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Normal lymphocyte responsiveness to lectins but impaired sensitivity to in vitro glucocorticoids in major depression

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Summary

The mitogen-induced lymphocyte proliferative response and its sensitivity to in vitro (10^{-10} – 10^{-6} M) dexamethasone (DEX) administration were investigated in 12 severely depressed patients and 13 healthy controls. Patients with major depressive disorder exhibited no impairment of lectin-induced blastogenesis, but a significantly weaker suppressive effect of in vitro DEX on 1.0 μ g/ml phytohemagglutinin A-induced proliferation. The inhibitory potency of in vitro DEX was inversely correlated with in vivo adrenal cortical hormone levels at 4.00 p.m. These effects were not observed with pokeweed mitogen- and concanavalin A-stimulated cells. There were no correlations with age, weight, sex or severity of depression. These results do not support the hypothesis of a primarily impaired cell-mediated immunity, but might be indicative of reduced glucocorticoid receptor sensitivity in major depressive disorder.

Key words: Major depression; Lymphocyte transformation test; Glucocorticoids; Glucocorticoid receptor; Cell-mediated immunity

Introduction

In recent years, knowledge about the interactions between the brain, behavior and immunity has expanded rapidly (Ader et al., 1990). One well-studied mechanism of nervous system influ-

ence on the immune system is regulation via adrenal cortical hormones. Both the experience of stressful life events and the syndrome of depression have been associated with alterations in circulating concentrations of glucocorticoids, as well as alterations in immune function (Calabrese et al., 1987; Irwin et al., 1987).

During acute depressive illness approximately 50% of patients were shown to have increased hypothalamic-pituitary-adrenal (HPA) axis activity, e.g., excess cortisol production and resistance

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to dexamethasone suppression (Carroll et al., 1968; Linkowski et al., 1985). Nevertheless, they fail to display clinical signs of Cushing's syndrome.

There might be a simple explanation for this phenomenon, such as a different duration and magnitude of hypercortisolism as compared to Cushing's syndrome. Since a variety of hormones are reported to be resistant to glucocorticoid-induced changes, e.g., adrenocorticotrophic hormone (ACTH), β -endorphin and prolactin (Fang et al., 1981; Rupprecht et al., 1987, 1988), a glucocorticoid receptor dysfunction in major depressive disorder might be hypothesized. This again should have some effects on immune system integrity, since peripheral blood mononuclear cells possess glucocorticoid receptors and are sensitive to glucocorticoids (Cupps and Fauci, 1982).

Usually *in vivo* pharmacologic doses of glucocorticoids inhibit antigen- and mitogen-induced T lymphocyte proliferation and lymphokine production. In contrast, B lymphocytes are known to be sensitive to the suppressive effects of glucocorticoids only in the early activation phase of the cell cycle (Cupps and Fauci, 1982; Bowen and Fauci, 1984).

Most frequently, the mitogen response assay was used to determine the capacity of T and B lymphocytes to respond to antigens, i.e., the integrity of cell-mediated immunity. The commonly used lectins concanavalin A (Con A) and phytohemagglutinin A (PHA) are known to stimulate T lymphocytes quite selectively, whereas pokeweed mitogen (PWM) induces an accessory cell-dependent B lymphocyte proliferation (Rosenberg and Lipsky, 1979; Cupps and Fauci, 1982). Some groups have reported a significantly lower response to these lectins in lymphocytes of depressed patients compared to various control groups (Schleifer et al., 1984; Calabrese et al., 1986; Kronfol et al., 1986; Cosyns et al., 1989). Other investigators were not able to replicate these findings (Albrecht et al., 1985; Darko et al., 1989; Schleifer et al., 1989) or even described an increase in response to PHA (Altshuler et al., 1989). Lowy et al. (1988) did not find any differences in basal lymphocyte responses between healthy controls, depressed and non-depressed psychiatric patients. Instead, they described a de-

creased glucocorticoid sensitivity of Con A-stimulated lymphocytes in depressed patients, who exhibited a positive dexamethasone suppression test (DST).

Despite its sensitivity to *in vivo* and *in vitro* glucocorticoids, the lectin-induced immune responses showed no correlation with the levels of circulating adrenal cortical hormones (Darko et al., 1989; Kronfol and House, 1989; Schleifer et al., 1989), nor with urinary free cortisol (Kronfol et al., 1986). But it has to be noticed that these studies differed quite considerably in methodology (e.g., concentrations of lectins, incubation period), as well as selection criteria of patients (e.g., diagnostic criteria, severity of depression, medication, age, sex).

This study addresses the following questions. (1) Is major depression (according to DSM-III-R) associated with an impaired cell-mediated immunity? (2) Is major depression associated with an impairment of normal sensitivity of cell-mediated immunity to glucocorticoids? (3) Are the results associated with circulating concentrations of adrenal cortical hormones, such as cortisol and ACTH? (4) Are the results affected by the severity of depression or by several other variables, such as gender, age, weight?

Methods

Patients

Twelve subjects (mean age \pm SD: 48.7 ± 15.1 years; mean body weight \pm SD: 72.5 ± 13.4 kg), admitted to the psychiatric clinic of the University of Würzburg with severe unipolar major depressive disorder or bipolar depression (according to DSM-III-R) and 13 non-hospitalized healthy controls, closely matched for age, sex and weight (mean age \pm SD: 47.4 ± 6.4 years; mean body weight \pm SD: 69.2 ± 8.6 kg) were studied. Four female depressives and the matched controls were tested during the midluteal phase of the cycle, the other females were postmenopausal.

All subjects were carefully screened for nutritional status and prevalence of medical illness, as well as alcohol or nicotine abuse and stressful life events that might influence pituitary-adrenal or immune system function, by medical history, physical examination and routine laboratory investigation.

All patients were drug-free for at least 4 days prior to investigation, and seven patients for more than 3 weeks. DSM-III-R (American Psychiatric Association, 1987) diagnoses were made independently by two psychiatrists, who were blind to laboratory investigations. Severity of depression was assessed on the day of laboratory investigation using the 21-item Hamilton Rating Scale for Depression (HRS-D; Hamilton, 1960).

Controls had no history of psychiatric disorder, nor had they relatives with a psychiatric illness. They were free of any medication and their HRS-D scores were below 5.

Written informed consent was obtained from all subjects prior to the investigation. This study was approved by the ethical committee of the University of Würzburg.

Assays

All subjects were admitted to a sleep laboratory unit at least 1 h prior to blood sampling. For determination of hormonal values and lymphocyte proliferation parameters 50 ml venous blood was collected at 4.00 p.m. into plastic tubes containing EDTA.

Preparation of cells

Peripheral blood mononuclear cells (PBMC) were prepared from EDTA blood under sterile conditions by sodium metrizoate-Ficoll density gradient centrifugation as described previously (Rupprecht et al., 1990). Briefly, cells were washed twice in phosphate-buffered saline (PBS) at $100 \times g$ for 10 min and incubated for 60 min at 37°C, followed by a third washing procedure. The final cell count was performed with a Coulter counter (Model S5, Coulter Electronics Ltd, England). The viability of cells exceeded 95%, as judged from their ability to exclude trypan blue (Pappenheimer, 1917). Contamination by granulocytes and monocytes was always less than 8%.

Cell culture

PBMC were suspended at a concentration of 2.5×10^4 /well in culture medium (see Chemicals) and incubated in triplicate in 96-well microtiter plates (0.2 ml/well; NUNC, Sweden) for 60 h to evaluate Con A- and PHA-generated T cell proliferation and for 132 h to evaluate

PWM-generated accessory cell-dependent B cell proliferation in a sterile, humidified atmosphere (5% CO₂/95% air). To determine lectin dose-response curves, Con A and PWM were added at final concentrations of 0.5, 1.0, 5.0, 10.0, 20.0, 40.0 and 50.0 µg/ml, PHA at 0.1, 0.5, 1.0, 2.5, 5.0, 10.0 and 20.0 µg/ml. Eventually, lectin-induced DNA synthesis in lymphocytes was analyzed at one optimal and one non-optimal concentration of each lectin (Table 2). To assess in vitro glucocorticoid sensitivity, 10^{-10} – 10^{-6} mol/l dexamethasone (DEX) was added to spontaneous (without lectin) and mitogen-stimulated lymphocyte cultures. Again one optimal and one non-optimal concentration of each lectin were used for mitogen-stimulated lymphocyte cultures.

To measure DNA synthesis 0.5 µCi [³H]thymidine was added 5 h prior to termination of the incubation period. Incorporation of [³H]thymidine was assessed by rapid filtration through Whatman GF/B filters (Whatman, Maidstone, U.K.) with a Titertek semi-automatic cell harvester (Flow Laboratories, Inc., Rockville, MD, U.S.A.) by a 11-s wash with distilled water at room temperature. The radioisotope was measured by standard liquid scintillation counting in a Beckman LS 5000 TD counter at about 54% efficiency. All samples were assayed in triplicate. The coefficient of variation was always less than 15%.

Serum hormone determinations

ACTH was measured by a newly developed immunoradiometric assay (IRMA) supplied by the Nichols Institute (San Juan Capistrano, CA, U.S.A.; Raff and Findling, 1989). The lower detection limit was 1.5 pmol/l, and the intra- and interassay coefficients of variation were 3% and 6.8%, respectively.

Cortisol was measured by a direct radioimmunoassay (RIA) (Stalla et al., 1981). The lower detection limit was 25 nmol/l, and the intra- and interassay coefficients of variation were 5% and 9%, respectively.

Chemicals

[³H]Thymidine was obtained from Amersham, U.K. Phosphate-buffered saline (PBS) was obtained from Boehringer (Mannheim, F.R.G.).

Concanavalin A (Con A), phytohemagglutinin A (PHA), pokeweed mitogen (PWM), dexamethasone and sodium metrizoate-Ficoll were obtained from Sigma (St. Louis, MO, U.S.A.), and Rotiszint 22 was obtained from Roth (Karlsruhe, F.R.G.). Con A, PHA, PWM and dexamethasone were diluted to the respective final concentrations with culture medium. The culture medium consisted of RPMI 1640 (Boehringer, Mannheim, F.R.G.) supplemented with 2 mM L-glutamate (Biochrom, Berlin, F.R.G.), 0.1 mg/ml gentamicin (Biochrom, Berlin, F.R.G.) and 10% fetal calf serum (FCS, Biochrom, Berlin, F.R.G.). The same lots of lectins and FCS were used throughout the whole investigation to limit methodological variation.

Statistical analysis

Lymphocyte proliferation was expressed in terms of a stimulation index (SI) defined by the ratio of stimulated (in the presence of lectin) counts per minute (cpm) to spontaneous (in the absence of lectin) cpm. The results are mean \pm SD, unless otherwise stated. Hormone values below the detection limit of our assays were recorded as the respective detection limits. Data were analyzed using Pearson's product-moment correlation, one-way analysis of variance

(ANOVA), and analysis of covariance (ANCOVA). All significance levels are two-tailed.

Results

Cell viability

Under the culture conditions of the lymphocyte proliferation assays, the mean cell viabilities at the time of harvest (day 3 and day 5) were $97.0\% \pm 1.3\%$ and $94.8\% \pm 2.5\%$, respectively.

Descriptive statistics

Table 1 summarizes the demographic and clinical data of the 12 depressive patients studied. There were no significant differences in age, weight or hormone values between the controls and depressed patients investigated.

No significant correlations could be seen between age, weight, HRS-D score and hormone values.

Cortisol and ACTH values were not significantly correlated in patients with major depressive disorder ($r = 0.387$, $P = 0.24$, $n = 12$), but nearly reached statistical significance in controls ($r = 0.520$, $P < 0.07$, $n = 13$). Due to the increased number of cases it reached significance in the whole cohort of subjects ($r = 0.451$, $P < 0.03$, $n = 25$).

TABLE 1
DEMOGRAPHIC AND CLINICAL DATA OF THE 12 DEPRESSIVE PATIENTS STUDIED

Age (years)	Sex	DSM-III-R	HRS-D	Depressive episodes	Drug-free period (days)	Medication prior to drug-free period
44	F	296.53	33	3	> 28	
66	F	296.33	36	2	> 28	
61	F	296.34	28	3	> 28	
64	F	296.33	28	4	> 28	
39	F	296.33	25	3	7	100 mg ami
60	M	296.33	24	3	5	100 mg ami
60	M	296.33	28	3	5	150 mg map
34	F	296.33	28	3	4	100 mg map
54	F	296.53	33	6	> 28	
18	F	296.23	26	1	> 28	
53	M	296.33	26	2	> 21	
34	F	296.33	39	3	5	150 mg ami
48.7 \pm 15.1			29.5 \pm 4.7	3.0 \pm 1.2		Mean \pm SD

ami, amitriptyline; map, maprotiline.

TABLE 2

HORMONE VALUES AND LYMPHOCYTE PROLIFERATIVE RESPONSE OF DEPRESSIVE PATIENTS AND CONTROLS

	Controls (n = 13)	Patients (n = 12)
Cortisol (pmol/l)	225.7 ± 77.3	273.1 ± 104.8
ACTH (pmol/l)	3.6 ± 0.8	4.2 ± 1.5
<i>Stimulation indices</i>		
optimal		
10.0 µg/ml PHA	28.0 ± 5.0	23.4 ± 3.9
10.0 µg/ml Con A	15.1 ± 2.4	14.7 ± 2.1
10.0 µg/ml PWM	17.5 ± 3.1	21.9 ± 4.2
non-optimal		
1.0 µg/ml PHA	9.2 ± 1.4	9.4 ± 1.6
1.0 µg/ml Con A	5.6 ± 0.9	4.0 ± 0.5
1.0 µg/ml PWM	11.7 ± 2.4	15.9 ± 2.7

Stimulation index: ratio of lectin-stimulated to spontaneous cpm.

Lectin-induced lymphocyte blastogenesis

Table 2 shows the hormone values and lymphocyte blastogenesis after stimulation with one optimal and one non-optimal concentration of each lectin. There was no statistically significant difference between depressed patients and controls in lymphocyte response to any concentration of the lectins tested.

No significant correlations could be observed between the lymphocyte responses to PWM, Con A or PHA and age, weight, HRS-D score or cortisol values. Only lymphocyte blastogenesis after stimulation with the non-optimal PHA concentration (1.0 µg/ml) was significantly associated with ACTH values in depressed patients ($r = -0.66$, $P < 0.04$).

Sensitivity of lymphocyte blastogenesis to in vitro added dexamethasone

In vitro added DEX produced a dose-dependent suppression of spontaneous, PHA- (Figs. 1, 2) and Con A-induced lymphocyte proliferative responses (not shown) in patients and healthy controls. In vitro DEX administration exhibited no suppressive effect on PWM-stimulated blastogenesis in either group (Fig. 3).

In all subjects serum cortisol levels at 4.00 p.m. were significantly associated with the inhibitory potency of in vitro DEX on 1.0 µg/ml PHA-

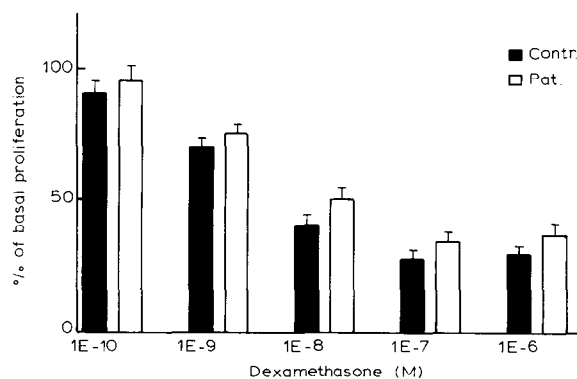


Fig. 1. Effect of 10^{-10} – 10^{-6} M in vitro dexamethasone on 1.0 µg/ml PHA-induced lymphocyte blastogenesis (arithmetic means ± SD; all assays were performed in triplicate). After covarying out serum cortisol levels, the differences between healthy controls ($n = 13$) and depressive patients ($n = 12$) were statistically significant ($P < 0.05$; see Results for details).

stimulated cells (Table 3). ACTH levels were only correlated with the inhibition of PHA-induced proliferation by 10^{-10} – 10^{-9} mol/l DEX (Table 3). The results with 10.0 µg/ml PHA-stimulated cells are shown in Table 3.

No significant correlations could be observed between levels of adrenal cortical hormones and DEX-induced inhibition of cells stimulated with optimal or non-optimal concentrations of Con A and PWM.

Since the in vitro PHA-stimulated lymphocyte proliferative response was correlated with the in vivo hormone levels (Table 3), it seemed useful to

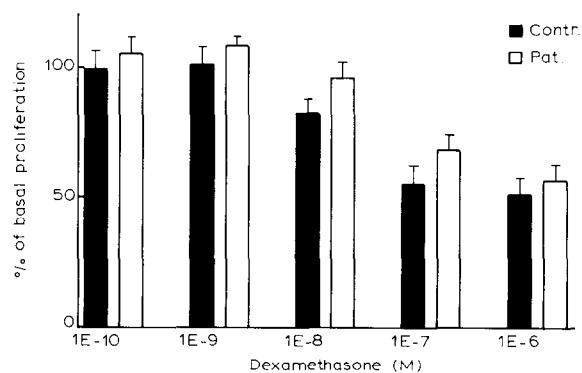


Fig. 2. Effect of 10^{-10} – 10^{-6} M in vitro dexamethasone on 10.0 µg/ml PHA-induced lymphocyte blastogenesis (arithmetic means ± SD; all assays were performed in triplicate). The differences between healthy controls ($n = 13$) and depressive patients ($n = 12$) were statistically not significant.

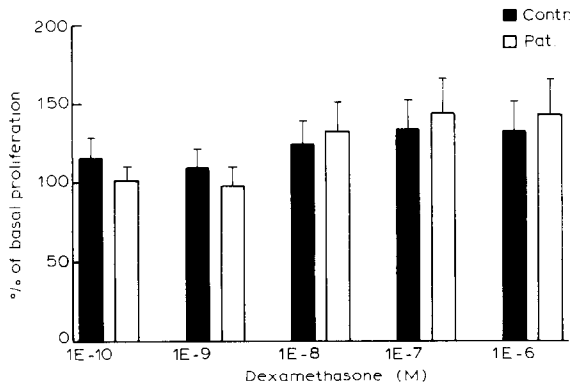


Fig. 3. Effect of 10^{-10} – 10^{-6} M in vitro dexamethasone on $10.0 \mu\text{g/ml}$ PWM-induced lymphocyte blastogenesis (arithmetic means \pm SD; all assays were performed in triplicate). The differences between healthy controls ($n = 13$) and depressive patients ($n = 12$) were statistically not significant.

weigh this influence statistically. Therefore, individuals' in vitro lymphocyte proliferation was adjusted for their in vivo cortisol levels (ANCOVA). This revealed that $1.0 \mu\text{g/ml}$ PHA-stimulated lymphocytes of depressed patients exhibited a significantly weaker suppression of blastogenesis after in vitro addition of 10^{-8} mol/l ($F = 4.49$, $P < 0.04$), 10^{-7} mol/l ($F = 6.09$, $P < 0.03$) and 10^{-6} mol/l DEX ($F = 6.61$, $P < 0.02$). The same results could be obtained after serum ACTH levels were covaried out (10^{-8} mol/l: $F = 5.11$, $P < 0.04$; 10^{-7} mol/l: $F = 3.99$, $P < 0.05$; 10^{-6} mol/l DEX: $F = 5.52$, $P < 0.03$). Only a non-significant trend ($P = 0.1$) could be seen with $10.0 \mu\text{g/ml}$ PHA-stimulated lymphocytes.

TABLE 3
CORRELATIONS (r) BETWEEN ACTH AND CORTISOL LEVELS AND PHA-STIMULATED LYMPHOCYTE BLASTOGENESIS

DEX [mol/l]	1.0 $\mu\text{g/ml}$ PHA		10.0 $\mu\text{g/ml}$ PHA	
	Cortisol	ACTH	Cortisol	ACTH
10^{-10}	-0.45 *	-0.52 *	-0.23	-0.34
10^{-9}	-0.42 *	-0.45 *	-0.32	-0.39 **
10^{-8}	-0.44 *	-0.37 **	-0.26	-0.35 **
10^{-7}	-0.50 *	-0.22	-0.39 **	-0.38 **
10^{-6}	-0.52 *	-0.34	-0.41 **	-0.37 **

$N = 25$.

* $P < 0.05$; ** $P < 0.1$.

No significant differences in DEX sensitivity of PWM- and Con A-stimulated lymphocytes could be detected between the two groups, even after covarying out the influence of in vivo hormone values.

Discussion

In accordance with Albrecht et al. (1985), Darko et al. (1989) and Schleifer et al. (1989) we were not able to corroborate previous findings of primarily impaired cell-mediated immunity in depressed patients by means of the lymphocyte transformation assay (Schleifer et al., 1984; Calabrese et al., 1986; Kronfol et al., 1986; Cosyns et al., 1989). There were no differences in PWM-, PHA- or Con A-induced proliferative responses between patients with major depressive disorder and healthy controls.

Schleifer et al. (1989) attributed impaired lectin-induced lymphocyte blastogenesis to factors such as age or severity of depression. However, in accordance with other groups (Albrecht et al., 1985; Kronfol et al., 1986; Cosyns et al., 1989) we were not able to confirm such an association. Moreover, in contrast to Schleifer et al. (1989), we could not observe a significant contribution of severity of depression, as assessed by the 21-item HRS-D, or of several independent variables, such as gender, age or weight.

These discrepancies might be due to our relatively homogeneous and small group of severely depressed patients, as well as to differences in the methodology applied.

Previous studies took blood samples in the morning hours, when the level of cortisol reaches its circadian peak (Ritchie et al., 1983). Glucocorticoid receptors are supposed to be down-regulated in the morning hours and up-regulated in the evening (Rupprecht et al., 1990). Therefore, glucocorticoid-induced effects might be more evident when blood samples are taken in the afternoon hours. This also indicates that single-point measurements of circulating hormones might not exactly reflect the blood concentrations over the previous several hours, which primarily determine receptor sensitivity.

The circadian variation of lymphocyte subpopulations is well established, as well as their differ-

ent sensitivity to glucocorticoids (Ritchie et al., 1983). Moreover, stimulation of T lymphocyte subpopulations is thought to depend partly on the lectin and on the concentrations used (Dwyer and Johnson, 1981). Therefore, the circadian rhythm of T cell subsets in the peripheral blood and their different sensitivity to glucocorticoids might be a reason for the discrepant results after stimulation with the T cell mitogens PHA and Con A.

Data in previous work (with the exception of Darko et al., 1989) have been expressed as stimulated (in the presence of lectin) cpm minus spontaneous (in the absence of lectin) cpm. In contrast, we analyzed lymphocyte proliferation in terms of a stimulation index, which weighs in vitro proliferation of in vivo stimulated cells more heavily and has a lower interassay coefficient of variation.

Although our patients with major depressive disorder tended to have higher cortisol values than healthy controls, this did not reach statistical significance. In accordance with other investigators (Albrecht et al., 1985; Kronfol et al., 1986) we were not able to observe a correlation between primary in vitro lymphocyte blastogenesis and in vivo levels of adrenal cortical hormones.

Interestingly, the DEX-induced inhibition of PHA-stimulated blastogenesis was inversely correlated with adrenal cortical hormone values. Consequently, high levels of circulating cortisol in vivo result in a desensitization of the glucocorticoid receptor. This supports previous findings from our and other laboratories that the glucocorticoid receptor is under autoregulatory control in humans (Lowy et al., 1988; Rupprecht et al., 1990). Moreover, after covarying out the influence of in vivo hormone levels, PHA-stimulated lymphocytes of depressed patients exhibited an impaired sensitivity to pharmacological as well as physiological concentrations of in vitro glucocorticoids.

These results are partly in contrast to those of Lowy et al. (1988), who also described a weaker DEX-induced suppression of lymphocyte blastogenesis, but only after stimulation with Con A. This discrepancy might be mainly attributed to the above-mentioned methodological differences (e.g., time of blood sampling and concentrations of lectins), as well as to differences in selection

criteria of patients (e.g., dexamethasone suppressors and non-suppressors).

Our data suggest that in depressed patients immune system integrity could usually be maintained by adaptation to the endogenous hypercortisolemia. But this fragile new homeostasis might be difficult to maintain if additional disturbances occur (e.g., pharmacologically induced or physiological shifts of in vivo hormonal balance). This might further support the hypothesis of a glucocorticoid receptor dysfunction in patients with major depressive disorder, since a variety of hormones are reported to be resistant to glucocorticoid-induced changes during the acute phase of the disease, as well (Fang et al., 1981; Rupprecht et al., 1987, 1988). Moreover, some recent reports have described that antidepressants elicit an increase of glucocorticoid receptor mRNA and immunoreactivity in neuronal cell cultures and in the locus coeruleus of rats (Pepin et al., 1989; Kitayama et al., 1988).

In conclusion, the elucidation of the exact mechanism of impaired glucocorticoid sensitivity (i.e., abnormalities in glucocorticoid receptor activation, translocation, genomic interaction, expression of proteins, etc.) may yield insights into the pathophysiology of major depressive disorder. Therefore, further analysis of glucocorticoid receptor function (e.g., by means of in vivo and in vitro challenge studies) is needed to more clearly delineate the relationship among the hypothesized impaired receptor plasticity, derangement of HPA axis activity and major depressive disorder.

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