemical changes. This inability to correct these abnormalities may be associated with the therapeutic failure of antidepressant treatment in these individuals who have committed suicide.

An important therapeutic implication of this model is the prediction that agents that can reduce the stress response, and/or decrease HPA activation, will be useful in the pharmacological treatment of anxiety, depression, and perhaps suicidal behavior. In this respect, the recently developed CRH receptor antagonists may provide us with a new therapeutic armamentarium to treat these patients.[121,122] These compounds could be used in conjuction with antidepressants, as adjuvants or augmenting agents, and may decrease treatment resistance. This agents may also be useful in monotherapy, because preventing hypercortisolemia may be translated into an improvement of monoaminergic receptor function. The use of modern biochemical and molecular neuroanatomical techniques in the postmortem human brain should allow us to test these hypotheses, first in animal models and then directly in psychiatric illness.

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