Regulation of Hippocampal 5-HT$_{1A}$ Receptor Gene Expression by Dexamethasone

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The effect of dexamethasone, a selective GR agonist, on hippocampal, 5-HT$_{1A}$ receptor mRNA expression and 5-HT$_{1A}$ binding was examined using in situ hybridization histochemistry and in vitro *receptor autoradiography. One week after adrenalectomy, both 5-HT$_{1A}$ receptor mRNA expression and 5-HT$_{1A}$ binding were increased throughout the hippocampus. Administration of dexamethasone at the time of adrenalectomy significantly attenuated the increases in 5-HT$_{1A}$ mRNA expression in all hippocampal subfields ($p < .05$, Fisher Test), although 5-HT$_{1A}$ mRNA levels remained significantly higher than sham levels in all subfields with the exception of CA$_1$. However, 5-HT$_{1A}$ binding levels were responsive to dexamethasone administration only within particular hippocampal subfields, CA$_1$, and dentate gyrus. We conclude that GR occupation negatively regulates 5-HT$_{1A}$ receptor mRNA expression within the hippocampus and that 5-HT$_{1A}$ receptor sites are most sensitive to modulation in those hippocampal subfields expressing higher levels of GR receptors.* ***[Neuropsychopharmacology 10:215–222, 1994]***

KEY WORDS: *5-Hydroxytryptamine; Receptors; mRNA; In situ hybridization; Corticosteroids; Hippocampus*

The hypothalamo-pituitary-adrenal (HPA) axis is instrumental in controlling adaptive responses to physiological and psychological stressors. The final products of the HPA axis, adrenal steroids, have been shown to have profound effects on mood and behavior (McEwen 1987). For example, Cushing's syndrome, which is characterized by elevated corticosteroid levels, is associated with a high incidence of depression (Kathol 1985). Indeed, a significant proportion of depressed patients exhibit symptoms indicative of an overstimulated HPA axis (Murphy 1991), providing a basis for the putative involvement of adrenal steroids in the pathophysiology of affective disease. The precise mechanisms by which corticosteroids may control affective state are unclear. However, it is likely that hormonal interactions with neurotransmitter systems involved in modulating limbic circuitry are important.

Animal studies have shown that corticosteroids can alter several elements of serotonergic neurotransmission, including serotonin (5-HT) metabolism and turnover (Curzon et al. 1972; Van Loon et al. 1981; Singh et al. 1990). However, it is also evident that adrenal steroids can act to directly regulate 5-HT receptors (Biegon et al. 1985; De Kloet et al. 1986), providing a direct means by which corticosteroids can act to modulate synaptic serotonergic activity. Such regulation is particularly evident within the hippocampal formation. Electrophysiological evidence indicates that 5-HT-induced responses within the hippocampus are sensitive to a brief application of corticosteroids (Joels et al. 1991), providing a functional coupling for steroid-5-HT receptor interactions in this region. Pharmacological studies indicate that at least two subtypes of corticosteroid receptors are found in the brain: the mineralocorticoid receptor (MR), which binds corticosterone with high affinity but low capacity, and the glucocorticoid recep-

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Received August 20, 1993; revised December 10, 1993; accepted January 23, 1994.

Published by Elsevier Science Inc.
655 Avenue of the Americas, New York, NY 10010

0893-133X/94/$7.00

tor (GR), which exhibits a lower affinity for the endogenous ligand but high capacity (Reul and De Kloet 1985). The hippocampus contains very high concentrations of both MR and GR receptors compared to other brain regions (Sarrieau et al. 1984; Fuxe et al. 1985). In situ hybridization histochemistry has confirmed the intrahippocampal synthesis of these sites and provided a clear picture of their respective distributions across hippocampal subfields (Herman et al. 1989a; Chalmers et al. 1993).

At least seven major families of 5-HT receptors are found in the brain; 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7 (Frazer et al. 1990; Ruat et al. 1993). Early investigations indicated that hippocampal 5-HT_1 receptors were specifically sensitive to corticosteroid modulation (Biegon et al. 1985; De Kloet et al. 1986). However, recent molecular cloning data indicates that at least six subtypes of 5-HT_1 receptors, 5-$HT_{1A\text{-}1F}$, can now be differentiated in the CNS (Albert et al. 1990; Julius et al. 1988; Hamblin et al. 1992; Lovenberg et al. 1993; Adham et al. 1993). Of these, the 5-HT_{1A} receptor is found in particularly high abundance within the hippocampus (Chalmers and Watson 1991; Pompeino et al. 1992). Using autoradiographic techniques, we and others have con-firmed the specific sensitivity of 5-HT_{1A} receptors to corticosteroid regulation within hippocampal subfields (Chalmers et al. 1993; Mendelson and McEwen 1992). Moreover, we have established that adrenal steroids selectively regulate 5-HT_{1A} receptors at the level of 5-HT_{1A} mRNA expression and that this effect may be mediated by MR receptors (Chalmers et al. 1993).

The present studies were designed to investigate the sensitivity of hippocampal 5-HT_{1A} receptors to selective GR activation using the synthetic GR agonist dexamethasone. We have employed a specific 5-HT_{1A} cRNA riboprobe and a selective 5-HT_{1A} ligand to allow simultaneous autoradiographic analysis of both 5-HT_{1A} mRNA and 5-HT_{1A} receptors in discrete anatomical subfields of the same brain.

METHODS

Animals

In all studies subjects were male Sprague-Dawley rats (250 to 300 g). Rats were housed six per cage in a 12 hour light:dark cycle and allowed free access to food and water.

Treatment Protocol

Rats were either adrenalectomized (ADX) (12) or sham-ADX (11) bilaterally using a dorsal approach. All surgeries were performed between 9:00 A.M. and 10:30 A.M. Post-surgery, ADX animals received 0.9% saline as drinking water. Five ADX animals received once daily injections of dexamethasone (50 μg 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 (six animals) or dexamethasone (five animals) in an identical fashion to ADX groups. After 1 week, all animals were killed by decapitation, brains were removed and frozen in isopentane. At the time of sacrifice, trunk blood was collected and assayed for corticosterone and adreno-cortico-trophic hormone (ACTH).

Riboprobe Design

5-HT_{1A} cRNA riboprobe was produced from a BalI-PvuII fragment of the rat 5-HT_{1A} receptor gene (Albert et al. 1990) ligated into HincII-cut pGEM blue (Promega). This fragment is composed of a 910 bp insert covering the sequence from the beginning of the second putative transmembrane domain to the middle of the extracytoplasmic domain found between transmembrane domains VI and VII, encompassing the entire sequence of the third cytoplasmic loop. This includes the region of least homology for G-protein coupled receptors. 5-HT_{1A} probe specificity was confirmed by absence of signal in both sections labeled with sense 5-HT_{1A} probe and sections pretreated with RNase prior to hybridization with antisense (cRNA) 5-HT_{1A} probe (Chalmers and Watson 1991). Riboprobes were produced using either SP6 or T7 transcription systems in a standard labeling reaction mixture consisting of: lug linearized plasmid, 5× SP 6 transcription buffer, 125 uCi ^{35}S-UTP, 150 uM NTP's, 12.5 mM dithiothreitol, 20 U RNAse inhibitor and 6 U of the appropriate polymerase. The reaction was incubated at 37°C for 90 minutes, with labeled probe separated from free nucleotides over a Sephadex G50-50 column.

In Situ Hybridization

Sections were removed from storage at −80°C and placed directly into 4% buffered paraformaldehyde at room temperature. After 60 minutes, slides were rinsed in isotonic phosphate buffered saline (10 minutes) and treated with proteinase K (1 μg/ml in 100 mM Tris/HCl, pH 8.0) for 10 minutes at 37°C. Subsequently, sections underwent successive washes in water (1 minute), 0.1 M triethanolamine (pH 8.0, plus 0.25% acetic anhydride) for 10 minutes and 2× SSC (0.3 mM NaCl, 0.03 mM sodium citrate, pH 7.2) for 5 minutes. Sections were then dehydrated through graded alcohols and air dried.

Post-fixed sections were hybridized with 1.0×10^6 dpm [^{35}S]UTP-labeled riboprobe in hybridization buffer containing 75% formamide, 10% dextran sulphate, 3× SSC, 50 mM sodium phosphate buffer pH 7.4), 1× Denhardt's solution, 0.1 mg/ml yeast tRNA and 10 mM dithiothreitol in a total volume of 25 μl. The diluted

probe was applied to sections on a glass coverslip that was sealed into place with rubber cement. Sections were hybridized overnight at 55°C in a humid environment.

Post-hybridization, the rubber cement was removed and sections were washed in 2× SSC for 5 minutes and then treated with RNase A (200 μg/ml in 10 mM Tris/HCl, pH 8.0, containing 0.5 M NaCl) for 60 minutes at 37°C. Subsequently, sections were washed in 2× SSC for 5 minutes, 1× SSC for 5 minutes, 0.5× SSC for 60 minutes at hybridization temperature, 0.5× SSC at room temperature for 5 minutes and then dehydrated in graded alcohols and air dried. For signal detection, sections were placed on Kodak XAR-5 X-ray film and exposed for 2 days at room temperature.

In Vitro Receptor Autoradiography

[^{3}H]-8-OH-DPAT binding was performed according to published methods (Palacios et al. 1987). Slide-mounted tissue sections were preincubated in 0.17 M Tris/HCl, pH 7.6, containing 4 mM $CaCl_2$ and 0.1% ascorbic acid for 30 minutes at room temperature. Subsequently, sections were incubated with 2 nM [^{3}H]8-hydroxy-2-(N, N-di-n-propylamino) tetralin) for 60 minutes at room temperature. Post-incubation, slides were washed in incubation buffer (2× 5 minutes) at 4°C and dried in a stream of cold air. Non-specific binding was determined in the presence of 2 μM 5-HT. Sections were apposed to tritium-sensitive Hyperfilm and exposed at room temperature for 10 days. As 2 nM has been determined as a saturating concentration for 8-OH-DPAT (Palacios et al. 1987), resulting autoradiograms are a measure of B_{max} for 5-HT$_{1A}$ receptors.

Microdensitometric Analysis

Autoradiograms generated from both in situ hybridization and in vitro receptor autoradiography were analyzed using an automated image analysis system (Dage camera, MACII/IMAGE program). Anatomical regions of interest were interactively selected and mean optical density measurements for each region were determined from at least six coronal sections. Hippocampal subfields were determined with reference to Nissl-stained sections and the anatomical atlas of Paxinos and Watson (1986). Nonspecific labeling of [^{35}S]-riboprobes was determined from an area of section exhibiting apparent lack of hybridization signal. For in vitro receptor autoradiograms, nonspecific binding was determined from adjacent sections incubated with [^{3}H]-8-OH-DPAT in the presence of 2 μM 5-HT. Optical density values from receptor autoradiograms were converted to pmoles/g tissue with reference to pre-calibrated tritium standards. Statistical differences were determined by one-way ANOVA and post hoc Fisher test.

RESULTS

Plasma Corticosterone and ACTH Levels

ADX animals possessed undetectable corticosterone values. The sham-saline group had a mean plasma corticosterone value of 1.2 ± 0.5 μg/dl whereas sham-dexamethasone animals exhibited undetectable levels. ACTH measurements were utilized to assess the effectiveness of dexamethasone administration; for ADX-saline and ADX-dexamethasone groups, these were 53.3 ± 25 fmoles/ml and 29.4 ± 12 fmoles/ml, respectively. For sham-saline and sham-dexamethasone groups, these were 16.6 ± 3 fmoles/ml and 20.6 ± 3 fmoles/ml, respectively.

Effects of Dexamethasone Administration

After 1 week ADX, 5-HT$_{1A}$ receptor mRNA expression was significantly increased in all hippocampal subfields (Figure 1A). ANOVA indicated significant effects of treatment in all subfields: CA_1–$F(3,17) = 10$, $p < .0004$; CA_2–$F(3,17) = 35$, $p < .0001$; $CA_{3/4}$–$F(3,17) = 36$, $p < .0001$; dentate gyrus–$F(3,17) = 28$, $p < .0001$. Administration of dexamethasone at the time of ADX significantly attenuated the increases in 5-HT$_{1A}$ mRNA expression in all subfields (Figure 1A). Post hoc analysis indicated significant differences between ADX-saline and ADX-dexamethasone groups in all subfields ($p < .05$, Fisher test). However, SHAM-saline and ADX-dexamethasone groups were also statistically different in all subfields with the notable exception of CA_1. Thus, it is apparent that 5-HT$_{1A}$ mRNA expression may be induced by ADX in most hippocampal subfields even in the presence of GR occupation. ADX treatment also induced significant increases in 5-HT$_{1A}$ binding in all hippocampal subfields, although these increments were proportionally smaller than 5-HT$_{1A}$ mRNA changes (Figure 1B). In each subfield increases in 5-HT$_{1A}$ binding were evident across strata oriens, radiatum, and moleculare in addition to the pyramidal cell layer. Unlike 5-HT$_{1A}$ mRNA expression, 5-HT$_{1A}$ binding levels were responsive to dexamethasone administration only within selective subfields, CA_1 and dentate gyrus (Figures 1B and 2). In these regions, post hoc analysis indicated significant differences between ADX-saline and ADX-dexamethasone groups ($p < .05$, Fisher test). 5-HT$_{1A}$ binding densities were not statistically different in both CA_2 and $CA_{3/4}$ subfields of ADX-dexamethasone and ADX-saline groups.

DISCUSSION

In vivo, dexamethasone principally acts through GR receptors (McEwen et al. 1990). Quantification of corticosteroid receptor occupation and activation levels af-

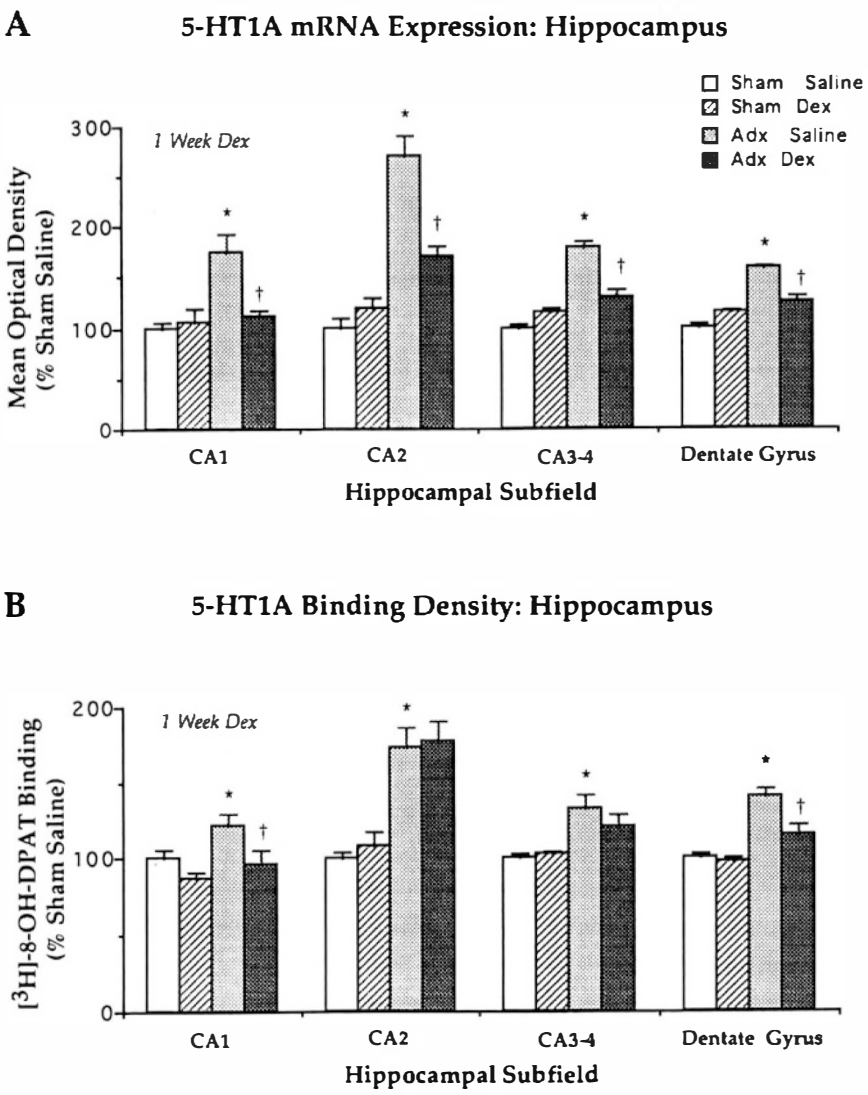

Figure 1. Densitometric analyses of 5-HT_{1A} receptor mRNA expression (**A**) and [^{3}H]-8-OH-DPAT binding (**B**) in hippocampal sections from *Sham Saline* (n = 5), *Sham Dex* (n = 5), *Adx Saline* (n = 5) and *Adx + Dex* (n = 6) animals, one week post-adrenalectomy. ANOVA indicated significant effects of treatment on both 5-HT_{1A} mRNA expression and [^{3}H]-8-OH-DPAT binding in each hippocampal subfield. Calculated pmoles/g tissue values for [^{3}H]-8-OH-DPAT binding in regions CA_1, CA_2, $CA_{3/4}$, and dentate gyrus, respectively were: 69 ± 4, 22 ± 1, 72 ± 2, 80 ± 3 for *Sham Saline*; 60 ± 3, 24 + 2, 74 ± 1, 78 ± 2 for *Sham Dex*; 84 ± 6, 38 ± 3, 96 ± 6, 112 ± 5 for *Adx Saline*; and 67 ± 6, 39 ± 3, 87 ± 5 and 92 ± 5 for *Adx Dex*. Analyses post hoc (Fisher Text) on both 5-HT_{1A} mRNA abundance and [^{3}H]-8-OH-DPAT binding confirmed significant increases when *Sham Saline* was compared to *Adx Saline* (* $p < .05$) and when *Adx Saline* was compared to *Adx Dex* († $p < .05$).

ter dexamethasone treatment indicates that dexamethasone selectively activates GR receptors (Spencer et al. 1990). Although dexamethasone also possesses affinity for MR receptors in vitro it does not appear to activate these sites in vivo (Spencer et al. 1990), this is especially evident within the hippocampus. Thus, with regard to corticosteroid receptor-mediated responses, dexamethasone can be regarded as a relatively selective GR agonist. The present studies were designed to investigate the effect of dexamethasone administration on hippocampal 5-HT_{1A} receptor modulation. In agreement with previous experiments (Mendelson and McEwen 1992; Chalmers et al. 1993), the present data indicate that hippocampal 5-HT_{1A} receptor mRNA expression and 5-HT_{1A} binding are sensitive to circulating corticosteroid levels. One week ADX treatment induced large increases in 5-HT_{1A} mRNA abundance across hippocampal subfields. These increases were significantly attenuated by dexamethasone administration at the time of ADX in all hippocampal subfields. The proportionally smaller increases in 5-HT_{1A} binding in response to ADX treatment were also attenuated by dexamethasone administration; however, this effect was only evident in specific hippocampal subregions, CA_1, and dentate gyrus. The results indicate that GR occupation negatively regulates hippocampal 5-HT_{1A} receptors at the level of 5-HT_{1A} receptor gene expression.

In previous studies we have shown that ADX-induced increments in hippocampal 5-HT_{1A} receptor mRNA levels could be prevented by administration of low levels of exogenous corticosterone at the time of ADX (Chalmers et al. 1993). The low mean plasma levels of corticosterone observed in these replaced animals suggested that the effects of corticosterone were mediated by high affinity MR receptors (De Kloet et al. 1986). Corticosterone administration prevented ADX-induced increases in 5-HT_{1A} mRNA across all hippocampal

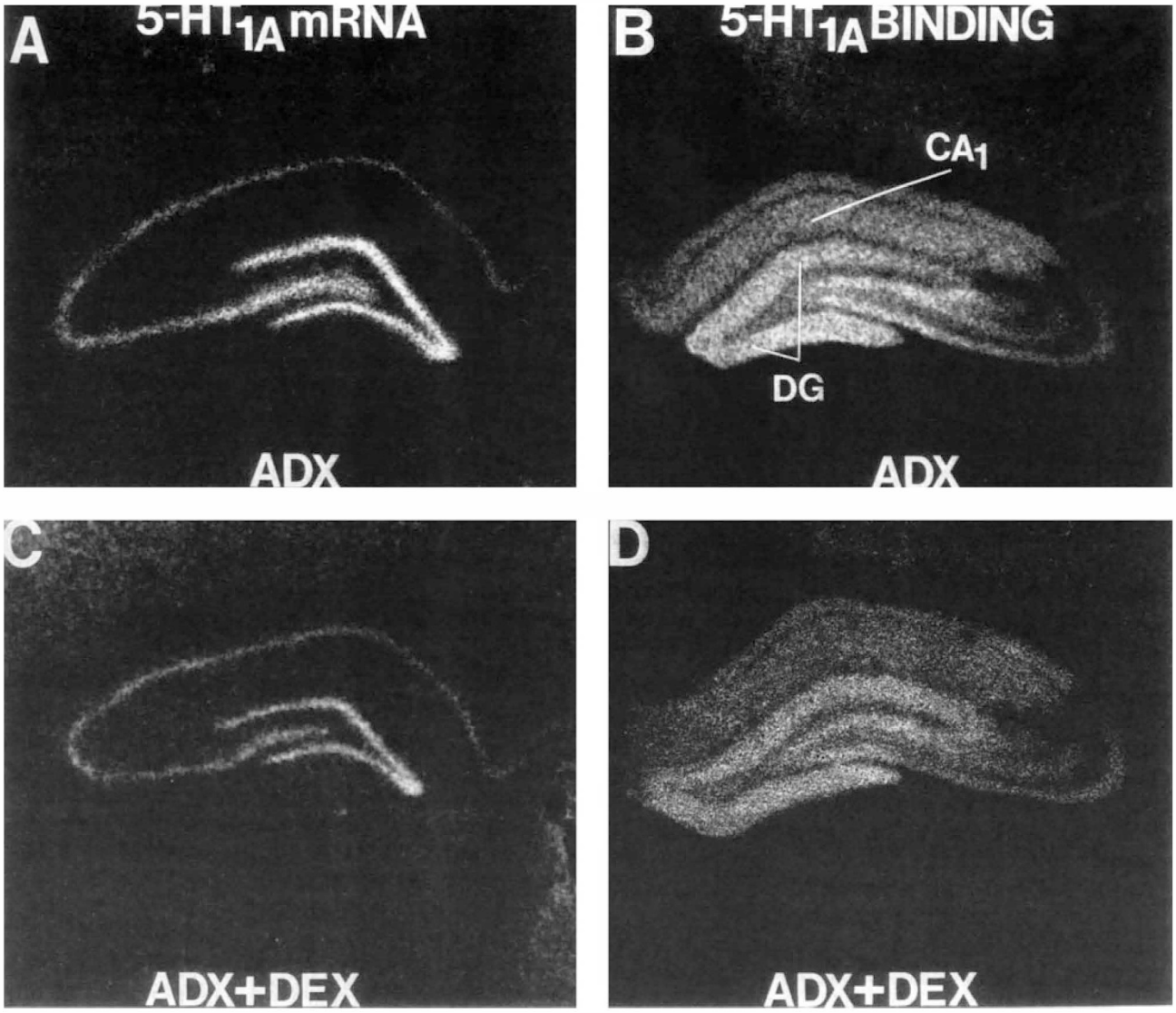

Figure 2. Photomicrographs of 5-HT$_{1A}$ receptor mRNA expression (**A** and **C**) and [^{3}H]-8-OH-DPAT binding (**B** and **D**) in hippocampal sections one week post-surgical treatment from *Adx* (**A** and **B**) and *Adx* + *Dex* (**C** and **D**) animals. Note the general reduction in hybridization signal throughout the hippocampus in *Adx* + *Dex* (**C**) in comparison to that obtained from *Adx* (**A**). In panel D, note the reduction in 5-HT$_{1A}$ binding across lamina oriens, radiatum, and moleculare of CA$_1$ and both blades of the dentate gyrus in comparison to that observed in B.

subfields (Chalmers et al. 1993). In the present study, dexamethasone administration also significantly attenuated increases in hippocampal 5-HT$_{1A}$ mRNA in response to ADX. However, unlike corticosterone replacement, dexamethasone administration was unable to maintain 5-HT$_{1A}$ mRNA levels at those of SHAM animals in most hippocampal subfields. Thus, although GR occupation negatively regulates 5-HT$_{1A}$ receptor mRNA expression, it appears to be less efficacious than MR occupation. Of course, based on the present in situ hybridization data, it is not possible to determine whether changes in 5-HT$_{1A}$ mRNA levels are due to changes in message transcription, mRNA stability, or both. Thus, it remains possible that MR and GR occupation may regulate 5-HT$_{1A}$ mRNA levels via differential molecular mechanisms. The contrasting effects of corticosterone and dexamethasone are also evident at the level of 5-HT$_{1A}$ receptor binding. Although low circulating corticosterone levels have been shown to prevent ADX-induced increases in 5-HT$_{1A}$ binding across most hippocampal subfields (Mendelson and McEwen 1992; Chalmers et al. 1993), the present study indicates that dexamethasone administration acts to regulate 5-HT$_{1A}$ binding in selective subfields, CA$_1$, and dentate gyrus. The reasons for this subfield selectivity are unclear. However, it may be relevant that the highest

levels of GR expression within the hippocampus are evident within CA_1 and dentate gyrus (Herman et al. 1989a), perhaps making these subregions more sensitive to glucocorticoids. Alternatively, it remains possible that 5-HT_{1A} receptors possess differential transcriptional/translational efficiencies with respect to hippocampal subfields. Thus, a longer time frame may be required to detect 5-HT_{1A} receptor binding changes in CA_2 and $CA_{3/4}$ in comparison to the dentate gyrus, despite comparable changes in 5-HT_{1A} mRNA levels in these regions.

The ability of both MR and GR receptors to negatively regulate hippocampal 5-HT_{1A} sites suggests that combined MR and GR receptor occupation may act to additively regulate 5-HT_{1A} receptors. Such a proposal is supported by the ability of high dose corticosterone, producing MR and GR occupation, to decrease hippocampal 5-HT_{1A} receptor binding to levels significantly lower than those observed with low dose corticosterone, producing only MR occupation (Mendelson and McEwen 1992). It is significant that this effect was detectable specifically within the dentate gyrus where high levels of both MR and GR receptors are found. In addition to regulation at the receptor level, it is likely that combined MR and GR occupation will result in significant alterations in hippocampal 5-HT_{1A} receptor mRNA expression. Indeed, studies in our laboratory indicate that chronic non-habituating stress paradigms, resulting in very high levels of circulating corticosterone, can reduce 5-HT_{1A} mRNA levels to below baseline in some hippocampal subfields (Lopez et al. 1993). However, it is possible that other stress-activated circuitry may contribute to this receptor response as well. In this regard it is of interest that a previous study found increases in hippocampal 5-HT_{1A} receptor binding in response to restraint stress (Mendelson and McEwen 1991). However, these studies were conducted over a relatively acute time frame (1 and 5 days) as opposed to our chronic studies (2 weeks), raising the possibility that hippocampal 5-HT_{1A} sites may respond biphasically to stress. It is also relevant that different stressors have been shown to differentially affect midbrain raphe neurons (Lee et al. 1987). Thus, the influence of raphe-hippocampal activity may be variable with respect to the particular stressor involved.

The hippocampal formation is a key limbic structure commonly recognized as participating in the modulation of cognition, mood, and behavior (Isaacson 1974). In addition, the presence of high concentrations of MR and GR receptors, as well as a multitude of physiological studies, implicate the hippocampus as an anatomical structure where corticosteroids act to modulate the activity of the limbic-HPA axis, thereby controlling their own release (Sapolsky et al. 1984; Herman et al. 1989b). Thus, the hippocampus represents an important anatomical interface between the HPA system and limbic circuitry. The ability of corticosteroids to regulate postsynaptic 5-HT_1A receptors within hippocampal subregions provides one molecular mechanism by which steroids can influence the effects of serotonergic input to the hippocampus and, consequently, modulate limbic function. Within the hippocampus, 5-HT_{1A} receptors on pyramidal cells mediate a hyperpolarizing response associated with an increase in K^+ conductance (Segal et al. 1989). Electrophysiological studies indicate that corticosteroids act via MR receptors to suppress 5-HT_{1A}-mediated responses, at least within the CA_1 subfield (Joels et al. 1991). Such studies are concordant with the ability of corticosteroids to negatively regulate 5-HT_{1A} receptor gene expression. However, within in vitro preparations, GR ligands apparently fail to suppress 5-HT_{1A}–mediated conductance changes (Joels et al. 1991). It is possible that the discrepancy between these in vitro studies and the present in vivo data relate to differential experimental time frames (1 to 4 hours for in vitro studies, 1 week for present study). If so, this suggests that 5-HT_{1A} receptors may be more acutely responsive to MR occupation but chronically responsive to GR occupation.

High doses of corticosterone have been shown to reduce 5-HT_{1A} related behavior in unstressed animals (Dickinson et al. 1985). In this study, corticosterone treated animals exhibited no apparent changes in regional 5-HT synthesis, raising the possibility that corticosteroid related changes in postsynaptic 5-HT sensitivity may be involved. Our data suggests that MR- (Chalmers et al. 1993) and/or GR-(present data) mediated reductions in hippocampal 5-HT_{1A} receptor gene expression could contribute to such behavioral responses. The sensitivity of brain 5-HT_{1A} receptors to corticosteroid regulation may be particularly relevant to the involvement of the HPA axis in modulating affective state as a wide body of evidence suggests a role for the 5-HT_{1A} receptor in the pathophysiology of mood disorders. For example, a number of antidepressant drugs (tricyclics and monoamine oxidase inhibitors) have been found to regulate 5-HT_{1A} receptor number and responsiveness, particularly within the hippocampus (Blier and De Montigny 1985; Blier et al. 1988; Welner et al. 1989). In addition, selective 5-HT_{1A} agonists, such as gepirone and buspirone, can be used to effectively treat generalized anxiety disorder (Rickels 1990), as well as major depression (Fabre 1990). Thus, chronic increases in corticosteroid secretion, such as those described in major depression (Kathol et al. 1989) and Cushing's Syndrome (Murphy 1991), may contribute to the development or maintenance of affective symptomatology via MR- and GR-mediated alterations in hippocampal 5-HT_{1A} receptor gene expression.

In sum, we have shown that dexamethasone administration suppresses the expression of 5-HT_{1A} receptor mRNA in the hippocampus and that this regula-

tion is accompanied by significant reductions in 5-HT$_{1A}$ receptor binding in the dentate gyrus and CA$_1$ subfields. These data indicate that hippocampal 5-HT$_{1A}$ sites are down-regulated under conditions of GR occupancy. Such modulation is highly relevant to stress-induced regulation of limbic circuitry and may ultimately contribute to putative corticosteroid-induced changes in affective state.

ACKNOWLEDGMENTS

This work was supported by a NARSAD fellowship to D.T.C., a R.W.J. Minority Medical Faculty Development grant to J.F.L., a NIDA award to D.V., and grants from NIMH (1 PO1 MH42251) and The Lucille P. Markey Charitable Trust (88-46) to H.A. and S.J.W.

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