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Functional effects of corticosterone on 5-HT_{1A} and 5-HT_{1B} receptor activity in rat brain: in vivo microdialysis studies

Eitan Gur, Eliyahu Dremencov, Bernard Lerer, Michael E. Newman *

Biological Psychiatry Laboratory, Department of Psychiatry, Hadassah-Hebrew University Medical Center, PO Box 12000, Jerusalem 91120, Israel

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Abstract

Glucocorticoid hormones are known to be elevated in depression, and to interact with serotonin 5-HT_{1A} receptors at both the presynaptic and postsynaptic levels. Since one of the presumed mechanisms of action of antidepressant drugs is induction of changes in sensitivity of 5-HT_{1A} and also 5-HT_{1B} receptors, the effects of repeated administration of corticosterone (50 mg/kg s.c. b.i.d. for 10 days) on activities of these receptors were determined using in vivo microdialysis in freely moving rats. Presynaptic 5-HT_{1A} receptor activity, as measured by the effect of a challenge dose (0.2 mg/kg s.c.) of the 5-HT_{1A} agonist 8-hydroxy-2 (di-*n*-propylamino) tetralin (8-OH-DPAT) to reduce 5-HT levels in the hypothalamus, was not affected by corticosterone administration. Presynaptic 5-HT_{1B} receptor activity, as measured by the effect of the 5-HT_{1B} receptor antagonist (*N*-[4-methoxy-3-(4-methyl-1-piperizinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazole-3-yl)[1,1′-biphenyl]-carboxamide (GR 127935) (5 mg/kg s.c.) to increase 5-HT levels, was increased in hypothalamus but not hippocampus of corticosterone-treated rats. Postsynaptic 5-HT_{1A} receptor activity, as measured by the effect of 8-OH-DPAT to increase cyclic AMP levels in the hippocampus, was not affected by corticosterone administration. The decrease in presynaptic 5-HT_{1B} receptor activity after chronic administration of antidepressant drugs complements the increases in 5-HT_{1B} receptor number observed in animal models of depression.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); Corticosterone; Hippocampus; cAMP

1. Introduction

An increase in basal plasma cortisol levels is one of the most consistent of the biochemical abnormalities found in depressed patients. This increase is usually ascribed to increased activity of the hypothalamic–pituitary–adrenal (HPA) axis, with hypersecretion of corticotrophin releasing factor (CRF) as the underlying cause (Nemeroff, 1999). There is abundant evidence for interaction between the hypothalamic–pituitary–adrenal axis and the noradrenaline and serotonin (5-hydroxytryptamine, 5-HT) neurotransmitter systems, both of which are also altered in depression (Chaouloff, 2000). Studies of the mechanism of action of antidepressant drugs (ADs) are normally performed in intact healthy animals, which are clearly not suffering from depression. Although many animal models of depression exist, the degree to which they parallel the human condition is questionable, and many investigators consider them to be more closely related to stress (Willner, 1991). In the present work we have treated animals with the glucocorticoid hormone corticosterone in order to mimic the high cortisol levels seen in human patients. We have examined serotonergic function in these animals, which may constitute a "biochemical model of depression", as a prelude to administering antidepressant drugs to such animals and determining whether any of the biochemical changes normally encountered on administration of these drugs to normal animals are also found in animals with high corticosterone levels.

A leading theory of the mechanism of action of antidepressants (Blier and de Montigny, 1994, 1998) suggests that these drugs increase serotonergic neurotransmission by inducing changes in sensitivity of 5-HT_{1A} receptors. These receptors are found both presynaptically as somatodendritic autoreceptors in the raphe nuclei, and postsynaptically in terminal areas, with a particularly high concentration in limbic areas such as the hippocampus. In

* Corresponding author. Tel.: +972-2-6777617; fax: +972-2-6439294.
E-mail address: newman@hadassah.org.il (M.E. Newman).

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electrophysiological studies, antidepressant drugs of the selective serotonin re-uptake inhibitor (SSRI) class were found to induce subsensitivity of the presynaptic 5-HT_{1A} receptors and also of presynaptic 5-HT_{1B} receptors, which are found on nerve terminals, while drugs of the tricyclic antidepressant (TCA) class as well as electroconvulsive shock (ECS) increased the sensitivity of postsynaptic 5-HT_{1A} receptors (Blier and de Montigny, 1994, 1998). Several studies have shown subsensitivity of postsynaptic 5-HT_{1A} receptors in depressed patients compared to control subjects. This has been determined by administration of a 5-HT_{1A} receptor agonist and subsequent measurement of either hypothermic or hormone responses to the agonist. Following earlier work using this technique by Lesch et al. (1990a,b), we have recently shown a significantly reduced cortisol response and a completely blunted hypothermic response to the 5-HT_{1A} receptor agonist ipsapirone in depressed patients compared to control subjects (Shapira et al., 2000).

In animal studies, administration of corticosterone has been shown to affect the functioning of both pre- and post-synaptic 5-HT_{1A} receptors. The hypothermic response to the 5-HT_{1A} receptor agonist 8-hydroxy-2 (di-*n*-propylamino) tetralin (8-OH-DPAT) was reduced by corticosterone both in rats (Young et al., 1992) and mice (Young et al., 1994a). In mice, the hypothermic response is mediated by presynaptic 5-HT_{1A} receptors while in rats the majority of the evidence indicates that it is mediated by postsynaptic 5-HT_{1A} receptors, a situation which probably also prevails in humans (Blier et al., 1994). The postsynaptically mediated behavioural response to 8-OH-DPAT in rats, consisting of flat-body posture, forepaw treading and hind-limb abduction, was unaffected by corticosterone in one study (Young et al., 1992) but was reduced in another (Takao et al., 1997). Human studies have also shown reduced sensitivity of post-synaptic 5-HT_{1A} receptors in the hypothalamus after glucocorticoid administration. In healthy male volunteers, a single 100 mg dose of cortisol reduced the growth hormone (GH) response to L-tryptophan (Porter et al., 1998). Administration of 20 mg cortisol twice daily for 10 days also reduced the hypothermic and cortisol responses to challenge with the 5-HT_{1A} receptor agonist buspirone (Young et al., 1994b). The same regimen of cortisol, however, had no effect on the prolactin response to the 5-HT releasing agent fenfluramine (Young et al., 1998), due to the lack of specificity of fenfluramine for 5-HT_{1A} receptors. 5-HT_{1A} receptor activity in animals after administration of glucocorticoids has also been investigated by ligand binding and electrophysiological methods. 5-HT_{1A} receptor number in hippocampus and frontal cortex was reduced by corticosterone administration both in normal and in adrenalectomised animals (Mendelson and McEwen, 1992; Crayton et al., 1996; Takao et al., 1997) and similar changes have been shown for 5-HT_{1A} receptor mRNA levels (Chalmers et al., 1993, 1994; Meijer and de Kloet, 1994, Zhong and Ciaranello, 1995). Mendelson and McEwen (1992) also showed decreases in 5-HT_{1B} receptor binding in the dentate and CA4 areas of the hippocampus, and in area 2 of the cortex, on administration of corticosterone to either intact or adrenalectomised animals. In vitro treatment of brainstem slices with corticosterone resulted in a decrease in the potency of 8-OH-DPAT to inhibit the discharge of serotonergic neurons, indicative of desensitization of somatodendritic 5-HT_{1A} autoreceptors in the raphe nuclei (Laaris et al., 1995). Acute application of corticosterone also reduced the 5-HT_{1A} receptor-mediated hyperpolarization in hippocampal pyramidal neurons (Joels et al., 1991; Beck et al., 1996).

In the present study, we have employed in vivo microdialysis to perform functional biochemical measurements of both pre- and post-synaptic 5-HT_{1A} receptor activity in animals administered corticosterone or vehicle for 10 days, and have also measured presynaptic 5-HT_{1B} receptor activity. Presynaptic 5-HT_{1A} receptor activity was measured by determining the ability of an s.c. injection of 8-OH-DPAT to lower 5-HT levels in the hypothalamus (Sharp et al., 1989), and post-synaptic 5-HT_{1A} function in the hippocampus was assessed by measuring the rise in cyclic AMP levels after 8-OH-DPAT administration. 5-HT_{1A} receptors are negatively coupled to adenylate cyclase when activity is measured in membrane preparations from the hippocampus (de Vivo and Maayani, 1986). However, when in vivo microdialysis is used to measure cAMP levels in hippocampus of living animals (Sijbesma et al., 1991; Cadogan et al., 1994), administration of 8-OH-DPAT results in an increase in cAMP levels. This effect occurs at the 5-HT_{1A} receptor since it is blocked by the antagonist *N-tert*-butyl 3-4-(2-methoxyphenyl)piperazine-1-yl-2-phenylpropanamide dihydrochloride (WAY-100135, Cadogan et al., 1994).We have recently shown (Newman et al., 2000) that the in vivo cAMP response to 8-OH-DPAT is increased after chronic administration to rats of clomipramine, in keeping with the increased 5-HT_{1A} receptor-mediated functioning as measured electrophysiologically in the hippocampus after treatment with this drug (de Montigny and Aghajanian, 1978). Presynaptic 5-HT_{1B} receptor function was measured by determining the ability of a s.c. injection of the 5-$HT_{1B/1D}$ receptor antagonist (*N*-4-methoxy-3-(4-methyl-1-piperizinyl)phenyl]-2′-methyl-4′-5-methyl-1,2,4-oxadiazole-3-yl)[1,1′-biphenyl]-carboxamide (GR 127935) to increase 5-HT levels both in hippocampus and hypothalamus.

2. Materials and methods

2.1. Treatment of animals

Male albino rats (Sabra strain) were used in all experiments. The rats were housed in a temperature-controlled environment (24°C) with a regular 12 h light/dark cycle. Food and water were freely available. Corticosterone was

suspended in a 1:9 mixture of ethanol:olive oil by sonication at a concentration of 50 mg/ml and injected s.c. twice daily for 10 days at a dose of 1 ml (50 mg)/kg. Control animals received injections of the ethanol:olive oil vehicle. The dose and time period of administration was chosen to match those at which subsensitivity of the 5-HT_{1A} receptor-mediated hypothermia and behavioral responses were obtained (Young et al., 1992; Takao et al., 1997). Administration of corticosterone to rats twice daily has been shown to lead to plasma levels of 20–30 μg/dl, which are maintained for up to 10 h. This level closely corresponds to the elevated cortisol levels seen in depressed patients (Shapira et al., 2000), which are often associated with a loss of diurnal variation. Injections were begun in a staggered manner so that a 3-day experimental period (1 day for implantation of guides and probes and 2 days for collection of fractions) was available for each pair of animals.

2.2. Implantation and perfusion of microdialysis probes

Animals were anaesthetised with a 17:3 mixture of ketamine (100 mg/ml) and xylazine (2%) and mounted in a stereotaxic apparatus. Guides for dialysis probes (CMA/12) were implanted into the ventral hippocampus at posterior 5.8 mm from bregma, 4.5 mm lateral and 4.0 mm vertical, and into the lateral hypothalamus at posterior 1.5 mm from bregma, 1.3 mm lateral and 7.0 mm vertical. Rats were maintained under anesthesia for approximately 1 h, after which they were freely moving and had unlimited access to food and water. Dialysis probes (4 mm for hippocampus, 2 mm for hypothalamus) were inserted into the guides towards the end of the period of anesthesia. The inlets of the probe were connected, through plastic tubing with an internal volume of 12 μl/m, to 2.5 ml gas-tight syringes mounted on a microinfusion pump. The inlet and outlet tubing of the probe were mounted to a flexible cable running from the head of the rat to a liquid swivel, allowing the animal to rotate and rear without entangling the fluid tubing. The probes were perfused with Ringer's solution containing 3 mM $CaCl_2$, 4 mM KCl, 130 mM NaCl and 10 μM citalopram, pH 6.5, at 0.2 μl/min overnight. The following morning the flow rate was increased to 0.5 μl/min, and 30-min fractions collected. After each experiment, the dialysis probes were removed under anesthesia, sterilised in alcohol, and if still intact re-inserted into new animals. The animal procedures outlined above received the approval of the Institutional Animal Care and Use Committee of the Hebrew University Faculty of Medicine and Dental Medicine and Hadassah Medical Organization.

2.3. 5-HT receptor challenges

On the second experimental day for each animal, fractions from the hippocampus were frozen at −20°C immediately after collection and kept for subsequent cyclic AMP analysis. Fractions from the hypothalamus were injected into the high performance liquid chromatography (HPLC) apparatus immediately after collection for measurement of 5-HT. Once stable baseline 5-HT levels had been obtained, usually after collecting four experimental samples, the 5-HT_{1A} receptor agonist 8-OH-DPAT (0.2 mg/kg) was injected s.c. A further six fractions were then collected before adjusting the liquid switch to allow perfusion of Ringer's solution containing 50 μM forskolin to the hippocampus for 30 min. A further six fractions were than collected from the hippocampus only. On the following day, fractions from both brain areas were used for 5-HT determination. Once stable baseline 5-HT levels had been obtained, the 5-$HT_{1B/1D}$ receptor antagonist GR 127935 (5 mg/kg) was injected s.c. and a further six fractions collected.

2.4. Determination of 5-HT levels

Concentrations of 5-HT were determined by a Bioanalytical systems (BAS) High Performance Liquid Chromatography (HPLC) system. Samples were injected immediately after collection using a Rheodyne 9125 injector with a 5-μl injection loop. The mobile phase was made up of 90 mM sodium dihydrogen phosphate, 10 mM NaCl, 0.5 mM EDTA, 0.15 g/l sodium octyl sulphate and 10.5% acetonitrile, pH 5, and was delivered by the HPLC pump at 1.0 ml/min. The mobile phase was passed through a flow splitter and pumped through a 10 cm C-18 5 mm reversed phase column at 0.1 ml/min. 5-HT content was analysed with a LC-4C electrochemical detector (BAS) with a glassy carbon working electrode set at 550 mV vs. an Ag/AgCl reference electrode. Concentrations of 5-HT were calculated by comparing peak levels from the microdialysis samples with those of external standards of known concentration of 5-HT. The detection limit was 0.5–1 fmol. The average of the first four baseline samples was taken as 100%.

2.5. Cyclic AMP determination

Cyclic AMP in the perfusates was measured using a commercially available (Amersham Pharmacia) radioimmunoassay kit using ^{125}I-cAMP, with a detection limit of 2 fmol. The average of the four baseline samples prior to administration of 8-OH-DPAT was taken as baseline and set to 100%.

2.6. Materials

(±)-8-OH-DPAT, 5-HT creatinine sulfate complex and sodium octyl sulfate were obtained from Sigma (St. Louis, MO, USA). GR 127935 was a gift of Glaxo Wellcome, Stevenage, UK. Citalopram was a gift of H. Lundbeck, Copenhagen, Denmark. HPLC grade acetonitrile was from

Frutarom, Haifa, Israel. All other chemicals were of analytical grade and were obtained from Merck-Darmstadt, Germany.

2.7. Data analysis

5-HT or cAMP levels expressed as percentages of the initial levels for each animal were analysed over the time course for each challenge by two-way analysis of variance, with treatment as a "between groups" variable and time (fraction number) as a "within groups" variable, i.e. as a repeated measure.

3. Results

Basal levels of 5-HT were not affected by administration of corticosterone. In hypothalamus, basal values were 22.0 ± 3.2 fmol/5 μl dialysate in vehicle-treated rats, and 26.6 ± 3.8 fmol/5 μl dialysate in corticosterone-treated rats (mean ± S.E.M. of 17 observations in each case). In hippocampus, basal values were 26.4 ± 8.9 fmol/5 μl dialysate (mean ± S.E.M. of observations from six animals) in vehicle-treated rats, and 22.8 ± 4.8 fmol/5 μl dialysate (mean ± S.E.M. of observations from nine animals) in corticosterone-treated rats.

Fig. 1 shows the effect of s.c. injection of 0.2 mg/kg 8-OH-DPAT on 5-HT levels in the hypothalamus of these rats. 8-OH-DPAT induced a 70–80% decrease in 5-HT levels, reaching a minimum 1 h after its administration, in both groups of rats. Two-way analysis of variance on the data from fractions 4 to 11, i.e. from injection of 8-OH-DPAT until 5-HT levels had returned to baseline, showed a significant effect of time (F [7,112] = 21.15, $P <$ 0.000001), but no effect of treatment (F [1,16] = 0.11, $P = 0.74$) or interaction between time and treatment (F [7,112] = 0.63, $P = 0.73$).

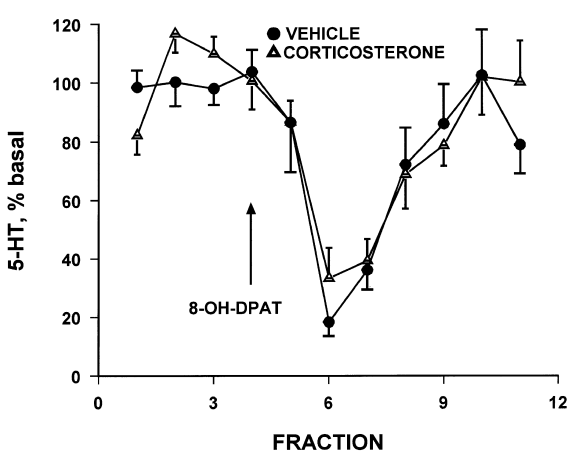

Fig. 1. Effect of 8-OH-DPAT (0.2 mg/kg s.c.) on 5-HT levels in hypothalamus. Results are mean ± S.E.M. of observations from nine vehicle-treated animals and nine corticosterone-treated animals.

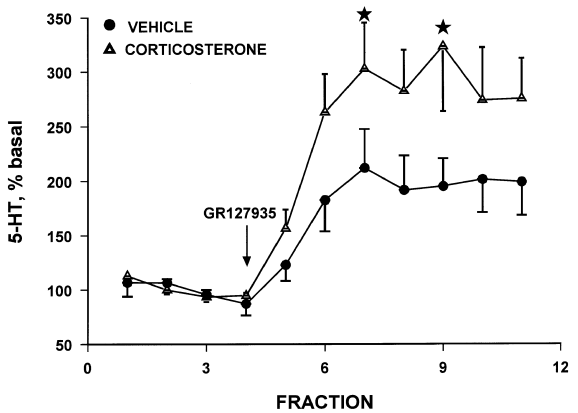

Fig. 2. Effect of GR 127935 (5 mg/kg s.c.) on 5-HT levels in hypothalamus. Results are mean ± S.E.M. of observations from nine vehicle-treated animals and eight corticosterone-treated animals. * Significant differences ($P < 0.05$) between vehicle-treated and corticosterone-treated rats by Newman–Keuls post-hoc test.

Fig. 2 shows the effect of s.c. injection of 5 mg/kg GR 127935 in the hypothalamus. In vehicle-treated rats, GR 127935 induced an approximately 2-fold increase in 5-HT levels which reached a maximum 90 min after injection and was maintained for the rest of the experiment, i.e. for a further 2 h. In corticosterone-treated rats, the time course of the increase in 5-HT levels was similar but a peak of approximately 3-fold of basal was reached. Two-way analysis of variance on the data from fractions 4 to 11 showed a significant effect of time (F [7,105] = 15.56, $P <$ 0.000001), and a significant effect of treatment (F [1,15] = 4.39, $P = 0.05$), but no interaction between time and treatment (F [7,105] = 1.41, $P = 0.21$). Post-hoc Newman–Keuls tests showed significant differences between vehicle-treated and corticosterone-treated rats at fractions 7 and 9, i.e. 1.5 and 2.5 h after injection of GR 127935.

Fig. 3 shows the effect of s.c. injection of 5 mg/kg GR 127935 in the hippocampus. The time course of the increase in 5-HT levels was similar to that in hypothalamus and the response in corticosterone-treated rats, to 2.5-fold

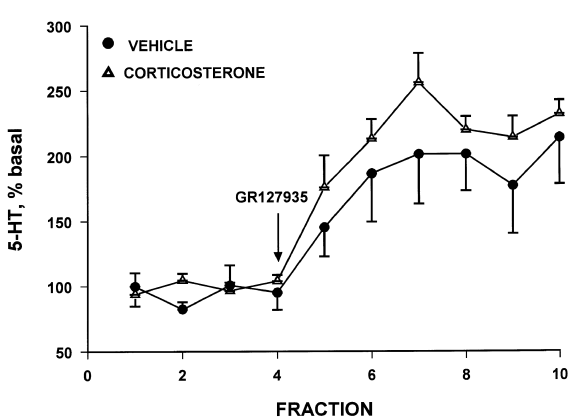

Fig. 3. Effect of GR 127935 (5 mg/kg s.c.) on 5-HT levels in hippocampus. Results are mean ± S.E.M. of observations from seven vehicle-treated animals and nine corticosterone-treated animals.

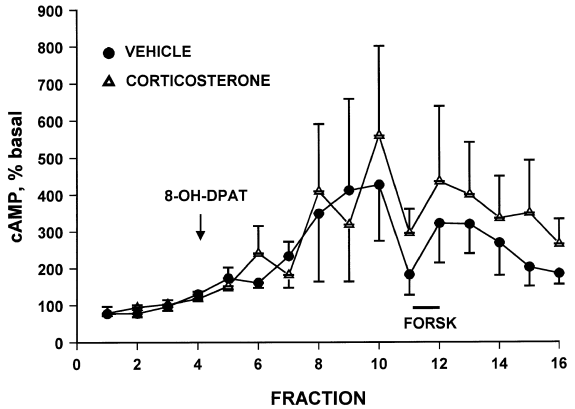

Fig. 4. Effect of 8-OH-DPAT (0.2 mg/kg s.c.) and forskolin (50 μM) on cyclic AMP levels in hippocampus. Results are mean ± S.E.M. of observations from 10 vehicle-treated animals and nine corticosterone-treated animals.

of basal, was slightly higher than that in vehicle-treated rats, to 2-fold of basal, but this difference was not statistically significant. Two-way analysis of variance on the data from fractions 4 to 10 showed a significant effect of time (F [6,84] = 12.12, $P < 0.000001$). There was no effect of treatment (F [1,14] = 1.51, $P = 0.24$), or interaction between time and treatment (F [6,84] = 0.33, $P = 0.92$).

Basal cyclic AMP levels in hippocampus of vehicle-treated rats were 2.84 ± 0.43 fmol/μl and were not significantly different from those in corticosterone-treated rats (2.19 ± 0.49 fmol/μl, mean ± S.E.M. of eight observations in each case). Fig. 4 shows the cyclic AMP responses to 0.2 mg/kg 8-OH-DPAT administered s.c. and to 50 μM forskolin, administered via the microdialysis probe, in both groups of rats. Both agents induced approximately 4-fold increases in cAMP levels in both groups of rats. Two-way analysis of variance of cAMP levels over fractions 4 to 11, the period from injection of 8-OH-DPAT until cAMP levels returned to baseline, showed a significant effect of time (fraction number) (F [7,112] = 4.07, $P = 0.0005$) but no significant effect of treatment (F [1,16] = 0.02, $P = 0.88$) or interaction between time and treatment (F [7,112] = 0.49, $P = 0.83$). Two-way analysis of variance of cAMP levels over fractions 11 to 16, from the period during which forskolin was infused until cAMP levels returned to baseline, also showed a significant effect of time (F [5,80] = 2.46, $P = 0.039$), but no significant effect of treatment (F [1,16] = 0.48, $P = 0.49$) or interaction between time and treatment (F [5,80] = 0.14, $P = 0.98$).

4. Discussion

There have been few studies of the effects of glucocorticoids on 5-HT levels in rat brain, and none involving the in vivo microdialysis procedure. Inoue and Koyama (1996) found that a single dose of corticosterone at 50 mg/kg decreased tissue 5-HT levels in four different brain regions, hippocampus, amygdala, striatum and medial prefrontal cortex, while repeated administration of the same dose for 12 days had no effect. Korte-Bouws et al. (1996) found using in vivo microdialysis that administration of the glucocorticoid synthesis inhibitor metyrapone reduced the effect of the monoamine oxidase inhibitor pargyline to increase 5-HT levels in rat hippocampus. It could be inferred from this result that endogenous glucocorticoids increase 5-HT levels and turnover, but direct evidence for this is lacking.

In the present work, chronic administration of corticosterone had no effect either on basal 5-HT levels in hippocampus or hypothalamus, or on the ability of the 5-HT_{1A} receptor agonist 8-OH-DPAT to reduce 5-HT levels in the hypothalamus, a measure of presynaptic 5-HT_{1A} receptor functioning. Although the concentration of 8-OH-DPAT employed in this experiment was high, a decrease in the reduction of 5-HT levels induced by this concentration of 8-OH-DPAT was recently observed in rat ventral hippocampus after chronic administration of citalopram (Cremers et al., 2000). Kreiss and Lucki (1995) used an even higher concentration of 8-OH-DPAT (1 mg/kg administered i.p.) and found subsensitivity of the response in both hippocampus and striatum after chronic fluoxetine, and in hippocampus only after chronic desipramine.

The reductions in 5-HT_{1A} receptor activity in animals observed after corticosterone administration in the literature are difficult to interpret since while they appear to parallel the situation in depressed patients, administration of antidepressants to intact animals also results in subsensitivity of these receptors. Thus, a reduction in the hypothermic response to 8-OH-DPAT was observed in mice after a variety of treatments including chronic electroconvulsive shock, tricyclic antidepressants and selective serotonin re-uptake inhibitors (Goodwin et al., 1985). In rats, similar effects were observed with electroconvulsive shock, monoamine oxidase inhibitors and with selective serotonin re-uptake inhibitors, but to a lesser extent with tricyclic antidepressants (Goodwin et al., 1987; Hensler et al., 1991; Stockmeier et al., 1992; Blier and Bouchard, 1992). There has been only one report of treatment with an antidepressant drug resulting in a reduction in the hypothermic response to a 5-HT_{1A} receptor agonist in depressed patients. Lesch et al. (1990c) found a reduction in the response to ipsapirone after administration of amitriptyline to depressed patients. After administration of chronic electroconvulsive therapy (ECT) to patients, however, we were unable to demonstrate any change in either the cortisol or hypothermic responses, both of which were already severely blunted (Shapira et al., 2000).

In the present work the 5-HT_{1B} receptor antagonist GR 127935 increased basal 5-HT levels in both brain areas studied. In hippocampus, the increase induced by this compound in corticosterone-treated rats was no different from that in vehicle-treated rats. However, in the hypo-

thalamus, GR 127935 produced a significantly greater increase in 5-HT in corticosterone treated rats, indicative of functional supersensitivity of the 5-HT_{1B} terminal autoreceptors in this region. This change in 5-HT_{1B} receptor activity may be a significant finding since there are two reports of increased 5-HT_{1B} receptors in an animal model of depression, while reduction of 5-HT_{1B} receptor mediated activity after chronic administration of antidepressants is a common finding. Edwards et al. (1991) showed that induction of learned helplessness in rats was associated with up-regulation of 5-HT_{1B} receptors in cortex, hippocampus and septum of rats, while Neumaier et al. (1997) found increased levels of 5-HT_{1B} receptor mRNA specifically in dorsal raphe of learned helpless rats, with no changes in hippocampus or frontal cortex. These results were interpreted as implying a specific increase in 5-HT_{1B} autoreceptors, since these are synthesized in the cell bodies of serotonergic neurons in the raphe nucleus and then transported to the nerve terminal areas, while postsynaptic 5-HT_{1B} receptors are synthesized in the cell bodies of non-serotonergic neurons in areas such as the hippocampus.

Desensitization of 5-HT_{1B} receptors after long-term administration of selective serotonin re-uptake inhibitors such as fluoxetine has also been shown both using electrophysiological methods (de Montigny et al., 1990), and in experiments in which 5-HT release was measured in incubated slice preparations from hippocampus or hypothalamus (Blier and Bouchard, 1994; El Mansari et al., 1995). In microdialysis experiments, Sayer et al. (1999) recently showed a reduction in the action of the 5-HT_{1B} receptor agonist RU 24969 to decrease 5-HT levels in hypothalamus after long-term administration both of the tricyclic antidepressant desimipramine and of the selective serotonin re-uptake inhibitor paroxetine. In recent work from this laboratory, we showed that the effect of GR 127935 to increase 5-HT levels in hippocampus was reduced after a 6-day administration of fluoxetine (Dremencov et al., 2000), while the same effect in hypothalamus was reduced after administration of the tricyclic antidepressant clomipramine for 4 weeks (Newman et al., 2000). The increased response to GR 127935 observed with corticosterone in the present study complements these findings and suggests that supersensitivity of presynaptic 5-HT_{1B} autoreceptors, leading to a decrease in 5-HT availability in the synaptic cleft, may be a characteristic of the depressed state possibly induced by high circulating glucocorticoid levels, while one of the actions of antidepressant drugs is to induce subsensitivity of presynaptic 5-HT_{1B} autoreceptors and thus increase the availability of 5-HT at synapses in forebrain areas.

It may be anticipated that as a result of the increase in 5-HT_{1B} autoreceptor sensitivity, basal 5-HT levels would be decreased in the hypothalamus of corticosterone-treated rats. That this was not found seems to indicate that there is little tonic activation of 5-HT_{1B} receptors under normal conditions. Similar results, showing desensitization of somatodendritic 5-HT_{1A} autoreceptors but no change in basal 5-HT levels in frontal cortex and ventral hippocampus, were recently obtained in two studies examining the effects of chronic administration of the antidepressants fluoxetine and citalopram, respectively (Dawson et al., 2000; Cremers et al., 2000). Similarly, in the studies of Sayer et al. (1999), both chronic paroxetine and desipramine induced subsensitivity of 5-HT_{1B} autoreceptors but no change in basal 5-HT levels in the hypothalamus.

The lack of effect of corticosterone on the post-synaptic 5-HT_{1A} receptor-mediated response, stimulation of cyclic AMP formation in the hippocampus by 8-OH-DPAT, does not agree with results of electrophysiological experiments in which either acute (Joels et al., 1991; Beck et al., 1996) or long-term (Karten et al., 1999) administration of corticosterone decreased post-synaptic 5-HT_{1A} receptor sensitivity in the hippocampus. Furthermore, measurements of post-synaptic 5-HT_{1A} receptor sensitivity in the hypothalamus by using the ability of 5-HT_{1A} receptor agonists to induce hypothermia or hormone secretion, have also indicated a reduction in response in both animal (Bagdy et al., 1989; Young et al., 1992, 1994a; Haleem, 1992; Takao et al., 1997) and human (Young et al., 1994b; Porter et al., 1998) studies after administration of cortisol or corticosterone. It should be noted, however, that in several of the studies in which glucocorticoid administration was found to lead to down-regulation of 5-HT_{1A} receptor binding sites in the hippocampus, this phenomenon was localised to certain hippocampal subfields. Thus, Mendelson and McEwen (1992) found that while low levels of corticosterone reversed adrenalectomy-induced rises in 5-HT_{1A} receptor binding in all hippocampal subfields except the CA1 area, high levels of corticosterone decreased binding below the levels seen in sham rats in the dentate gyrus only. Chalmers et al. (1994) found that 5-HT_{1A} receptor binding only responded to dexamethasone in the CA1 subfield and in the dentate gyrus, while Meijer and de Kloet (1994) found a negative correlation between plasma corticosterone levels and 5-HT_{1A} receptor mRNA levels specifically in the dentate gyrus only. In addition, Karten et al. (1999) were unable to show any changes in 5-HT_{1A} receptor mRNA levels in CA1 pyramidal cells after long-term corticosterone administration. In our experiments, probes were positioned so that sampling took place from both the CA1 and CA3 areas. Since the CA1 area in particular seems to be insensitive to corticosterone-induced changes in 5-HT_{1A} receptor number, this could explain the lack of change in 8-OH-DPAT-induced cyclic AMP formation.

In the present work, the effect of corticosterone on cAMP formation induced by the adenylate cyclase activator forskolin was measured in order to determine whether the glucocorticoid affected the activity of the catalytic unit of adenylate cyclase. Previous studies on the effects of glucocorticoids and other mediators involved in the hy-

pothalamic–pituitary–adrenal axis on cyclic AMP levels using ex vivo brain preparations have not given consistent results. Stone et al. (1987) administered corticosterone to rats at the same dose as used here for 8–10 days and obtained a reduction in α-adrenoceptor-induced potentiation of the cAMP response to β-adrenoceptor agonist stimulation in cortical slices. A similar effect was seen after repeated injections of adrenocorticotrophic hormone (ACTH). Duman et al. (1989) administered the synthetic steroid dexamethasone to rats for varying time periods and obtained an initial decrease in α-agonist potentiation of β-agonist-induced stimulation of cAMP formation, followed by increases in the responses to isoproterenol, 2-chloroadenosine and forskolin in cerebral cortex slices, indicative of increased post-receptor stimulation, after administration of either dexamethasone for 14 days or ACTH for 33 days. The discrepancy between these results and the present findings in which forskolin-induced cAMP formation was unaffected by repeated corticosterone administration probably relate to the differences in the experimental preparations used.

In conclusion, the present results demonstrate that animals treated repeatedly with corticosterone develop supersensitivity of presynaptic 5-HT_{1B} receptors specifically in the hypothalamus. Since the number of these receptors appears to be elevated in learned helpless rats, while 5-HT_{1B} receptor activity is reduced after chronic treatment with antidepressants, the activity of these receptors may be closely related to clinical depression.

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