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Corticosteroids regulate 5-HT_{1A} but not 5-HT_{1B} receptor mRNA in rat hippocampus

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Abstract

The role of mineralocorticoid and glucocorticoid receptors (MR and GR, respectively) in the regulation of serotonin receptors has not been clearly delineated. There is no consensus regarding the regulation of 5-HT_{1A} receptors, and corticosteroid regulation of 5-HT_{1B} mRNA has not been previously studied. We compared the effects of long-term (two week) adrenalectomy (no MR or GR activation) and several hormone replacement protocols designed to stimulate MR selectively (ALDO), MR and GR (HCT), and continuous MR with cyclical GR activation (SHAM adrenalectomy). 5-HT_{1A} and 5-HT_{1B} mRNAs were measured by in situ hybridization in hippocampus and raphe nuclei. None of the experimental manipulations altered 5-HT_{1B} mRNA levels in the hippocampus or dorsal raphe, and also had no effect on 5-HT_{1A} mRNA in dorsal or median raphe. However, 5-HT_{1A} mRNA levels were regulated in a complex manner in the different subfields of hippocampus. We conclude that both MR and GR play an integrated role in regulating 5-HT_{1A} mRNA levels in hippocampus while having no effect on 5-HT_{1B} mRNA levels under these conditions.

Keywords

Adrenalectomy; Corticosteroid receptor; Mineralocorticoid receptor; Glucocorticoid; Hippocampus; Dorsal raphe nucleus; Serotonin receptor

1. Introduction

The adrenal corticosteroids (CTs), aldosterone, cortisol (in man) and corticosterone (in rat), are very important in maintaining homeostasis and in regulating the response to stressful stimuli. Normally CTs are released in a circadian rhythm, with low basal levels during periods of inactivity, and a surge in steroid levels during active periods [13,14]. CT levels also increase in response to the presentation of a stressful stimulus [37]. CT secretion into the plasma is controlled by the HPA axis [13], and CT binds to receptors in the brain leading to a feedback inhibition of the release of adrenocorticotrophic hormone from the pituitary. There are two types of CT receptors, the mineralocorticoid (MR, Type I) and the glucocorticoid (GR, Type

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II) receptors [14]. GR have low occupancy by CTs at the nadir of their circadian fluctuation, but do become largely occupied during circadian or stress provoked surges. By contrast, MR have about 10-fold higher affinity for CT, which results in nearly full MR receptor occupancy throughout the day under normal physiological conditions [14].

The serotonin (5-hydroxytryptamine, 5-HT) neurotransmitter system and the HPA axis have a reciprocal regulatory function in the feedback management of an organism's response to stress. Serotonergic cell bodies are located in the raphe and project to many brain areas, including the hippocampus, hypothalamus and cortex. Serotonin neurotransmission is regulated, in part, by inhibitory autoreceptors that limit the release of 5-HT. The 5-HT_{1A} receptor is a somatodendritic autoreceptor in raphe neurons that mediates a reduction in firing rate. The 5-HT_{1A} receptor is also found postsynaptically in brain nuclei receiving 5-HT axon projections, such as the hippocampus [21]. The 5-HT_{1B} receptor in the rat is found on presynaptic serotonergic terminals in many brain areas including the raphe and hippocampus and inhibits the synthesis and release of 5-HT from axon terminals [22]. However, the large majority of 5-HT_{1B} receptors are located on presynaptic terminals of non-serotonergic neurons throughout the CNS where they regulate the release of several different neurotransmitters from axon terminals [6].

Elevated plasma cortisol or corticosterone levels and a dampening of inhibitory feedback mechanisms are thought to be important factors in the development of depression, whereas antidepressant treatment leads to a normalization of cortisol levels and HPA axis feedback inhibition [27]. An alteration in 5-HT function in hippocampus has been proposed as a mechanism underlying these changes in negative feedback. Chronic CT treatment does alter 5-HT_{1A} mediated responses in the hippocampus [3,32]. 5-HT_{1A} and 5-HT_{1B} knockout studies have demonstrated that these receptors play an important role in regulating behavioral responses to stress [24]. However, it is not clear if this is an effect mediated directly in hippocampus or if it also involves altered presynaptic 5-HT function.

Modulation of some component of the 5-HT_{1A} or 5-HT_{1B} receptor-effector cascade could be one mechanism by which chronic stress or high levels of plasma CT could alter serotonergic function. The highest density of both MR and GR receptors is in the hippocampus [28,35] whereas only GR receptors are found in the dorsal and median raphe [17,35,38]. We hypothesize that the differential expression of MR and GR is an important determinant of the regionally specific regulation of 5-HT_{1A} and 5-HT_{1B} receptors. Corticosteroids appear to alter the number of 5-HT receptor binding sites; however, methodological differences between studies have led to inconclusive results and no study has directly compared changes in the raphe and the hippocampus. Previous studies have examined the effects of corticosteroids on the number of 5-HT_{1A} binding sites and/or mRNA levels in the hippocampus and/or the dorsal raphe [9,11,25,29,39]. In these studies, the treatment paradigms included rats that had been adrenalectomized for periods from 1 day to 1 week; rats were treated by replacement with no CT, basal levels of CT, aldosterone, GR selective ligands or high levels of CT for periods from 1 day to 1 week. These studies with varying protocols have not yielded consistent results. In our present study we used a sufficiently long treatment period (2 weeks) to examine the stable effects of these manipulations as well as to discriminate the contribution of MR and GR to 5-HT_{1A} and 5-HT_{1B} receptor regulation in hippocampal and raphe neurons. Furthermore, to measure receptor regulation, we used in situ hybridization which, unlike radioligand receptor binding studies, has the ability to distinguish presynaptic versus postsynaptic receptors by detecting hybridization signal in serotonergic (e.g. raphe) vs. nonserotonergic (e.g. hippocampal) neurons.

2. Materials and methods

2.1. Animal procedures

All procedures were approved by the animal care committee at our institutions. Male Sprague–Dawley rats (Harlan) 75–100 g were used for all treatment groups. Four different treatment groups were used for the experiments in this investigation (Table 1). The adrenalectomy was performed as described previously [3–5,32,33]. Bilateral adrenalectomies were performed on all treatment groups, except the SHAM group, to remove circulating corticosteroids. The animal was anesthetized with halothane, a small incision was made into the abdominal cavity, just below the rib cage, and the adrenal glands were carefully removed. The muscle wall was then sutured and the skin closed using wound clips. The ADX group received no further treatment. The ALDO group had an osmotic minipump containing aldosterone (Alzet model 2002, Alza Corp., Palo Alto, CA), implanted subcutaneously at the time of adrenalectomy. The minipump delivered 10 µg/h aldosterone (Steraloids, Inc., Wilton, NH) dissolved in propylene glycol to stimulate MR selectively. For the HCT group of animals, MR and GR were both stimulated by subcutaneously implanting 200 or 300 mg corticosterone pellets (2 or 3 week release, respectively, Innovative Research, Toledo, OH), in the back of the neck at the time of adrenalectomy. The SHAM group of animals was produced by visualizing the adrenal glands but leaving them intact. After surgery the animals were maintained on a standard 12 h light/dark cycle and rat chow, ad libitum. SHAM animals were given standard drinking water while the ADX, ALD, and HCT animals were given 0.9% NaCl drinking water ad libitum. At the end of 14 days, the animals were sacrificed in the morning, and the brains were removed and frozen on dry ice. At the time of sacrifice, trunk blood was collected to determine the plasma corticosterone levels by radioimmunoassay [7]. Any ADX or ALDO treated animals with corticosterone levels greater than 0.5 µg/dl were excluded from the study.

2.2. In situ hybridization histochemistry

The protocol for in situ hybridization has been previously described [31]. Tissue sections (20 µm) were prepared in the frontal plane using a cryostat, then thaw mounted on silanized glass slides. The sections were stored at –70°C until processed for in situ hybridization. In brief, tissue sections were thawed at room temperature and fixed in cold 4% paraformaldehyde. After rinsing in phosphate-buffered saline, sections were treated with acetic anhydride (0.25% in 0.1 M triethanolamine), dehydrated, delipidated and air dried.

Oligonucleotide probes were designed on the basis of low sequence homology to other known receptor mRNA sequences. 5-HT_{1A} mRNA was detected with an oligonucleotide probe corresponding to residues 1110–1151 [8]. For the 5-HT_{1B} probes, three oligonucleotides corresponding to residues 1343–1382, 1630–1668, and 1790–1829 of the rat 5-HT_{1B} clone, MG11B [19], were synthesized as previously described [31]. The probes were individually labeled with [³³P]-dATP (Andotek, Irvine, CA) using terminal deoxyribonucleotidyl transferase (Gibco, Gaithersburg, MD) and purified on NENSORB columns (Dupont NEN Research Products, Boston, MA). The specific activity of each oligonucleotide probe was 3–7 µCi/pmol.

The labeled probes were diluted (2 pmol/ml) in a hybridization buffer containing 50% formamide, 10% dextran sulfate, 0.3 M sodium chloride, 10 mM Tris (pH 8.0), 1 mM EDTA, 1× Denhart's (0.2% each bovine serum albumin, Ficoll, and polyvinylpyrrolidone), 0.4 mg/ml yeast tRNA, and 10 mM dithiothreitol. Fifty microliters of the hybridization mixture was applied to each slide and the sections were covered with silanized coverslips. The slides were incubated in moist, covered trays at 37°C overnight. Following the hybridization reaction, coverslips were removed and the slides were washed in 0.5×(5-HT_{1A}) or 1×SSC (5-HT_{1B}) (150 mM NaCl in 15 mM sodium citrate) for 1 h at 55°C, and again in 1×SSC at room

temperature for 1 h. The slides were rinsed in distilled water, dehydrated through a series of graded alcohol rinses containing 300 mM ammonium acetate, and air dried. Autoradiography was performed using Hyperfilm β max (Amersham, Arlington Heights, IL) with 4 to 14 day exposures. The distribution of 5-HT_{1A} and 5-HT_{1B} in situ hybridization signal detected agrees with previously published reports [10,31].

2.3. Data analysis

In situ hybridization signal was quantified using a computer based densitometry system (MCID, Imaging Research, Inc., St Catherines, ON); the optical density (OD) of hybridization signal was quantified using ¹⁴C-plastic standard sections, which yields a linear relationship between tissue radioactivity and measured signal intensity. This was in turn converted into equivalent units of ³³P dpm/mm² using the method of Eakin et al. [15]. The rater was blinded to treatment condition of brains during sectioning, hybridization, and densitometry. Tissue sections from each brain were matched anatomically to correspond to plates 33 (hippocampus) and 49 (dorsal and median raphe nuclei) from the rat brain atlas by Paxinos and Watson [34]. In the hippocampus, individual subfields in the digitized sample were selected for analysis by automatic edge detection. In the midbrain, oval or diamond-shaped templates were used to measure the median raphe nucleus and medial subnucleus of the dorsal raphe, respectively. OD was determined in the regions of interest of two consecutive autoradiographs corresponding to these coordinates. These were averaged and tissue background OD was subtracted for each region in each brain. After the treatment condition of the brains was uncoded, regional mean values were calculated for each treatment group. Treatment group averages were evaluated for statistical significance using ANOVA and Fisher's least square post-hoc test. A $P < 0.05$ was used to determine significance.

3. Results

The effectiveness of the bilateral adrenalectomy and hormone replacement was confirmed by measuring corticosterone levels (Table 1). SHAM, ALDO and ADX animals, had undetectable or low morning corticosterone levels; HCT animals, receiving high dose corticosterone treatment, had levels of serum corticosterone similar to that seen following the presentation of stressful stimuli [13,35,36].

5-HT_{1A} and 5-HT_{1B} mRNA were detected in hippocampus and midbrain at the level of the anterior dorsal and median raphe by in situ hybridization histochemistry (Fig. 1). The anatomical distribution of each mRNA species conformed to previously published descriptions [8,30]. As previously reported [31], 5-HT_{1B} mRNA density was too low in median raphe to quantify reliably (Fig. 1D). 5-HT_{1B} hybridization signal was strong in CA1 pyramidal neurons, but is not expressed in CA3 and dentate gyrus (DG) (Fig. 1C).

Adrenalectomy and replacement steroid treatment for 2 weeks differentially regulated the 5-HT_{1A} and 5-HT_{1B} mRNA levels in the hippocampus, dorsal raphe and median raphe. In general, the 5-HT_{1A} receptor mRNA was significantly altered by CT treatment (Figs. 2 and 3), whereas the 5-HT_{1B} receptor mRNA was not altered in any of the brain areas under investigation (Figs. 4 and 5). In particular, significant regulation of the 5-HT_{1A} mRNA levels was apparent in the hippocampus. In contrast, there was no impact of adrenalectomy or CT treatment on dorsal or median raphe 5-HT_{1A} mRNA hybridization signal (Fig. 3). Within the hippocampus, the effects of corticosterone on 5-HT_{1A} hybridization varied among the three subfields examined, CA1, CA3 and the dentate gyrus. The most dramatic differences were apparent in the DG, whereas the differences in CA1 and CA3 were more subtle (Fig. 2).

In the absence of endogenous CT, 5-HT_{1A} mRNA was increased in CA1 and DG, i.e. ADX>SHAM by 32% (CA1) and 23% (DG) (Fig. 2). There was no difference between ADX

and SHAM in area CA3. There was no significant difference between ALDO and SHAM, or ALDO and ADX groups in the levels of 5-HT_{1A} mRNA in any of the three hippocampal subfields. Continuous activation of MR by aldosterone in the ALDO group produced levels of 5-HT_{1A} mRNA that were in between those produced in the ADX and SHAM groups; however ALDO induced changes were not significantly different from SHAM or ADX in any brain region.

Continuous activation of both MR and GR by high dose corticosterone (HCT) for 2 weeks significantly decreased 5-HT_{1A} mRNA in all three hippocampal subfields as compared to ADX (Fig. 2). In areas CA3 and DG, the mRNA levels for the HCT group were also significantly less than those in the ALDO group. The 5-HT_{1A} mRNA levels in the HCT group were significantly decreased as compared to SHAM only in the DG.

4. Discussion

The principal findings of this study were that (1) corticosteroid manipulations differentially altered mRNA levels for postsynaptic 5-HT_{1A} receptors in each subfield of hippocampus, (2) corticosteroid treatment had no effect on mRNA for 5-HT_{1A} autoreceptor in median raphe or dorsal raphe, and (3) the levels of mRNA for the 5-HT_{1B} receptor whether pre- or postsynaptic, were not altered by corticosteroid manipulations in the raphe or hippocampus.

Animals received treatment for 2 weeks to investigate the long-term consequences of these manipulations, which may be relevant to chronic stress and depression. To our knowledge, the results obtained from this study are the first to use these treatment manipulations to assess the effects of the stress hormone corticosterone on 5-HT_{1A} and 5-HT_{1B} mRNA regulation in any brain region. The treatment groups were chosen to produce activation of neither MR nor GR (ADX), only the MR (ALDO), or both the MR and GR (HCT). The SHAM group retains normal cyclical occupation of both MR and GR during the diurnal surge.

The treatment groups were chosen to produce activation of neither MR nor GR (ADX), only the MR (ALDO), or both the MR and GR (HCT). The SHAM group is a normal control that provides information on MR activation with cyclical occupation of both MR and GR during the diurnal surge. When the data from the SHAM group is compared to that of the ALDO group, the influence of the transient occupation of GR can be assessed. Animals received treatment for 2 weeks to investigate the long-term consequences of these manipulations, which may be relevant to chronic stress and depression. To our knowledge, the results obtained from this study are the first to use these treatment manipulations to assess the effects of the stress hormone corticosterone on 5-HT_{1A} and 5-HT_{1B} mRNA regulation in any brain region.

Although in situ hybridization measurement of receptor mRNA levels provides an indirect index of receptor synthesis, this technique also provides important information about receptor regulation in discrete populations of neurons that often cannot be obtained with radioligand binding. Chronic treatments such as those used in this study are likely to modulate receptor systems on both pre- and post-transcriptional levels [23]. Therefore, measurement of mRNA levels provides information that complements results obtained using binding site analysis or functional assays of receptor activity.

One aim of the present study was to attempt to reconcile the disparate results obtained by other laboratories that have examined 5-HT_{1A} regulation by CT. The data obtained in this study are compared to results obtained in previous studies in Table 2. In all studies in which both mRNA and receptor binding were measured identical results were obtained. In the majority of the studies, adrenalectomy increased 5-HT_{1A} receptor and/or mRNA number, whether the treatment was for 1 day or 3 weeks. Long-term adrenalectomy, i.e. 2 months, resulted in a decrease in 5-HT_{1A} mRNA levels in the dentate gyrus.

Treatment with the relatively selective MR agonist aldosterone produced levels of 5-HT_{1A} receptor and/or mRNA that were not significantly different from those measured in the SHAM treatment group. Treatment with low doses of corticosterone produced similar results to those obtained using aldosterone. In five of the nine studies reviewed the levels of 5-HT_{1A} receptor and/or mRNA were not significantly different between the ALDO/LCT treatment groups and the SHAM and ADX treatment groups; the levels of 5-HT_{1A} receptor and/or mRNA levels were less than that seen in the ADX group, but greater than that seen in the SHAM group. In the other four studies aldosterone or low doses of corticosterone brought the levels of 5-HT_{1A} receptor and/or mRNA back to SHAM levels, and therefore they were significantly lower than the levels found in the ADX treatment group. In general it appears that treatment with aldosterone or low doses of corticosterone reverses the increase in 5-HT_{1A} receptor and/or mRNA number produced by the removal of the endogenous hormone corticosterone by adrenalectomy.

The effect of treatment with high stress levels of corticosterone produced similar results to those seen with aldosterone or low doses of corticosterone in the CA1 and CA3 subfield, but not in the dentate gyrus. High corticosterone treatment reversed the effects of adrenalectomy so that 5-HT_{1A} receptor and mRNA levels were not significantly different from those levels found in SHAM-treated rats in the CA1 and CA3 subfields. However, in all cases in which treatment with high doses of corticosterone was for at least 1 week, the levels of 5-HT_{1A} receptor and mRNA levels were reduced as compared to both SHAM and ADX-treated rats in the dentate gyrus. This effect appears to be dependent upon the co-activation of both MR and GR, since GR activation alone did not reduce 5-HT_{1A} receptor levels below SHAM or ADX; in fact in the one study that specifically looked at the effects of GR activation without concomitant activation of MR found an increased number of 5-HT_{1A} receptors as compared to SHAM.

Therefore, based on the review of studies measuring the effects of corticosterone treatment on 5-HT_{1A} receptor and/or mRNA levels in the hippocampus, three general conclusions can be made. In the absence of the endogenous stress hormone corticosterone, 5-HT_{1A} receptor and mRNA levels are increased in CA1 and dentate gyrus. In the CA3 subfield, the effect is not as pronounced with some reporting a significant increase and others finding an increase that was not significant in 5-HT_{1A} receptor and/or mRNA. MR activation by the MR selective agonist aldosterone or low doses of corticosterone reverses the effect of adrenalectomy back to control levels. Treatment with stress levels of corticosterone, maximally occupying both MR and GR, does not produce any further regulation of 5-HT_{1A} receptor and/or mRNA levels in the CA1 and CA3 subfields, but produces a further reduction in 5-HT_{1A} receptor and/or mRNA levels below that seen in SHAM-treated animals in the dentate gyrus.

We found no effects of CT on 5-HT_{1B} mRNA in hippocampal CA1 pyramidal layer or in the dorsal raphe. Previously, Mendelson and McEwen (1992) described increased cyanopindolol binding in the dentate gyrus after adrenalectomy that was reversed by corticosterone treatment. Since 5-HT_{1B} receptors are transported to axonal terminals in most if not all cells that express this receptor, including CA1 pyramidal cells [6], it is difficult to determine the cellular source of the binding sites in radioligand binding assays. It is possible that the increased binding noted by Mendelson and McEwen (1992) represented 5-HT_{1B} binding sites on terminals projecting from an unknown brain region into dentate gyrus. These considerations reinforce the utility of mRNA detection as compared to binding when interpreting the cell source involved in a 5-HT_{1B} effect.

CT treatment did not alter the levels of 5-HT_{1A} mRNA in the dorsal or median raphe. Previous studies have also reported that the levels of 5-HT_{1A} receptor and/or mRNA are not altered in the dorsal raphe [20,26] or the median raphe [39] by adrenalectomy. However, 5-HT_{1A} receptor

number is reported to be decreased 2 weeks following adrenalectomy, an effect that was not reversed by corticosterone treatment [39].

To summarize our findings, corticosterone had tissue- and receptor-specific effects on regulation of 5-HT_{1A} and 5-HT_{1B} receptor mRNA levels in the hippocampus and median and dorsal raphe. Absence of the stress hormone by adrenalectomy leads to an upregulation of 5-HT_{1A} mRNA levels in CA1 and the dentate gyrus subfields of the hippocampus. Replacement with aldosterone or high stress levels of corticosterone reverses the upregulation and brings 5-HT_{1A} receptor levels back to normal in CA1. In the dentate gyrus treatment with high doses of corticosterone leads to a further reduction in 5-HT_{1A} mRNA levels. Manipulation of corticosterone plasma levels does not regulate 5-HT_{1A} mRNA levels in the median or dorsal raphe. Also, corticosterone treatment does not alter 5-HT_{1B} mRNA levels in the dorsal raphe or the hippocampus. These findings are important because they point to the selective receptor mRNA regulation by corticosterone across brain regions.

This selective regulation of 5-HT receptor mRNA levels by corticosterone may be explained in part by the differential distribution of the MR and GR mRNA and protein in the hippocampal subfields and in the dorsal and median raphe. Previous studies have shown that MR mRNA and protein are found in CA1, CA3 and the DG, but GR mRNA and protein are primarily localized in CA1 and DG [17,18,41]. Only GR is present in the median and dorsal raphe [16]. It is possible that in the dentate gyrus, where both MR and GR are present in high density, activation of MR and GR heterodimers confers additional transcriptional control [40].

Regulation of 5-HT_{1A} and 5-HT_{1B} receptors by other drug treatments has been reported. Both 5-HT_{1A} and 5-HT_{1B} receptors serve as autoreceptors in serotonergic cells and heteroreceptors in other neurons. 5-HT_{1B} autoreceptors are selectively regulated by fluoxetine [1,30] and chemical axotomy with 5,7-dihydroxytryptamine [31]. 5-HT_{1A} receptors are also regulated in a cell specific manner. Antidepressants have opposing effects on presynaptic and postsynaptic 5-HT_{1A} function [12] and on the magnitude of CA1 versus CA3 5-HT_{1A} receptor-mediated hyperpolarization [2]. Furthermore, corticosteroids have been shown to alter hippocampal G-protein expression [33] and the direct activation of G-proteins by bypassing the receptor [32]. Thus, corticosteroid regulation of 5-HT_{1A} function in hippocampus is complex and may involve regulation of protein transcription, receptor density, and the regulation of the efficacy of signal transduction.

Based on the results collected in this study we conclude that CT treatment selectively alters 5-HT_{1A} but not 5-HT_{1B} mRNA levels within subfields of the hippocampus. Furthermore, activation of MR alone reverses the effects of adrenalectomy in the hippocampus. Co-activation of both MR and GR by high stress levels of corticosterone results in a further decrease in 5-HT_{1A} mRNA in the dentate gyrus as compared to SHAM treated rats. Continued investigation of corticosteroid regulation of 5-HT_{1A} receptor mRNA, protein levels, and effector coupling and how these differ in brain nuclei may facilitate understanding of these receptor effects in the pathogenesis and treatment of clinical depression.

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Fig. 1. Distribution of 5-HT_{1A}, 5-HT_{1B} mRNA in hippocampus, raphe. 5-HT_{1A} mRNA (A, B), 5-HT_{1B} (C, D) detected by in situ hybridization in sections containing hippocampus (A, C), raphe nuclei (B, D). 5-HT_{1A} expression is apparent in each major subfield of hippocampus: CA1, CA3, dentate gyrus (dg, A), both dorsal, median raphe (drn, mrn, respectively; B). 5-HT_{1B} MRNA was detected in CA1 (C), dorsal raphe (D); hybridization signal was very weak in median raphe. The hybridization signal was somewhat lower than usual in the 5-HT_{1B} assay, but was still easily distinguishable from background. Computer-assisted densitometry was used to measure in situ hybridization signal intensity (see Methods). Scale bar=51 μ m.

Fig. 2.

5-HT_{1A} mRNA levels in hippocampus following manipulation of CT levels. Hybridization signal from in situ hybridization was detected as described in the Methods section. Optical density was measured over the CA1, CA3 pyramidal layers, the dentate granular layer in hippocampus, over the medial area of dorsal raphe nucleus, the entire median raphe nucleus. The means of 7 to 9 animals/group are shown (see Table 1 for *n*-values for each group). The error bars represent S.E.Ms. In the indications of statistical significance above the bars; solid lines over bars are compared to bars indicated with dashed lines. In CA1, adrenalectomy increased 5-HT_{1A} mRNA relative to SHAM or HCT ($P < 0.05$, $F_{3,29} = 3.32$). In CA3, 5-HT_{1A} mRNA was higher in ADX animals than HCT, ($P < 0.05$, $F_{3,29} = 3.03$), but the trend did not reach significance when compared to SHAM. In dentate gyrus, ADX increased 5-HT_{1A} mRNA ($P < 0.05$) while 5-HT_{1A} mRNA in HCT animals was reduced from SHAM levels ($P < 0.01$, $F_{3,29} = 13.53$). 5-HT_{1A} mRNA in HCT animals was much less than adrenalectomized animals ($P < 0.001$). ALDO levels of 5-HT_{1A} mRNA were intermediate to ADX, SHAM treatment results, but the differences did not reach statistical significance.

Fig. 3. 5-HT_{1A} mRNA levels in dorsal, median raphe following manipulation of CT levels. The means of 7 to 9 animals/group are shown (see Table 1 for *n*-values for each group). The error bars represent S.E.Ms. There were no differences between any treatment groups in dorsal or median raphe.

Fig. 4.

Density of 5-HT_{1B} mRNA in hippocampus following experimental treatments. 5-HT_{1B} mRNA was measured in CA1 pyramidal layer. The means of 7 to 9 animals/group are shown (see Table 1 for *n*-values for each group). The error bars represent S.E.Ms. There were no significant changes in 5-HT_{1B} mRNA level between any treatment groups.

Fig. 5. Density of 5-HT_{1B} mRNA in dorsal raphe nucleus following experimental treatments. 5-HT_{1B} mRNA was measured in CA1 pyramidal layer. The means of 7 to 9 animals/group are shown (see Table 1 for *n*-values for each group). The error bars represent S.E.Ms. There were no significant changes in 5-HT_{1B} mRNA level between any treatment groups.

Table 1

Serum corticosterone concentrations determined at sacrifice

Treatment	Corticosterone ($\mu\text{g/dl}$) $\pm$ S.E.M.	<i>n</i>
SHAM	0.28 $\pm$ 0.18	6/8 ^a
ADX	0.00 $\pm$ 0.00	9
ALDO	0.26 $\pm$ 0.14	7
HCT	26.50 $\pm$ 1.90 ^{***}	9

^a2/8 sham animals did not have serum corticosterone determined.

^{***} $P < 0.001$, significantly different than the other three treatment groups ($F_{3,27}=181$).

Table 2

Corticosterone effects on 5-HT_{1A} receptor mRNA in CA1, CA3 and dentate gyrus of hippocampus^a

	ADX	ALDO	DEX or GR agonist	LCT	HCT	Treatment length
Meijer et al., 1995 [31] (mRNA)	CA1 DG	nc ↑		CA1 DG	(nc) (↓)	6 hours
Chalmers et al., 1993 [11] (mRNA, receptor)	CA1 CA3 DG	↑ ↑ ↑		CA1 CA3 DG	nc nc (↓)	1 day
Meijer et al., 1995 [31] (mRNA)	CA1 CA3 DG	nc nc ↑		CA1 CA3 DG	(nc) (nc) (↓)	2 days
Meijer and de Kloet, 1995 [44] (mRNA)	CA1 CA3 DG	nc nc ↑		CA1 CA3 DG	(nc) (nc) (↓)	1 week
Kuroda et al., 1994 [25] (receptor)	CA1 CA3 DG	nc ↑ ↑	nc CA1 CA3	(nc) nc (↓)	CA1 CA3 DG	1 week
Chalmers et al., 1993 [11] (mRNA, receptor)	CA1 CA3 DG	↑ ↑ ↑	↑ DG	(nc) (nc) (nc)	CA3 DG nc	1 week
Tejani-Butt and Labow, 1994 [39] (receptor)	CA1 CA3 DG	nc nc ↑		CA1 CA3 DG	nc nc (↓)	1 week
Zhong and Ciaranello, 1995 [46] (mRNA)	CA1 CA3 DG	↑ ↑ ↑		CA1 CA3 DG	nc nc (↓)	1 week
Le Corre et al., 1997 [26] (mRNA)	CA1 CA3 DG	nc ↑ ↑		CA1 CA3 DG	nc nc (↓)	1 week
Mendelson and McEwen, 1992 [45] (receptor)	CA1 CA3 DG	nc ↑ ↑		CA1 CA3 DG	nc nc (↓)	1 week

	ADX	ALDO	DEX or GR agonist	LCT	HCT	Treatment length
Neumaier et al. (this study) (mRNA)	CA1 ↑	CA1 nc	(nc)		CA1 nc	(↓) 2 weeks
	CA3 nc	CA3 nc	(nc)		CA3 nc	(↓)
	DG ↑	DG nc	(nc)		DG (↓)	(↓)
Tejani-Butt and Labow, 1994 [43] (receptor)	CA1 ↑			CA1 nc		3 weeks
	CA3 ↑			CA3 nc		
	DG ↑			DG nc		
Liao et al., 1993 [42] (mRNA)	DG ↑		DG ↓	(↑)		2 months ADX
						dex 3 days

^a Increase, decrease or no change (nc) relative to SHAM-operated controls is indicated by symbols outside of parentheses; increase, decrease or no change relative to ADX is indicated by symbols inside of parentheses.