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Corticosteroids Regulate Brain Hippocampal 5-HT_{1A} Receptor mRNA Expression

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Using *in situ* hybridization techniques, the expression of 5-HT_{1A} receptor mRNA was measured within the hippocampal formation after bilateral adrenalectomy (ADX). After 24 hr ADX, 5-HT_{1A} receptor mRNA expression was significantly increased in all hippocampal subfields in ADX animals relative to sham-operated controls (SHAM). The magnitude of the increase was most pronounced within CA2 (127%) and CA3/4 (94%) subfields of dorsal hippocampus, intermediate in the dentate gyrus (73%), and least within CA1 (60%). Administration of exogenous corticosterone (CORT) at the time of ADX maintained the level of 5-HT_{1A} receptor mRNA expression within the range of SHAM animals. *In vitro* receptor autoradiographic analysis of 5-HT_{1A} receptors in adjacent sections from the same animals indicated a simultaneous increase in 5-HT_{1A} binding throughout the hippocampus in response to ADX. 5-HT_{1A} binding increased to a similar extent (~30%) in CA subfields and dentate gyrus but remained within SHAM levels in CORT-replaced animals. 5-HT_{1A} receptor mRNA levels were also increased in hippocampal subregions of 1 week ADX animals relative to SHAM animals. Within both CA1 and CA2 subfields, the increments were approximately double those observed after 1 d ADX. 5-HT_{1A} receptor binding was increased in every hippocampal subfield to a similar extent as that observed after 1 d ADX. Increases in both 5-HT_{1A} receptor mRNA expression and 5-HT_{1A} receptor binding were preventable by administration of exogenous CORT at the time of ADX. Hippocampal 5-HT1C receptor mRNA and D_1 receptor mRNA expression were not significantly altered by either acute or chronic ADX treatment. These data indicate that adrenal steroids may selectively regulate hippocampal 5-HT_{1A} receptors at the level of 5-HT_{1A} receptor mRNA expression.

[Key words: 5-HT_{1A} receptors, mRNA, corticosteroids, hippocampus, receptor regulation,* in situ *hybridization]

The ability of peripherally secreted adrenal steroids to enter the CNS and alter mood and behavior is well established (McEwen, 1987). Documented abnormalities of hypothalamo–pituitary–adrenal axis (HPA) function in depressed subjects (Sachar et. al., 1973; Rubin et. al., 1987; Young et. al., 1991) forms the basis for the putative involvement of corticosteroids in the etiology of depressive illness and other affective disorders. In addition to neuroendocrine function, serotonergic transmission is implicated in normal and abnormal mental states (Eison, 1990), and selective serotonergic compounds are clinically effective antidepressants and anxiolytics (Cohn and Wilcox, 1985; Traber and Glaser, 1987). Thus, it is likely that the functional interactions between the HPA system and the central serotonergic system may be significant, providing a biochemical basis for hormonally induced alterations in CNS circuits.

Corticosteroids have been shown to alter several elements of serotonergic neurotransmission. Removal of circulating corticosteroids by adrenalectomy (ADX) results in anatomically specific decreases in indices of 5-HT metabolism, while stressful procedures, which raise corticosteroid levels, produce corresponding increases in 5-HT turnover (Curzon et al., 1972; Van Loon et al., 1981). Activity of tryptophan hydroxylase, the rate-limiting 5-HT biosynthetic enzyme, appears to be sensitive to circulating corticosteroid levels (Singh et al., 1990). However, corticosteroids may also act to modulate serotonergic neurotransmission directly by regulating 5-HT receptors. Autoradiographic studies (Biegon et al., 1985) first identified increased 5-HT_1 receptor binding in the rat hippocampal formation 1 week after bilateral ADX. Subsequent investigations have confirmed the sensitivity of 5-HT_1 receptors to circulating corticosteroid levels (De Kloet et al., 1986; Martire et al., 1989) 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 steroids (Joels et al., 1991), establishing a functional coupling for steroid–5-HT receptor interactions within the hippocampus.

The complex displacement of high-affinity ^{3}H-5-HT binding by spiperone (Peroutka and Snyder, 1979) first confirmed the heterogeneity of 5-HT_1 receptors, which have been further pharmacologically classified into at least four receptor subtypes, 5-HT_{1A}–5-HT_{1D}. Recent molecular cloning of 5-HT receptors is supportive of this original classification (Julius et al., 1988; Albert et al., 1990). Although original ADX studies did not differentiate between 5-HT_1 receptor subtypes, autoradiographic data using subtype-specific ligands (Pazos and Palacios, 1985; Palacios et al., 1987) and *in situ* hybridization histochemistry (Chalmers and Watson, 1991; Miquel et al., 1991; Pompeiano et al., 1992) indicate that the predominant postsynaptic hippocampal 5-HT_1 receptor is of the 5-HT_{1A} type, suggesting that 5-HT_{1A} receptors may be regulated by adrenal steroids. The reported alteration in hippocampal 5-HT_{1A} receptor binding in response to restraint-induced stress (Mendelson and McEwen, 1991), and the observed sensitivity of 5-HT_{1A} receptors to ADX (Mendelson and McEwen, 1990) are certainly supportive of such

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a conclusion. The possibility that the 5-HT_{1A} receptor may be under corticosteroid control is, in itself, of great interest, as this site apparently fails to adapt to long-term changes in serotonergic transmission induced by neurotoxic lesion (Verge et al., 1986) or serotonergic drugs (Hensler et al., 1991).

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 (Yamamoto, 1985). Pharmacological studies have defined at least two subtypes of corticosteroid receptors, which differ in their affinity for corticosterone (CORT; Reul and De Kloet, 1985): the mineralocorticoid receptor (MR), which resembles the peripheral kidney MR, binds CORT with high affinity, while the glucocorticoid receptor (GR) exhibits a three- to fivefold lower affinity for the endogenous ligand. Both autoradiographic and immunohistochemical studies (Sarrieau et al., 1984; Fuxe et al., 1985) indicate that the hippocampus contains particularly high concentrations of both MRs and GRs 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 (Herman et al., 1989a). 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.

Previous studies of 5-HT_{1A} receptors in ADX animals have relied solely on receptor binding analysis, providing little mechanistic insight into this putative corticosteroid–5-HT receptor interaction. The present studies were designed to investigate primarily the possibility of altered hippocampal 5-HT_{1A} receptor mRNA expression in response to acute or chronic corticosteroid deprivation. Using a specific 5-HT_{1A} receptor cRNA riboprobe, semiquantitative *in situ* hybridization allowed high-resolution anatomical analyses of 5-HT_{1A} receptor mRNA expression in hippocampal subfields in response to ADX. In addition, 5-HT_{1A} receptor sites were measured using *in vitro* receptor autoradiography, providing a combined autoradiographic method of assessing both 5-HT_{1A} mRNA expression and 5-HT_{1A} receptor in discrete anatomical regions of the same brain.

Materials and Methods

Animals. Subjects were male Sprague–Dawley rats (250–300 gm). Rats were housed six per cage in a 12:12 hr light/dark cycle and allowed free access to food and water.

Treatment protocols. Rats were either ADX or sham-ADX (SHAM) bilaterally using a dorsal approach. All surgeries were performed between 9:00 A.M. and 10:30 P.M. Postsurgery ADX animals received 0.9% saline as drinking water. For acute ADX studies, 11 animals were ADX under chloral hydrate anesthesia, and five subsequently received 5 mg of corticosterone (in sesame oil vehicle) intraperitoneally at 3:00 P.M. on the same day. A further five animals underwent sham procedure, involving anesthesia and bilateral exposure and manipulation of the adrenals. At 24 hr postsurgery, animals were killed by decapitation and brains were rapidly removed and frozen in isopentane cooled to −42°C on dry ice. For 1 week studies, 12 animals were ADX, 6 of which were subsequently given a subcutaneous implant of corticosterone (25% w/w) at the time of surgery. A further six animals underwent sham-ADX procedure as described above. Seven days later, animals were killed by decapitation and their brains frozen in isopentane. All brains were stored at −80°C prior to processing. Subsequently, brains were sectioned at 15 μm on a cryostat maintained at −20°C and thaw mounted onto polylysine-coated microscope slides. Sections were stored at −80°C. At the time of death, trunk blood samples from both 1 d and 1 week survival animals were collected in tubes containing EDTA and spun at 1500 rpm for 10 min. Subsequently, plasma samples were transferred to tubes containing 0.5 ml of HCl and assayed for corticosterone using competitive protein binding assay. ADX animals from both 1 d and 1 week studies possessed undetectable corticosterone levels; corticosterone-replaced animals from 1 d and 1 week survival times exhibited plasma corticosterone values of 4 ± 1 μg/dl and 1 ± 1 μg/dl, respectively, which were not statistically different from sham-operated groups at each time point. The higher mean corticosterone levels in 1 d replaced animals results from the intraperitoneal injection of corticosterone as opposed to pellet implantation in 1 week replaced animals.

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 base pair (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 represents 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). 5-HT_{1C} cRNA probe was synthesized from a 500 bp HincII fragment of the 5-HT_{1C} cDNA pSR-1c (Julius et al. 1988) ligated into pGEM 3Z. This insert corresponds to the third putative cytoplasmic loop of the 5-HT_{1C} receptor and exhibited low sequence homology with both 5-HT_{1A} and 5-HT_2 receptor cDNA. Probe specificity was confirmed by absence of signal in brain sections labeled with sense 5-HT_{1C} probe and pretreated with RNase prior to antisense 5-HT_{1C} probe hybridization (data not shown). Dopamine D_1 receptor cRNA probe was synthesized from a 480 bp fragment spanning putative transmembrane domains III–VI of D_1 cDNA (Zhou et al., 1990). Probe specificity has been previously confirmed (Mansour et al., 1992). GR mRNA was visualized using a cRNA probe synthesized from a 456 bp fragment of GR cDNA (provided by Keith Yamamoto) subcloned into the XbaI-EcoRI site of pGEM 4, while the MR cRNA probe was synthesized from a 347 bp PstI-EcoRI fragment of MR cDNA (Patel et al., 1989) ligated into pGEM 3. The specificity of these GR and MR riboprobes has been previously confirmed (Herman et al., 1989a). Riboprobes were produced using either SP6 or T7 transcription systems in a standard labeling reaction mixture consisting of 1 μg of linearized plasmid, 5× SP6 transcription buffer, 125 μCi of ^{35}S-UTP, 150 μM NTPs, 12.5 mM dithiothreitol, 20 U of RNase inhibitor, and 6 U of the appropriate polymerase. The reaction was incubated at 37°C for 90 min, labeled probe being 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 min, slides were rinsed in isotonic phosphate-buffered saline (10 min) and treated with proteinase K (1 μg/ml in 100 mM Tris-HCl, pH 8.0) for 10 min at 37°C. Subsequently, sections underwent successive washes in water (1 min), 0.1 M triethanolamine (pH 8.0, plus 0.25% acetic anhydride) for 10 min, and 2× SSC (0.3 mM NaCl, 0.03 mM sodium citrate, pH 7.2) for 5 min. Sections were then dehydrated through graded alcohols and air dried.

Postfixed sections were hybridized with 1.0 × 10^6 dpm ^{35}S-UTP–labeled riboprobes in hybridization buffer containing 75% formamide, 10% dextran sulfate, 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, which was sealed into place with rubber cement. Sections were hybridized overnight at 55°C in a humid environment.

Posthybridization, the rubber cement was removed and sections were washed in 2× SSC for 5 min and then treated with RNase A (200 μg/ml in 10 mM Tris-HCl, pH 8.0, containing 0.5 M NaCl) for 60 min at 37°C. Subsequently, sections were washed in 2× SSC for 5 min, 1× SSC for 5 min, 0.5× SSC for 60 min at hybridization temperature, and 0.5× SSC at room temperature for 5 min, 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 d at room temperature.

In vitro *receptor autoradiography.* ^{3}H-8-hydroxy-2-(*N,N*-di-*n*-propylamino) tetralin)) (^{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 min at room temperature. Subsequently, sections were incubated with 2 nM ^{3}H-8-OH-DPAT for

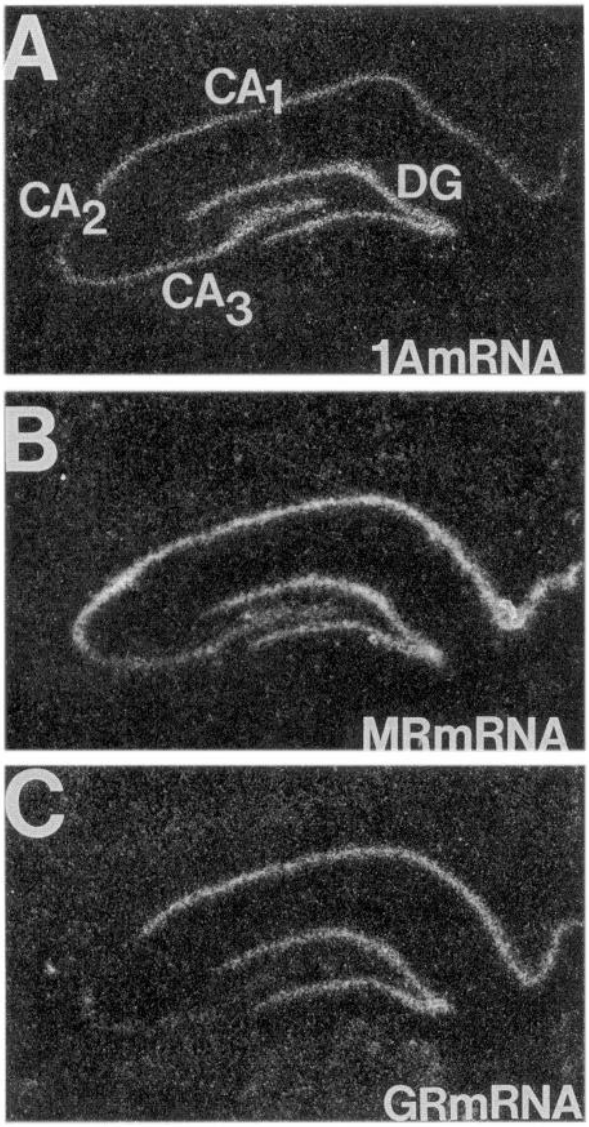

Figure 1. Photomicrographs illustrating relative anatomical distributions of mRNAs for 5-HT$_{1A}$ receptor (*A*), MR (*B*), and GR (*C*) within the hippocampus. *CA$_1$*, field CA1 (Ammon's horn); *CA$_2$*, field CA2 (Ammon's horn); *CA$_3$*, field CA3 (Ammon's horn); *DG*, dentate gyrus. Note the lack of hybridization signal in CA2 subfield in *A* and the heterogeneous distribution of MR and GR mRNA in *B* and *C*, respectively.

60 min at room temperature. Postincubation, slides were washed in incubation buffer (2 × 5 min) at 4°C and dried in a stream of cold air. Nonspecific 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 d. 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. Statistical differences between sham, ADX, and Cort-replaced groups were determined by one-way ANOVA and post hoc Fisher test.

Results

Relative distributions of hippocampal 5-HT$_{1A}$ receptor, MR, and GR mRNA. Figure 1 illustrates the relative distributions of 5-HT$_{1A}$ receptor, MR, and GR mRNA expression within the hippocampus. 5-HT$_{1A}$ receptor mRNA was very abundant within the pyramidal cell layer of CA1 and CA3 subfields and dentate gyrus granule cells with lower levels of expression within the CA2 subfield. Both MR and GR mRNA were evident throughout the hippocampus, although each displayed a distinctive pattern of abundance: GR mRNA was most abundant within CA1 cells while MR mRNA was most densely labeled across CA2 and the CA2/3 border (Fig. 1). Thus, although differentially distributed, steroid receptor mRNAs are expressed in all subfields in which 5-HT$_{1A}$ receptor mRNA is evident.

Effect of acute ADX treatment. At 24 hr post-ADX, 5-HT$_{1A}$ receptor mRNA expression was significantly increased in all hippocampal subfields of ADX animals (Fig. 2). Analysis of subfield data by one-way ANOVA indicated highly significant differences in all regions: CA1, $F(2,13) = 25.68$, $p < 0.0001$; CA2, $F(2,13) = 24.40$, $p < 0.0001$; CA3/4, $F(2,13) = 66.41$, $p < 0.0001$; dentate gyrus, $F(2,13) = 88.30$, $p < 0.0001$. Significant differences between SHAM and ADX groups were confirmed by post hoc analysis. The magnitude of the increase in 5-HT$_{1A}$ mRNA expression was most pronounced within CA2 (127%) and CA3/4 (94%) subfields of Ammon's horn and least within the CA1 subfield. These increases were evident from visual inspection of the autoradiograms (see Fig. 4). In all subfields, administration of exogenous CORT at the time of ADX resulted in levels of 5-HT$_{1A}$ receptor mRNA expression within the range of SHAM animals (see Figs. 2, 4). There were no statistically significant differences in 5-HT$_{1A}$ receptor mRNA expression within hippocampal subfields of SHAM and CORT-replaced groups. Analysis of 5-HT$_{1A}$ receptors in adjacent sections from the same animals indicated a simultaneous increase in ^{3}H-8-OH-DPAT binding throughout the hippocampus (Fig. 2). In all subfields, post hoc analysis revealed significant ($p < 0.05$) differences between SHAM and ADX groups. However, unlike the heterogeneous increases in 5-HT$_{1A}$ mRNA expression across hippocampal regions, 5-HT$_{1A}$ receptor binding was altered to a similar extent (~30%) in each subfield (Fig. 2). Within CA subfields, increases in 5-HT$_{1A}$ receptor binding were not confined to the pyramidal cell layer but were also evident across strata oriens radiatum and moleculare. Similarly, in dentate gyrus, both granule cell and molecular layers exhibited increased densities of 5-HT$_{1A}$ binding in response to ADX (see Fig. 4). Administration of exogenous CORT to ADX animals resulted in hippocampal 5-HT$_{1A}$ receptor binding levels similar to those of the SHAM group.

Effect of chronic ADX treatment. In a similar fashion to the 1 d ADX study, large increases in hippocampal 5-HT$_{1A}$ mRNA abundance were evident after 1 week ADX treatment (Fig. 3). One-way ANOVA indicated a highly significant effect of treatment in all subfields: CA1, $F(2,15) = 36.6$, $p < 0.0001$; CA2, $F(2,15) = 25.5$, $p < 0.0001$; CA3/4, $F(2,15) = 36.9$, $p < 0.0001$; dentate gyrus, $F(2.15) = 38.0$, $p < 0.0001$. The magnitude of the increase in 5-HT$_{1A}$ mRNA expression at 1 week ADX was similar to that observed at 1 d ADX in CA3/4 and dentate gyrus; however, within both CA1 and CA2 subfields, the incre-

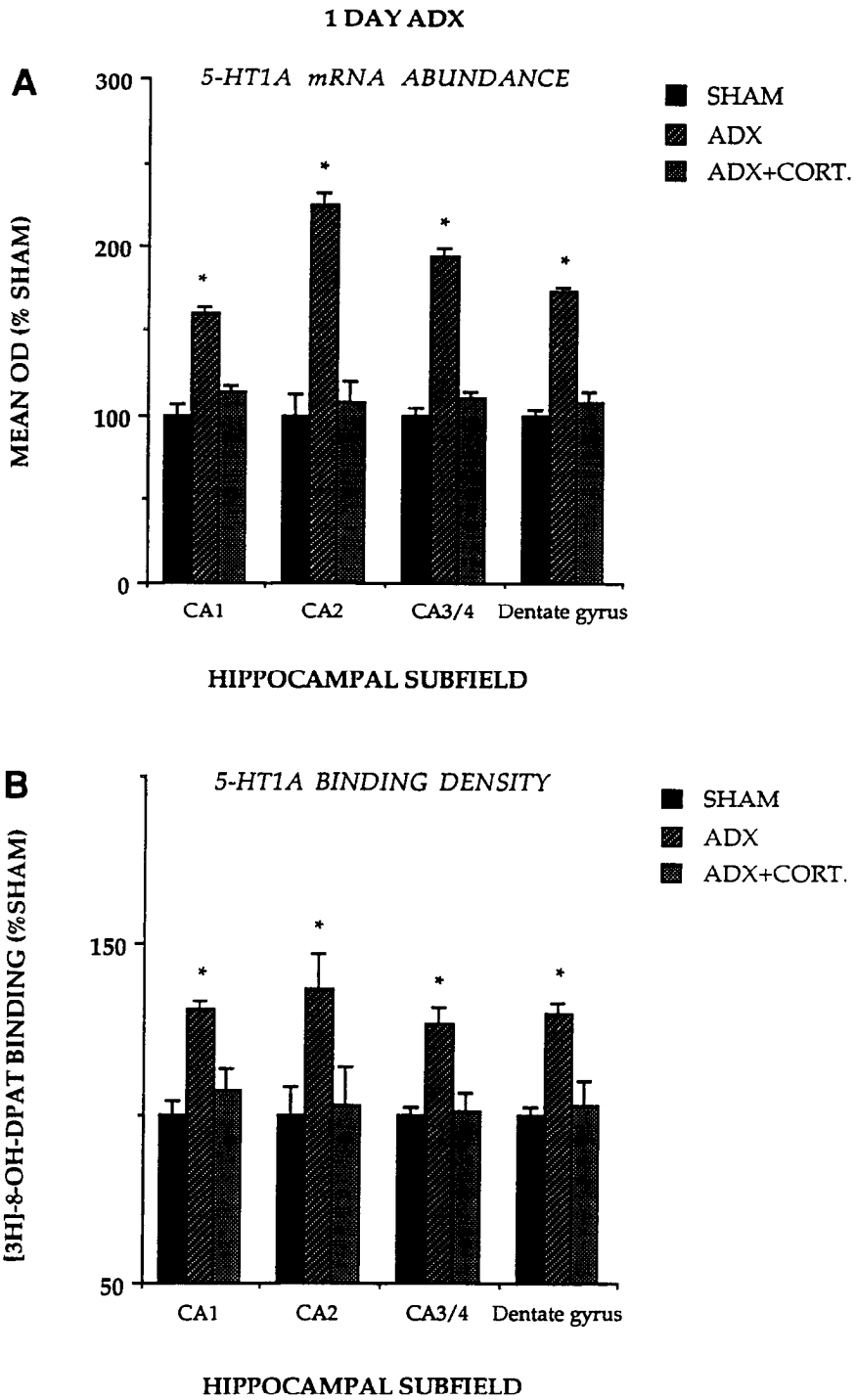

Figure 2. Densitometric analyses of 5-HT_{1A} receptor mRNA expression (*A*) and ^{3}H-8-OH-DPAT binding (*B*) in hippocampal sections from SHAM ($n = 6$), ADX ($n = 5$), and ADX + CORT ($n = 5$) animals after 1 d ADX. Overall one-way ANOVA indicated significant effects of treatment on both 5-HT_{1A} mRNA expression and ^{3}H-8-OH-DPAT binding in all hippocampal subfields. Post hoc analysis (Fisher test) confirmed significant increases (*, $p < 0.05$) in both 5-HT_{1A} mRNA abundance and ^{3}H-8-OH-DPAT binding in all hippocampal subfields in ADX groups relative to SHAM.

ments were approximately double those observed at 1 d (Fig. 3). Administration of CORT returned 5-HT_{1A} mRNA levels to within the range of those in SHAM animals in all subfields, although a statistically significant difference between SHAM and CORT-replaced groups was detected specifically within the CA1 subfield ($p < 0.05$, Fisher test). Again, in a similar fashion to 1 d ADX, increases in hippocampal 5-HT_{1A} mRNA expression were accompanied by increases in ^{3}H-8-OH-DPAT binding (Fig. 3). Interestingly, the largest increases in ^{3}H-8-OH-DPAT binding were evident within the CA2 subfield (43%), corresponding to the subregion exhibiting the most pronounced increment in 5-HT_{1A} mRNA expression (Fig. 3). Post hoc analysis confirmed significant differences in binding densities between SHAM and ADX groups in all subfields. Aside from CA2 region, the magnitude of the increase in ^{3}H-8-OH-DPAT binding was similar in all subfields to that observed in the 1 d animals and was preventable by CORT at the time of ADX.

Molecular specificity of ADX effect. In brain sections adjacent to those used for 5-HT_{1A} receptor measurements, both 5-HT_{1C} receptor mRNA and D_1 receptor mRNA expression were measured after acute and chronic ADX treatment. The 5-HT_{1C} receptor represents another, presently defined, postsynaptic hip-

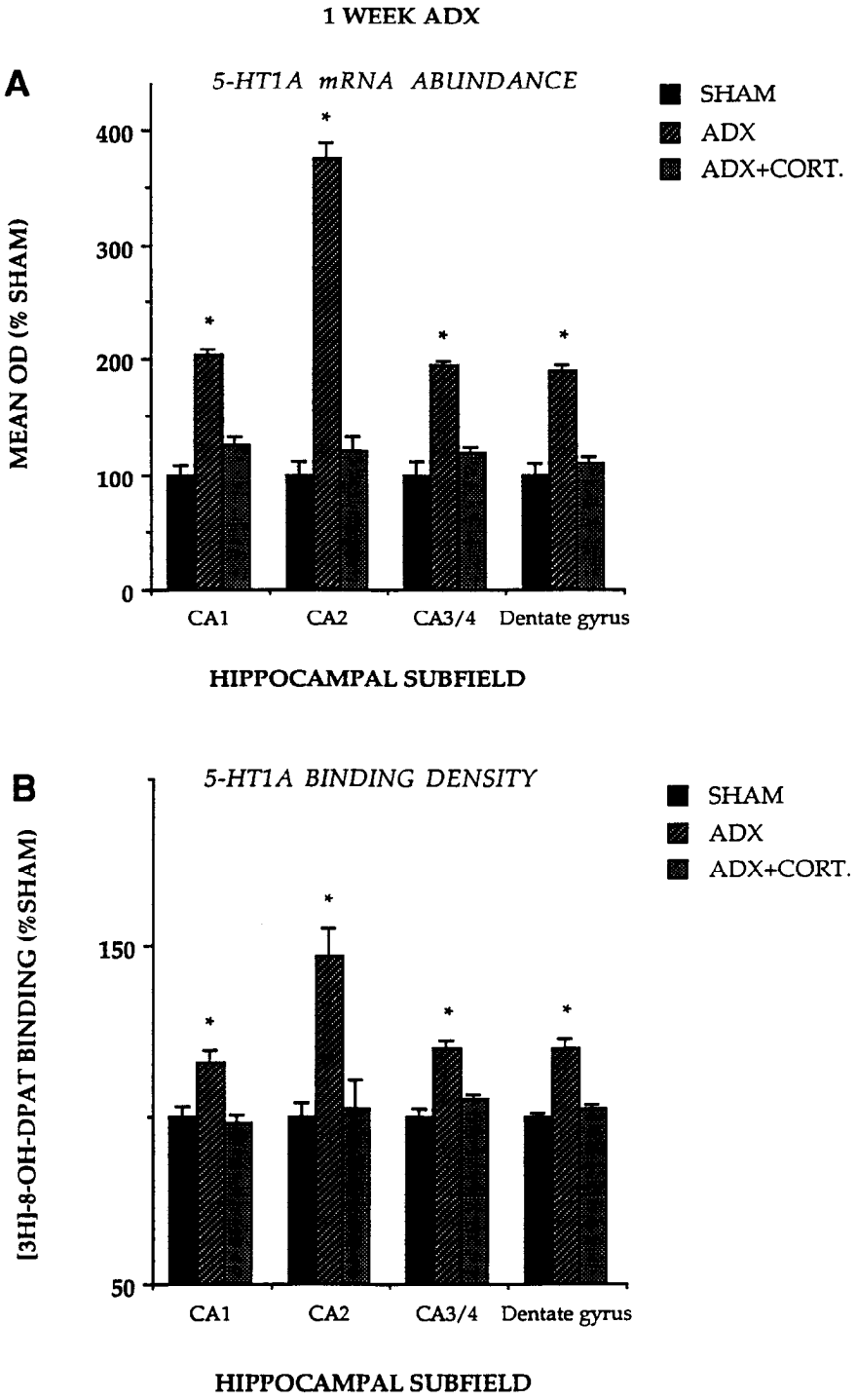

Figure 3. Densitometric analyses of 5-HT$_{1A}$ mRNA expression (*A*) and ^{3}H-8-OH-DPAT binding (*B*) in hippocampal sections from SHAM ($n = 6$), ADX ($n = 6$), and ADX + CORT ($n = 6$) animals after 1 week ADX. Overall one-way ANOVA indicated significant effects of treatment on both 5-HT$_{1A}$ mRNA expression and ^{3}H-8-OH-DPAT binding in each hippocampal subfield. Post hoc analysis (Fisher test) confirmed significant increases (*, $p < 0.05$) in both 5-HT$_{1A}$ mRNA abundance and ^{3}H-8-OH-DPAT binding in all hippocampal subfields in ADX groups relative to SHAM.

pocampal 5-HT$_1$ receptor subtype, while the D$_1$ receptor serves as a convenient nonserotonergic monoamine receptor expressed within the hippocampal formation. Hippocampal 5-HT$_{1C}$ receptors are not highly expressed within dorsal hippocampus but are found in high abundance within the ventral subiculum and ventral CA1 subfield. Measurements of hippocampal 5-HT$_{1C}$ mRNA expression were therefore confined to a ventral subicular/CA1 region. No significant alterations in 5-HT$_{1C}$ mRNA abundance were evident in this area after either 1 d or 1 week ADX treatment [$F(2,13) = 0.47$, $p < 0.63$, and $F(2,9) = 1.56$, $p < 0.27$, for 1 d and 1 week, respectively]. Comparison of experimental groups by post hoc analysis revealed no statistically significant differences (Fig. 5). Thus, hippocampal regulation of 5-HT$_{1A}$ receptor mRNA abundance in response to ADX is 5-HT$_1$ receptor subtype specific. Hippocampal D$_1$ receptor mRNA expression is found exclusively within dentate gyrus granule cells (Mansour et al., 1992) and, consequently, was measured only within this subregion. In a similar fashion to 5-HT$_{1C}$ mRNA, D$_1$ receptor mRNA expression was unaltered in dentate gyrus at both 1 d and 1 week ADX [$F(2,15) = 1.289$, $p < 0.30$, and $F(2,15) = 1.94$, $p < 0.18$, for 1 d and 1 week, respectively].

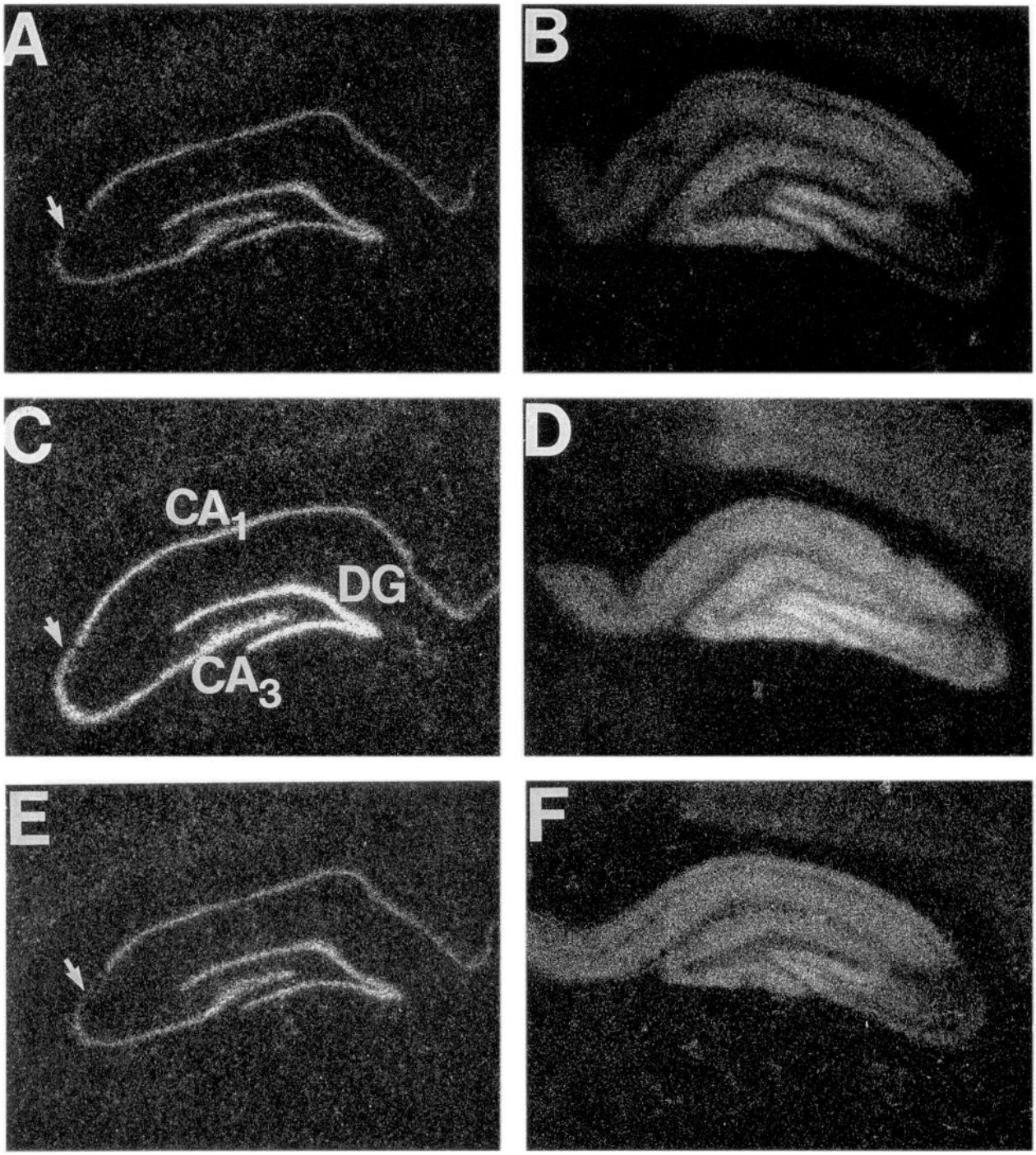

Figure 4. Photomicrographs of 5-HT_{1A} receptor mRNA expression (*A, C, E*) and ^{3}H-8-OH-DPAT binding (*B, D, F*) in hippocampal sections from 1 d SHAM (*A, B*), 1 d ADX (*C, D*), and 1 d ADX + CORT (*E, F*) animals. After ADX (*C*), note the general increase in hybridization signal throughout the hippocampal region in comparison to both SHAM (*A*) and CORT-replaced (*E*) sections. 5-HT_{1A} mRNA expression is induced in CA2 subfield after ADX (*arrow, C*) in comparison to SHAM (*arrow, A*) and CORT-replaced (*arrow, E*) levels. From densitometric analysis, mean gray level values in regions CA1, CA2, CA3/4, and dentate gyrus, respectively, were 20 ± 1, 9 ± 1, 32 ± 1, 43 ± 1 for SHAM; 32 ± 1, 21 ± 2, 62 ± 3, 75 ± 2 for ADX; and 23 ± 1, 10 ± 1, 35 ± 1, and 47 ± 3 for ADX+CORT. *B, D,* and *F* illustrate the parallel changes in ^{3}H-8-OH-DPAT binding in response to ADX. Note the general increases in binding density within the hippocampus after ADX (*D*) in comparison to levels in SHAM (*B*) and CORT-replaced (*F*) sections. Within CA fields, note the increase in binding across all hippocampal layers after ADX (*D*) and the increase in density across CA2 and CA3 subfields in comparison to SHAM (*B*) and CORT-replaced (*F*) sections.

Discussion

The present study indicates that alterations in circulating corticosteroid levels results in parallel changes in hippocampal 5-HT_{1A} receptor mRNA expression and 5-HT_{1A} receptor binding. Both short-term (24 hr) and chronic (1 week) ADX treatment induced large increases in 5-HT_{1A} mRNA abundance in pyramidal cells of hippocampal CA subfields and dentate gyrus granule cells that were prevented by CORT administration at the time of ADX. Changes in 5-HT_{1A} mRNA expression in

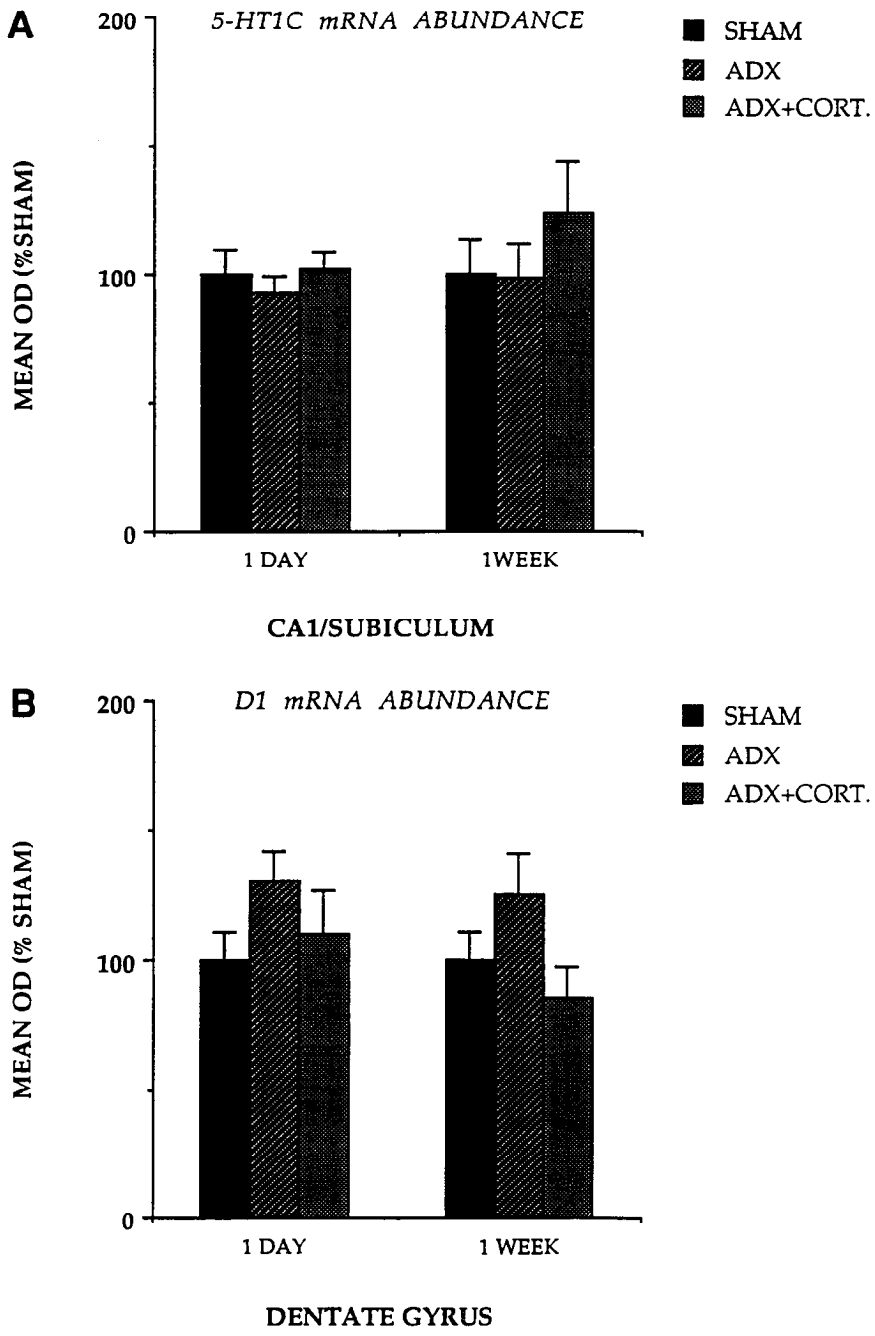

Figure 5. Densitometric analyses of 5-HT$_{1C}$ receptor mRNA expression (*A*) and dopamine D$_1$ receptor mRNA expression (*B*) in hippocampal sections from SHAM, ADX, and ADX + CORT after 1 d and 1 week ADX. Overall one-way ANOVA indicated no significant effects of treatment on either 5-HT$_{1C}$ mRNA abundance or D$_1$ receptor mRNA abundance at either 1 d or 1 week ADX.

response to ADX were accompanied by simultaneous, though proportionally smaller, increases in 5-HT$_{1A}$ receptor binding throughout the hippocampal formation that were also preventable by CORT administration. Hippocampal 5-HT$_{1C}$ receptor mRNA expression and dentate gyrus D$_1$ receptor mRNA levels were not significantly altered by either acute or chronic ADX treatment, indicating some degree of specificity to the corticosteroid-mediated regulation of hippocampal 5-HT$_{1A}$ receptor mRNA expression.

Previous ADX studies have shown that ADX treatment results in increases in total hippocampal ^{3}H-5-HT binding that were prevented by CORT administration (Biegon et al., 1985; De Kloet et al., 1986; Martire et al., 1989). Although 5-HT$_{1A}$ receptors represent the predominant hippocampal 5-HT$_1$ receptor, only one recent study indicates a specific increase in 5-HT$_{1A}$ receptors within hippocampal regions in response to ADX (Mendelson and McEwen, 1990). The results of the present study, utilizing the 5-HT$_{1A}$-selective ligand 8-OH-DPAT, confirms the sensitivity of hippocampal 5-HT$_{1A}$ sites to adrenal steroid regulation. As 8-OH-DPAT was employed at a saturating concentration of ligand (Palacios et al., 1987), it is likely that changes in binding densities in response to ADX + CORT replacement reflect changes in 5-HT$_{1A}$ receptor numbers. The simultaneous and substantial increments in 5-HT$_{1A}$ receptor mRNA expression in the same anatomical subfields after ADX are certainly supportive of increased 5-HT$_{1A}$ receptor synthesis. Based on these results, therefore, it seems probable that adrenal steroids modulate hippocampal 5-HT$_{1A}$ receptors by modulat-

ing receptor synthesis at the level of mRNA expression. Theoretically, such a molecular regulation may occur in several possible ways: (1) a corticosteroid-mediated alteration in 5-HT_{1A} receptor gene transcription (although there is, as yet, no published information regarding the presence of steroid-responsive elements on the 5-HT_{1A} receptor gene), (2) altered efficiency in processing of 5-HT_{1A} receptor primary transcript in response to corticosteroid, or (3) a corticosteroid-mediated control of 5-HT_{1A} receptor mRNA $t_{1/2}$ as reported for other steroid-responsive genes (McKnight and Palmiter, 1979; Guyette et al., 1987). Regardless of the precise molecular mechanism involved, it seems likely from the present studies that increases in 5-HT_{1A} mRNA abundance in response to ADX result in increased 5-HT_{1A} mRNA translation as reflected in higher 5-HT_{1A} receptor binding densities. In this regard, it is noteworthy that the very large increases in 5-HT_{1A} mRNA abundance (>100%) induced by ADX treatment result in only 20–30% increases in 5-HT_{1A} receptor binding densities within the same anatomical subfields. Although this may be a reflection of translational efficiency for this receptor protein within this region, it is also possible that increased receptor degradation in response to ADX could account for such a disparity. Alternatively, it remains possible that 5-HT_{1A} binding detects not only intrinsic hippocampal 5-HT_{1A} receptors but also those 5-HT_{1A} receptors that may be present on terminals of afferent serotonergic fibers. If presynaptic 5-HT_{1A} receptors remain unaltered by ADX treatment, this may act to effectively "mask" increases in postsynaptic 5-HT_{1A} sites.

Hippocampal 5-HT_{1A} receptors, as assessed by ligand binding, have proven to be remarkably resistent to homologous regulation, even after complete loss of 5-HT stimulation as a result of neurotoxic lesion (Verge et al., 1986; Hensler et al., 1991). Similarly, significant depletion of hippocampal 5-HT appears to produce only relatively small, or no, changes in 5-HT_{1A} receptor mRNA within hippocampal subfields (Brousseau et al., 1991). It is therefore most likely that the rapid changes in both 5-HT_{1A} mRNA expression and 5-HT_{1A} binding observed in response to ADX do not result from ADX-induced changes in serotonergic activity but rather reflect a direct effect on hippocampal neurons. Although hippocampal cells express high levels of both MRs and GRs (Fig. 1), the reversal of ADX effects on 5-HT_{1A} mRNA abundance and 5-HT_{1A} receptor binding by administration of low levels of exogenous CORT (mean plasma CORT, 4 μg/dl and 1 μg/dl for 1 d and 1 week replaced animals, respectively) suggests that steroid-induced reductions in hippocampal 5-HT_{1A} sites may be mediated by high-affinity MRs (De Kloet et al., 1986). Such a proposal is in agreement with *in vitro* electrophysiological studies in hippocampal slice preparations, which indicate that MR but not GR ligands act to suppress 5-HT_{1A}–mediated hyperpolarizations in CA1 pyramidal cells (Joels et al., 1991). In addition, the observed slow onset (hours after steroid application) and persistence of this electrophysiological effect are consistent with a genomic steroid effect. However, it is as yet unclear if this electrophysiological effect can be directly related to the present *in vivo* results, as only a small reduction in 5-HT_{1A} receptor B^{max} was evident within hippocampal slices after steroid application (Joels et al., 1991).

As removal of circulating corticosteroids acts to induce 5-HT_{1A} receptor mRNA expression, we can assume that, under normal circumstances, circulating adrenal steroids act to suppress hippocampal 5-HT_{1A} receptor expression. The ability of corticosteroids to regulate gene transcription negatively has been established for many genes including pro-opiomelanocortin, prolactin, and α_1-fetoprotein (Guertin et al., 1988; Sakai et al., 1988; Drouin et al., 1989). Identification of specific consensus sequences in these genes required for steroid-mediated transcriptional repression raises the possibility that a negative steroid responsive element may exist on the 5-HT_{1A} receptor gene. Furthermore, the ability of low-dose CORT replacement to block the effects of ADX in the present study suggests that any putative steroid responsive element on the 5-HT_{1A} gene is sensitive to an MR–steroid complex.

The present data indicate that receptor mRNA regulation is not a general response for all hippocampal neuronal membrane receptors, as both hippocampal 5-HT_{1C} receptor and D_1 receptor mRNA expression were unaltered by ADX treatment. The significance of this molecular specificity is presently unclear. However, from a hippocampal cell standpoint, suppression of inhibitory 5-HT_{1A} receptor expression would act to limit the inhibitory influence of serotonergic raphe–hippocampal inputs, presumably providing a means of controlling hippocampal cell "excitability." We may only speculate as to the possible physiological importance of such modulation; however, the numerous reports indicating a role for the hippocampal formation in neuronal regulation of the HPA axis (Dunn and Orr, 1984; Sapolsky et al., 1984; Herman et al., 1989b) certainly provide one possible underlying rationale for corticosteroid control of 5-HT_{1A} receptor expression in this region. With respect to HPA hippocampal feedback control, however, it is curious that restraint-induced increases in circulating corticosteroid levels have been reported to produce qualitatively similar changes in hippocampal 5-HT_{1A} receptor binding as ADX (Mendelson and McEwen, 1991). This observation again confirms the sensitivity of 5-HT_{1A} receptors to circulating corticosteroids but raises the apparent paradox that both absence of corticosteroids (ADX) and excessive release of corticosteroids (stress) act to increase hippocampal 5-HT_{1A} receptor binding. Although these observations are not easily reconciled, it is certain that the high levels of circulating corticosteroids, induced by restraint stress, will result in significant occupation of *both* MRs and GRs within the hippocampus (Spencer et al., 1990). Thus, while removal of low levels of circulating corticosteroids (ADX) will abolish the influence of tonic MR occupation on 5-HT_{1A} receptor expression, it is conceivable that GR occupation (stress) regulates 5-HT_{1A} receptors by an independent, and presumably overriding, mechanism to that of MR. It is also possible that stress activated circuits regulate hippocampal 5HT_{1A} receptors independently of corticosteroid regulation. Future studies examining 5-HT_{1A} receptor regulation in response to selective MR and GR occupation in a nonstress paradigm will be useful in relation to this issue.

The 5-HT_{1A} receptor is one of at least four 5-HT_1 receptor subtypes present within the mammalian brain (Peroutka, 1988). However, the 5-HT_{1A} receptor is of particular interest because of its high level of expression in limbic brain structures (Chalmers and Watson, 1991; Miquel et al., 1991; Pompeiano et al., 1992) and putative involvement in affective disorders such as anxiety and depression. Azapirones, buspirone and its analogs, which exhibit high agonist affinity for 5-HT_{1A} receptors, have been shown to be clinically effective anxiolytics (Traber and Glaser, 1987), and selective 5-HT_{1A} receptor activation produces antidepressant effects in proposed animal models for depression (Giral et al., 1988). From these observations, it is clear that regulation of 5-HT_{1A} receptors may consequently lead to

modulation of particular aspects of mood and behavior. The present studies indicate that alterations in circulating corticosteroid levels regulate 5-HT_{1A} receptor mRNA expression within the hippocampal formation, a central anatomical structure in limbic circuitry, thus providing one possible molecular mechanism by which adrenal steroids may act to influence circuitry associated with affective state. This interaction may prove to be highly relevant to the body of evidence indicating abnormalities in HPA axis function in both endogenous depressive illness and anxiety disorders (Berger, 1980; McEwen, 1987).

In summary, we have found that removal of circulating corticosteroids induces large increases in hippocampal 5-HT_{1A} receptor mRNA expression that are preventable by low-level corticosteroid replacement. The increases in 5-HT_{1A} mRNA levels occur rapidly (24 hr post-ADX) across all hippocampal subfields and are accompanied by parallel changes in 5-HT_{1A} receptor binding that are also reversible by corticosteroid replacement. The magnitude of the increases in 5-HT_{1A} receptor mRNA expression and 5-HT_{1A} receptor binding are generally similar after both 1 d and 1 week ADX treatment, suggesting an all-or-none type response. These data confirm the specific sensitivity of 5-HT_{1A} receptors to adrenal steroid regulation and indicate that corticosteroids act to modulate these sites at the level of 5-HT_{1A} receptor mRNA expression within this anatomical region. Controlling hippocampal 5-HT_{1A} receptor mRNA expression is one means by which corticosteroids may act to decrease sensitivity to 5-HT within the brain and consequently alter central serotonergic neurotransmission. Further investigations of this mechanism may provide valuable insight into affective disorders in which aberrant adrenocortical function is evident.

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