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An Investigation of Serotonergic Involvement in the Regulation of ACTH and Corticosterone in the Olfactory Bulbectomized Rat

ANNE MARCILHAC, MAXIME FAUDON, GENEVIEVE ANGLADE, FRANCIS HERY AND PHILIPPE SIAUD

Laboratoire des Interactions Fonctionnelles en Neuroendocrinologie, U-501 INSERM, Faculté de Médecine de Marseille-Nord, Boulevard Pierre Dramard, 13916 Marseille Cedex 20, France

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MARCILHAC, A., M. FAUDON, G. ANGLADE, F. HERY AND PHILIPPE SIAUD. *An investigation of serotonergic involvement in the regulation of ACTH and corticosterone in the olfactory bulbectomized rat.* PHARMACOL BIOCHEM BEHAV **63(**4) 599–605, 1999—The bilateral olfactory bulbectomy resulted in significantly higher plasma concentration of corticosterone, but not of ACTH in basal conditions and much higher plasma ACTH and corticosterone concentrations after 15 min of immobilization stress than were observed in sham-operated animals. Daily treatment with fluoxetine—a specific serotonin reuptake inhibitor—(15 mg/kg/day) had no effect on basal ACTH and corticosterone concentrations in OB rats. Fluoxetine treatment caused lower levels of ACTH, but not of corticosterone secretion, in response to immobilization stress. Bulbectomy significantly reducing 5-HT concentration in the amygdala. Stress increased serotonergic activity in the hypothalamus but not in the amygdala of OB rats. Chronic fluoxetine treatment of both unstressed and stressed OB rats resulted in a lower turnover rate in the two structures. Our results suggest that the hypercorticosteronemia observed after bulbectomy in unstressed OB rats is independent of the serotonergic system in both hypothalamus and amygdala. In contrast, they also demonstrate hypothalamic 5-HT changes in the HPA hyperactivity of OB rats in response to stress. Chronic fluoxetine treatment may normalize pituitary ACTH secretion in response to stress, possibly desensitization of the 5-HT receptors in the hypothalamus due to 5-HT being move available at the synapses. © 1999 Elsevier Science Inc.

Olfactory bulbectomy Adrenocorticotropin (ACTH) Corticosterone Stress Serotonin (5-HT) Hypothalamus Amygdala Fluoxetine Rat

THE activation of the hypothalamo–pituitary–adrenocortical (HPA) axis has been studied in bilateral olfactory bulbectomized (OB) rats [for review, see (38)]. Such studies have shown that plasma concentrations of corticosterone are high in stress-free conditions (16,18,41), the normal circadian patterns of corticosterone secretion are disrupted (43,56), and glucocorticoid levels rise to the extreme values in the presence of a stressor (16,46) in these rats. Many factors have been reported to affect these features of OB rats, including housing conditions (16), time of day of measurement (43,56), and number of days following surgery (9). Bilateral olfactory bulbectomy in rat also has drastic biochemical and behavioral effects [for review, see (11,39)]. The behavioral changes observed include increased irritability, mouse killing (42), and poor performance in passive avoidance tasks (55,57). Treatment with antidepressant drugs, such as imipramine, viloxazine, and mianserine, improves learning passive avoidance in bulbectomized animals (14,53,60). Other reports have shown that serotonin mediates the normalizing effects of certain antidepressants in OB rats (8,12). Indeed, acute administration of single doses of several drugs believed to facilitate serotonergic activity (fluoxetine, fenfluoramine, and quipazine) overcomes the passive avoidance deficit but not the locomotive hyperactivity of OB rats, whereas chronic administration overcomes both the passive avoidance deficit and locomotive hyperactivity (8,9,36,40). Several studies have also suggested that the amygdala may be involved in producing some of the effects of antidepressant drugs in OB rats (23,28,32,33). Imip

Requests for reprints should be addressed to Dr. Ph. Siaud, Laboratoire des Interactions Fonctionnelles en Neuroendocrinologie, INSERM U501, Faculté de Médecine de Marseille-Nord, boulevard Pierre Dramard, 13916 Marseille Cedex 20, France.

ramine or amitriptyline, injected into the amygdala, inhibits mouse-killing in normal and bulbectomized rats (33,61). Given the strong serotonin innervation of the amygdala [for review, see (52)], this suggests that antidepressant treatments may act by modifying 5-HT activity in the amygdala (29). In OB rats, this notion is supported by the fact that microinjection of serotonin, but not of noradrenaline, into the amygdala improves passive avoidance learning (28). However, there is considerable evidence that HPA function in patients with depression and in animal models of depression, such as OB rats, is altered, and that antidepressant treatments normalize the initial HPA dysregulation (1,7,19,20,30).

Thus, antidepressant drugs probably have some inhibition effects on the HPA axis of OB rats via changes in the serotonergic activity of the hypothalamus and the amygdala. We addressed these issues by treating OB rats daily for 2 weeks with fluoxetine, a specific inhibitor of serotonin reuptake, and studying the effects of this treatment on concentrations of adrenocorticotropin (ACTH) and corticosterone in the plasma in stress-free or stress conditions. Serotonin activity in the hypothalamus and amygdala was determined at the same time.

METHODS

Animals

Forty adult Sprague–Dawley male rats (C.E. Depré, St. Doulchard, France), weighing 180–200 g each at the start of the experiment, were housed singly in a soundproof facility with a controlled temperature (21 ± 1°C) and light schedule (50 × with 12 h light from 0600 and 12 h dark). Animals were habituated to this lighting schedule for 2 weeks before surgery. They received commercial food pellets (U. A. R., Paris, France) and water ad lib.

The experimental protocols used conformed to French laws on laboratory animals and were approved by the managing committee of the Center for Laboratory Animals, which houses all animals under experimental investigation on the campus.

Surgery

Rats were randomly assigned to two groups: sham-operated (SO; $n = 14$) and bulbectomized (OB; $n = 30$). Before surgery, rats were anesthetized using an Equithesin solution, a mixture of 4.6% chloral hydrate, 1% pentobarbitone, and 95% ethanol in saline, injected intraperitoneally (0.33 ml/100 g b. wt.) An incision was made in the skin overlying the skull, and a hole of 3 mm in diameter was drilled into the skull through the frontal suture. The olfactory bulbs were removed via the hole by gentle aspiration with a suction pump. The resulting cavity was filled with a hemostatic sponge. The lesions were checked by visual inspection after the rats were killed. Bulbectomized rats were excluded from the study if the bulb was not completely removed or if damage extended into the frontal cortex. Sham-operated rats were removed from the study if there was evidence of damage to the olfactory bulbs or to the frontal cortex. Approximately 5% of animals were excluded from each group for these reasons. The animals were kept in individual cages after surgery.

Drug Treatment

The day after surgery OB rats were randomly assigned to two groups and treated either with vehicle (DMSO; $n = 16$) or fluoxetine (*F*; $n = 14$), injected once per day throughout the experimental period. Fluoxetine was a gift from Eli Lilly and Co. (Indianapolis, IN), and was injected at the classically used dose of 15 mg/kg body weight (13). It was dissolved in dimethyl sulfoxide (DMSO) and was injected subcutaneously at 1700 h daily, in a volume of 0.15 ml, beginning 14 days before the stress procedure. The subcutaneous route of injection was chosen to minimize interaction with corticotropic function, intraperitoneal and per os routes of injection being much more stressful.

Stress Procedure

All animals were left undisturbed until 0830 h on the 15th day after surgery. The animals of the stressed groups (SO; $n = 7$; OB: $n = 8$; OB + F: $n = 6$) were then taken randomly and placed in Plexiglas restrainers (6 cm wide and 3.8 cm high) with a time interval of 10 min between the removal of successive animals. The rats were kept in these restrainers for 15 min, and were then removed and immediately decapitated. The unstressed groups (SO: $n = 7$; OB: $n = 8$; OB + F: $n = 8$) were decapitated at the same time. Every 5 min one animal from each group (stressed or unstressed) was killed. The last animal was decapitated at 1100 h. Trunk blood (4 ml per rat) was collected in centrifuge tubes containing 0.2 ml of 5% ethylenediaminetetra–acetic acid (EDTA) in saline. The mixture was centrifuged, and plasma samples were collected and stored at −30°C until required.

HPLC Assays of 5-HT and 5-HIAA in Hypothalamus and Amygdala

Brain were excised, chilled, and dissected on ice as quickly as possible (1 min). Tissue samples were frozen in dry ice within 2 min of death. The 5-HT and 5-HIAA content of tissue samples was assayed by high-performance liquid chromatography (HPLC) with an electrochemical detector. Tissue samples were collected in ice-cold 0.2 M HCl, briefly sonicated, and the mixture centrifuged at 16,000 *g* for 20 min. The recycling mobile phase, consisting of 0.1 M sodium acetate, 0.57 mM EDTA pH 4.5, with 7% methanol by volume, was pumped at 1 ml/min through a Superspher 00-RP 18 (250 × 4 mm) column. A Waters 460 electrochemical detector was used, equipped with a high-sensitivity analytical cell set, and an oxidizing potential of +0.65 V. Chromatograms were obtained using a Delsi-Enica 21 integrator. Indolamines were identified and quantified by comparison of their retention times and peak areas with those of standard solutions purchased from Sigma Chemical Co.

Radioimmunoassays of ACTH and Corticosterone in Plasma

Rat plasma ACTH was assayed using RIA kits (CEA-Oris, Saclay, France) with synthetic human 1–39 ACTH as the antigen and a standard hormone. The lowest detectable plasma ACTH concentration was 5 pg/ml, and the intraassay coefficient of variation (CV) was 5%, whereas the interassay CV was 9%. Corticosterone concentration was determined in 20-μl samples by radioimmunoassay (21). The intraassay CV was 6%, and the interassay CV was 8%. The sensitivity of the assay was 0.5 ng/ml.

Statistics

Concentrations are given as means ± SEM. All data were analyzed by multivariante analysis of variance (ANOVA) for factorial measurements. If significant differences were indicated by this test, post hoc analysis with Fisher's PLSD test was used to determine which groups differed from each another.

RESULTS

For plasma ACTH levels (Fig. 1.A), statistical analysis by three-way ANOVA showed no significant effect of the operation, $F(1, 64) = 0.6$, $p = 0.40$, but revealed that stress increased, $F(1, 64) = 5.0$, $p = 0.02$, and fluoxetine treatment decreased, $F(1, 64) = 5.02$, $p = 0.02$, plasma ACTH levels and in overall significant interaction, $F(5, 60) = 22.17$, $p < 0.001$. Further analysis revealed that OB rats exposed to no stressful procedure other than daily handling had similar basal plasma ACTH concentration to SO rats. Immobilization stress applied to SO and OB rats resulted in significantly higher plasma concentration of ACTH than were observed for the nonstressed control groups ([respectively, $F(1, 12) = 32.5$, $p < .001$; and $F(1, 36) = 54.7$, $p < 0.001$]). Stressed bulbectomized rats had significantly higher plasma concentrations of ACTH than stressed SO rats, $F(1, 23) = 4.8$, $p < 0.03$. In unstressed OB rats, basal concentrations of ACTH in plasma were similar in vehicle- and fluoxetine-treated animals. Fluoxetine-treated bulbectomized rats secreted much less ACTH in response to stress stimuli than vehicle-treated rats, $F(1, 21) = 11.1$, $p = 0.003$.

Plasma corticosterone levels (Fig. 1B) were also significantly affected by interactions between factors, $F(5, 44) = 116$, $p < 0.001$. Stress had a significant effect, $F(1, 40) = 32.3$, $p < 0.001$, whereas lesioning and pharmacological treatment did not; OB rats had significantly higher circulating corticosterone concentrations than did SO rats in basal condition, $F(1, 11) = 14.1$, $p = 0.003$. Immobilization stress applied to SO and OB rats gave significantly higher plasma corticosterone levels than were observed for nonstressed control groups ([SO, $F(1, 10) = 135.8$, $p < 0.001$; and OB, $F(1, 22) = 82.9$, $p < 0.001$]). Stressed bulbectomized rats had significantly higher plasma concentrations of corticosterone than did stressed SO rats, $F(1, 16) = 13.8$, $p < 0.001$. In unstressed OB rats, basal concentrations of corticosterone in plasma were identical in both vehicle- and fluoxetine-treated animals. In stressed bulbectomized rats, there was no significant difference the plasma corticosterone concentration between vehicle-treated and antidepressant-treated animals after 15 min of immobilization stress.

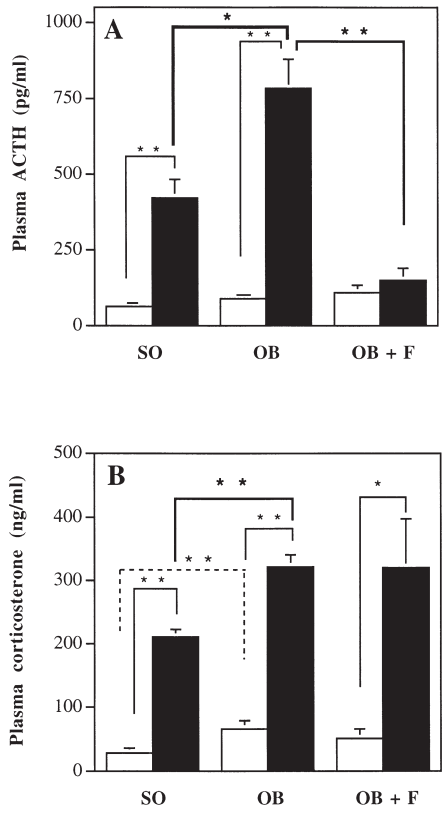

FIG. 1. Comparison of mean concentrations in plasma of ACTH (A) and corticosterone (B) in sham-operated (SO), bulbectomized (OB), and bulbectomized treated with fluoxetine (OB + F) rats unstressed (open bars) or subjected to an immobilization stress procedure for 15 min (black bars). Values are expressed as means ± SEM for six to eight animals. $*p < 0.05$, $**p < 0.01$.

Statistical analysis by a three-way ANOVA showed significant effects of interaction between three factors in the hypothalamus (bulbectomy, stress, and pharmacological treatment) on serotonin, $F(3, 28) = 4.85$, $p = 0.025$, 5-hydroxyindolacetic acid (5-HIAA), $F(5, 29) = 22.5$, $p < 0.0001$, content and the [5-HIAA]/[5-HT] ratio, $F(5, 27) = 22.3$, $p < 0.0001$. Further analysis showed that (a) bulbectomy increased 5-HT content, $F(1, 32) = 4.81$, $p = 0.03$, and decreased 5-HIAA content, $F(1, 33) = 8.51$, $p = 0.006$, and the [5-HIAA]/[5-HT] ratio, $F(1, 31) = 4.71$, $p = 0.03$; (b) stress increased 5-HIAA content, $F(1, 33) = 9.83$, $p = 0.003$, and the 5-HT activity, $(1, 31) = 11.73$, $p = 0.001$; and (c) fluoxetine treatment increased 5-HT, $F(1,32) = 17.74$, $p = .0005$, and decreased 5-HIAA content, $F(1, 33)$ 30.70, $p < .0001$, and the [5-HIAA]/[5-HT] ratio, $F(1, 31) = 45.30$, $p < 0.0001$. The concentrations of 5-HT and 5-HIAA and the turnover rate of 5-HT expressed as the [5-HIAA]/[5-HT] ratio in the basal condition were not changed by bulbectomy (Fig. 2A–C). After 15 min of immobilization stress more 5-HT was used in both SO and OB rat groups [(SO, $F(1, 20) = 6.33$, $p = 0.03$, and OB, $F(1, 11 = 24.44$, $p = 0.0004$)] (Fig. 2C). This was due to a significant increase in 5-HIAA concentration [SO, $F(1, 10) = 6.20$, $p = 0.03$, and OB, $F(1, 11) = 48.35$, $p < 0.0001$] (Fig. 2B). 5-HT concentration was unchanged by stress. The 5-HT activity measured in the hypothalamus of OB rats after stress was higher than that of SO rats in the same stress conditions, $F(1, 10) = 6.20$, $p = 0.03$. In OB rats, fluoxetine treatment caused an increase in 5-HT concentration in the hypothalamus in stress conditions, $F(1, 9) = 16.46$, $p = 0.002$, but not in basal condition, a decrease in 5-HIAA level [control, $F(1, 8) = 5.69$, $p = 0.04$, and stress, $F(1, 9) = 135.55$, $p < 0.0001$], consequently giving a fall in the [5-HIAA]/[5-HT] ratio values in both control, $F(1, 8) = 53.42$, $p < 0.0001$, and stressed, $F(1, 9) = 79.78$, $p < 0.0001$, animal groups (Fig. 2A–C).

Analysis of the serotonergic system in the amygdala also showed significant effects of interaction between to three factors on 5-HT, $F(5, 29) = 6.61$, $p = 0.003$, and 5-HIAA, $F(5, 27) = 23.21$, $p < 0.0001$, and on the [5-HIAA]/[5-HT] ratio, $F(5, 29) = 4.85$, $p = 0.025$. This analysis showed that (a) fluoxetine treatment increased 5-HT content, $F(1, 33) = 14.33$, $p = 0.006$, and decreased 5-HIAA content, $F(1, 31) = 23.21$, $p < 0.0001$, and the 5-HT turnover, $F(1, 33) = 91.74$, $p = 0.0001$; and (b) bulbectomy also decreased 5-HT use, $F(1, 33) = 10.35$, $p = 0.002$, in the amygdala. The concentration of 5-HT, $F(1, 9) = 5.99$, $p = 0.05$, was lower after bulbectomy (Fig. 3A). In sham-operated rats, immobilization did not affect the concentration of 5-HT, 5-HIAA, or the [5-HIAA]/[5-HT] ratio (Fig. 3A–C). In OB rats, the concentrations of 5-HT, $F(1, 11) = 23.43$, $p = 0.0005$, and 5-HIAA, $F(1, 11) = 8.52$, $p = 0.01$, were significantly higher in stressed than in unstressed OB rats (Fig. 3A–B), with no change in the [5-HIAA]/[5-HT] ra-

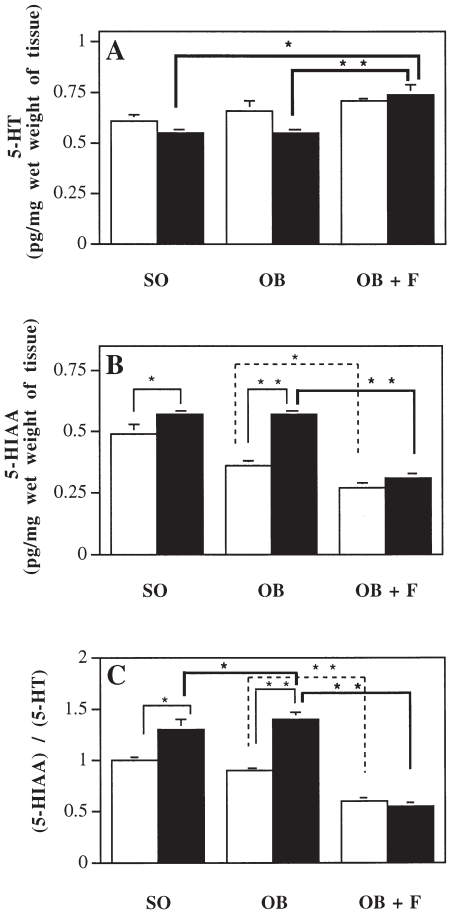

FIG. 2. Concentration of 5-HT (A) 5-HIAA (B), and [5-HIAA]/[5-HT] ratio (C) in the hypothalamus of sham-operated (SO), bulbectomized (OB), and bulbectomized treated with fluoxetine (OB + F) rats unstressed (open bars) or subjected to immobilization stress for 15 min (black bars). Values are expressed as means ± SEM for six to eight animals. $*p < 0.05$, $**p < 0.01$.

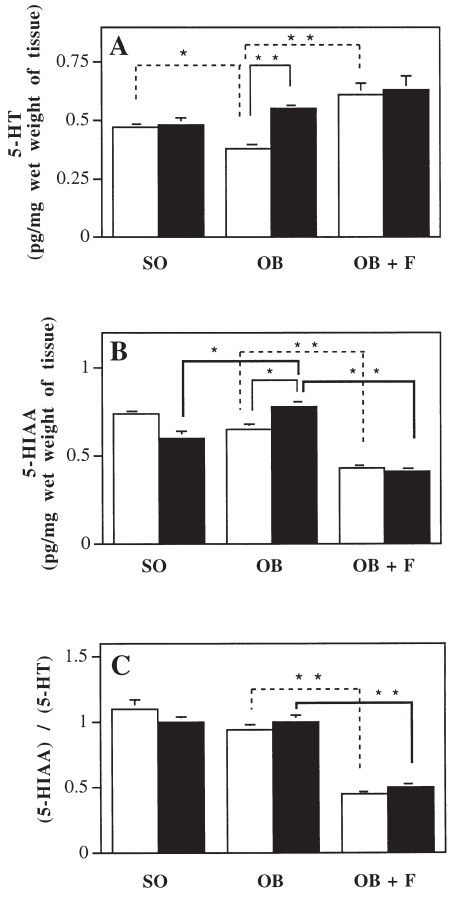

FIG. 3. Concentration of 5-HT (A) 5-HIAA (B), and [5-HIAA]/[5-HT] ratio (C) in the amygdala of sham-operated (SO), bulbectomized (OB), and bulbectomized treated with fluoxetine (OB + F) rats unstressed (open bars) or subjected to immobilization stress for 15 min (black bars). Values are expressed as means ± SEM for six to eight animals. $*p < 0.05$, $**p < 0.01$.

tio (Fig. 3C). After fluoxetine-treatment, the concentration of 5-HT in the amygdala of nonstressed bulbectomized rats was higher than that in untreated rats, $F(1, 8) = 18.85$, $p = 0.002$, returning to the levels observed in SO rats (Fig. 3A). The concentration of 5-HIAA was much lower in fluoxetine-treated rats [unstressed, $F(1, 8) = 23.57$, $p = 0.001$, and stressed, $F(1, 8) = 71.71$, $p < 0.0001$] (Fig. 3B). The rate of 5-HT turnover shows that antidepressant drug treatment significantly reduced the activity of the serotonergic system in the amygdala of unstressed, $F(1, 8) = 82.04$, $p < 0.0001$, and stressed $F(1, 9) = 44.96$, $p < 0.0001$ bulbectomized rats (Fig. 3C).

DISCUSSION

Our results show that bulbectomy significantly increased the basal concentration of circulating plasma corticosterone in unstressed rats without changing ACTH concentration. It is unclear why there was no significant change in ACTH level in OB rats given the clear changes in corticosterone concentration. However, ACTH has a much shorter plasma half-life than corticosterone. So the corticosterone peak lasts longer than the ACTH peak that caused it. Thus, a small increase in ACTH concentration could cause an accumulation of plasma corticosterone, resulting in a significant peak of plasma corticosterone concentration, when the plasma ACTH peak is no longer detectable. This notion is supported by Pfohl and coworkers, who demonstrated a significant increase in ACTH concentration in depressed patients by sampling blood every 20 min over 24 h (50,51). There may also be dissociation of the circadian synchronization of ACTH and corticosterone. Previous studies have shown that the rhythm of adrenocortical function is only partly dependent on a daily rhythm in the concentration of circulating ACTH (22). This may be due to there being a direct nerve pathway from the hypothalamus to the adrenals glands via the splanchnic nerve, which acts to stimulates corticosterone secretion independently of plasma ACTH levels (35). Immobilization stress resulted in higher concentrations of ACTH and corticosterone in the plasma of OB rats than in that of SO rats. These results are consistent with those of Cairncross and co-workers, who reported that bilateral olfactory bulbectomy results in an increase in both basal and stress-induced plasma corticosterone concentrations (16). The large increase in ACTH and corticosterone se-

cretions in response to stress may be due to a decrease in the number or sensitivity of glucocorticoid receptors (GR) in the anterior pituitary caused by the typically high basal corticosterone levels of OB rats. This downregulation of the GR by corticosterone in OB rats may decrease the efficiency of glucocorticoid negative feedback on ACTH and corticosterone secretion in response to stress. Alternatively, glucocorticoids may stimulate rather than inhibit ACTH release in pituitary cells, as has been demonstrated in chronically stressed rats (13,14,63,64). This would suggest functional changes in the interaction of the glucocorticoid receptor with the regulatory elements of the POMC gene, dependent on glucocorticoid levels. An additional corticosterone-independent mechanism, overriding glucocorticoid feedback, probably operates, as has been demonstrated in chronically stressed rats (44). HPA hyperactivity, as shown for behavioral disturbances in OB rats, may result from changes in serotonin activity in the hypothalamus and amygdala, both of which are involved in the regulation of corticotropic function (10,17,25–27).

Group by group analysis showed that bulbectomy did not affect 5-HT and 5-HIAA concentrations or 5-HT turnover rate in the hypothalamus, consistent with previous studies (34,59). In contrast, bulbectomy results in significantly lower 5-HT concentrations in the amygdala than were observed with SO rats, with no difference in 5-HT activity. The serotonin activity of the hypothalamus and amygdala were assessed using the [5-HIAA]/[5-HT] ratio as an indicator of the activity of this neurochemical system. This method has been used successfully in other studies (2,5). It does not disturb serotonin metabolism secondary to drug action, and 5-HT turnover can be estimated in the same animals used to assess neuroendocrinological parameters. This lack of change in 5-HT activity in the hypothalamus and amygdala after bulbectomy is consistent with the unchanged ACTH secretion observed. The lower concentration of 5-HT in the amygdala may be caused by changes in the 5-HT content in the terminal nerve fibers caused by corticosterone, as demonstrated for the hippocampus (4,31,37) and may be associated with the aggressive behavior observed in OB rats (24).

In both SO and OB rats, a 15-min period of immobilization led to higher serotoninergic activity in the hypothalamus. This result confirms that the stress-induced ACTH release from the pituitary is stimulated by hypothalamic 5-HT neuronal pathways (54,58). The higher level of 5-HT neuronal activity observed probably stimulates the release of CRH from the hypothalamus, which then acts on the pituitary, causing the release of ACTH. The higher level of 5-HT turnover in the hypothalamus of stressed OB rats than in stressed SO rats suggests that the hypothalamic serotoninergic system may be responsible for the HPA hyperreactivity to stress of OB rats. No change in 5-HT activity was detected in the amygdala of OB and SO rats after stress, despite significantly higher 5-HT and 5-HIAA concentrations in the amygdala of OB rats. This result is consistent with the findings of Beaulieu and co-workers (5), showing that immobilization does not affect serotoninergic activity in any amygdaloid areas studied.

Fluoxetine treatment has no effect on basal plasma ACTH and corticosterone levels. In contrast, fluoxetine reduced the ACTH secretion induced by stress in bulbectomized rats without affecting plasma corticosterone concentration. These results are consistent with stress increasing the effects of some antidepressant drugs (62), as previously suggested by Nankai and co-workers, who showed that restraint stress increases the downregulation by desipramine of serotonin transporter-binding sites in the prefrontal cortex and hypothalamus (45). This lack of plasma corticosterone changes despite a large decrease in circulating ACTH concentration in stressed OB rats after fluoxetine treatment may exist for several reasons: (a) the changes in concentration of the two plasma hormones follow different reactional time courses after fluoxetine treatment; (b) corticosterone secretion may be independent of circulating ACTH levels in this model, and may be driven by other pituitary hormonal or nerve factors insensitive to fluoxetine treatment; and (c) fluoxetine treatment changes adrenal sensitivity to ACTH. Treatment with other antidepressants have normalized circulating corticosterone concentration in OB rats (14,15,18).

However, in other experimental models, differential effects of antidepressant treatments on ACTH and corticosterone concentrations have also been observed in rats (3,6). Fluoxetine treatment led to higher 5-HT concentrations and lower 5-HIAA concentrations in the hypothalamus and amygdala of both unstressed and stressed OB rats. This higher 5-HT concentration may be due to lower levels of release of 5-HT caused by fluoxetine itself, in parallel with lower levels of 5-HT degradation due to specific reuptake inhibition, involving in fine, an increase in 5-HT availability at the synapses (13). Therefore, in basal conditions, the normalization of 5-HT content in the amygdala, with no change in plasma ACTH and corticosterone levels, suggest that the changes in 5-HT content in the amygdala after bulbectomy have no effect on circulating corticosterone. In contrast, the increase in 5-HT content of the amygdala of OB rats after fluoxetine treatment may only be responsible for behavioral normalization, as previously described (36,59). Therefore, the lower ACTH concentrations caused by fluoxetine treatment in stressed OB rats may be due to the desensitization of hypothalamic 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors or pituitary 5-HT_2 receptors by the repeated treatment, which decreases the efficiency of 5-HT synaptic transmission. Indeed, long term fluoxetine treatment inhibits the HPA system, in contrast to its acute effects (30). The observed decrease in ACTH secretion in OB rats after fluoxetine treatment may also be caused by a reduction in the synthesis of CRH mRNA or an increase in the efficacity of negative feedback by glucocorticoids. These notions are supported by the findings of Brady and co-workers (6), who observed lower levels of the CRH mRNA in the PVN and higher levels of mRNA for both mineralo- and glucocorticoid receptors in the hippocampus of rats after repeated fluoxetine treatment. A direct effect of fluoxetine on the HPA axis cannot be exclude because Pepin and co-workers demonstrated that antidepressants may increase the number of glucocorticoid receptors independently of their effects on biogenic amine metabolism or receptors, and that they may normalize the activity of the HPA axis (47–49).

Thus, the hypercorticosteronemia observed in unstressed rats at the trough of the circadian rhythm of the HPA axis seems to be independent of the serotonergic system in both hypothalamus and amygdala. The decrease in 5-HT content in the amygdala, caused by bulbectomy, may be a secondary effect of high corticosterone levels. Stressing OB rats resulted in a large increase in hypothalamic 5-HT activity higher than SO rats. This provides evidence of a stimulatory role for 5-HT in the control of HPA axis secretion. It also shows that hypothalamic 5-HT changes are induced in the HPA hyperactivity of the OB rats in response to stress. Chronic fluoxetine treatment may normalize pituitary ACTH secretion in response to stress by desensitizing the 5-HT receptors due to an increase of the greater availability of 5-HT to the synapses.

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