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Characterization and functional significance of glucocorticoid receptors in patients with major depression: modulation by antidepressant treatment

G. Calfa [a,1], S. Kademian [a,1], D. Ceschin [a], G. Vega [b], G.A. Rabinovich [c], M. Volosin [a,*]

[a] *Departamento de Farmacología, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. Haya de la Torre esq. Medina Allende. Ciudad Universitaria. (5000) Córdoba, Argentina*

[b] *PSIQUE Consultorios Integrales de Asistencia, Docencia e Investigación en Neurociencias, Av. Colón 350, (5000) Córdoba, Argentina*

[c] *División de Inmunogenética. Hospital de Clínicas "José de San Martín", Facultad de Medicina. Universidad de Buenos Aires. Córdoba 2351 3er Piso. (1120) Buenos Aires, Argentina*

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Abstract

Hyperactivity of the hypothalamic pituitary adrenal (HPA) axis in patients with major depression is one of the most consistent findings in biological psychiatry. Experimental data support the idea that glucocorticoid-mediated feedback via glucocorticoid receptors (GR) is impaired in major depression. The aim of the present work was to assess the putative changes in GR density of peripheral blood mononuclear cells (PBMCs) in a group of patients with major depression and to determine modulation of these GR sites by antidepressant treatment. In addition, susceptibility of PBMCs to glucocorticoid effects was also studied using a functional end-point analysis in vitro, such as cortisol inhibition of mitogen-induced lymphocyte proliferation. Cortisol levels were also measured before and after dexamethasone suppression test (DST). The results showed a decrease in GR density in depressed patients compared with healthy subjects, mainly in those patients that showed basal cortisol levels in the upper normal range and were refractory to DST. Regarding the functional significance of this variation, two representative groups emerged from our study: a) free-medication patients with GR function

* Corresponding author. Tel.: +54 351 4334437 (extension 163); fax: +54 351 4334420.
E-mail address: mlv@fcq.unc.edu.ar (M. Volosin).

[1] G. Calfa and S. Kademian contributed equally to this work.

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comparable to healthy controls, and b) patients showing diminished GR activity. These results suggest a lack of relationship between GR density and cortisol-induced inhibition of lymphocyte proliferation. Patients treated with different antidepressant drugs showed a marked increase in the number of GR sites per cell compared to non-treated. Interestingly, this increase was even higher than in normal subjects. Hence, restoration of GR density after an efficient antidepressant treatment could be an index of an effective modulatory action of drugs on GR expression and highlights the possibility that GR levels might be used as markers of a successful treatment.

Keywords: Gucocorticoid receptors; Cortisol; Major depression; Antidepressant treatment; Hypothalamus-pituitary-adrenal axis; Lymphocyte stimulation

1. Introduction

Patients with major depression exhibit alterations at different levels of the hypothalamus-pituitary-adrenal (HPA) axis. Research over the last few years revealed that alterations of the HPA axis are mainly a consequence of hypersecretion of corticotropin releasing hormone (CRH), a key peptide in HPA axis regulation. Although most attention has been focused on the potential neurotransmitter pathways that may be involved in increased CRH activity, one mechanism responsible for CRH hypersecretion might be an altered feedback inhibition of CRH release by glucocorticoids (GCs). Endogenous GCs serve as potent negative regulators of the expression and release of CRH through binding to glucocorticoid receptors (GRs) in brain areas that regulate HPA axis activity (Reul and DeKloet., 1985; McEwen et al., 1986).

Evidence supporting the notion that GCs-mediated feedback inhibition is impaired in major depression has been demonstrated by a number of studies showing no suppression of cortisol secretion following the administration of dexamethasone (DEX) (Carroll, 1982). Further evidence supporting this hypothesis has been raised by the lack of inhibition of ACTH response to CRH following DEX pre-treatment and the altered response to DEX-CRH test in these patients (Gold et al., 1986; Holsboer et al., 1986; von Bardeleben and Holsboer, 1989).

On the other hand, it has been hypothesized that antidepressants exert effects that are not necessarily mediated by the well-characterized signalling pathways, but might also act by improving GR function. In this context, it has been demonstrated that pharmacologically different antidepressants attenuate HPA activity and increase GR levels at the mRNA and protein levels (Pepin et al., 1989; Reul et al., 1993). These effects might be good predictors of therapeutic efficacy.

Since the impaired inhibitory response to DEX in patients with major depression might reflect alterations in GR sites, a number of studies have been conducted to examine GR expression and function. Since studies on the regulation of corticosteroid receptors in the human brain are not feasible; indirect approaches have been attempted. In fact, most of the studies have used readily accessible peripheral blood mononuclear cells (PBMCs), which express relatively high amounts of GR. While decreased GR binding was observed in depressed patients (Gormley et al., 1985;

Whalley et al., 1986; Yehuda et al., 1993), a number of reports showed no significant changes (Schlechte and Sherman, 1985; Wassef et al., 1990; Rupprecht et al., 1991a; Maguire et al., 1997).

Therefore, the present study was aimed to clarify these controversial results using a population of untreated depressed patients. More importantly, we analyzed for the first time GR density in PBMCs from patients after successful treatment with different antidepressants.

A correlation between lymphocyte proliferation and GC sensitivity has been demonstrated (Sauer et al., 1995), showing that the inhibitory influence of cortisol on thymidine incorporation by PBMCs could be a reliable marker of GR function. Moreover, patients with major depression have shown various immunological abnormalities, such as reduced NK and T-cell activity and increased lymphocyte apoptosis (Eilat et al., 1999). To explore this issue and correlate biochemical and functional data, we also analyzed the immunosuppressive effects of GCs on phytohemagglutinin (PHA)-activated lymphocytes from patients suffering from depression.

The results presented in this study are consistent with the hypothesis that a diminished number of GR underlies the HPA hyperactivity in patients with major depression. Furthermore, we postulate that the ability of antidepressants to modulate GR number in mononuclear cells from these patients might be a good predictor of their therapeutic efficacy.

2. Methods

2.1. Subjects

Twenty-nine out of 50 patients referred to the psychiatric facilities affiliated with the Psique Center, Córdoba, Argentina, meeting DSM-IV (Diagnostic and Statistical Manual) criteria for major depression episodes were enrolled in the study. Only patients with a 21-item "Hamilton Depression Rating Scale" (HDRS) score of 20 or greater were included. The HDRS was used because it represents an established index of overall severity of depressive episodes and has been factored into several relatively independent dimensions of depressive symptomatology (Rhoades and Overall, 1983). Those patients who had been treated with different pharmacological agents underwent a two-week drug washout period, under close clinical supervision, before taking the first blood sample. All subjects had normal physical examinations and underwent normal screening laboratory tests including complete blood count, thyroid function tests, urine analysis, urine pregnancy test and urine toxicology screening. Subjects were instructed not to drink alcohol during the last 24 h preceding blood collection. In order to control the impact of hormonal changes of the menstrual cycle on the HPA axis activity, blood samples were taken in the early follicular phase. Besides, women included in these studies were not taking oral contraceptives or receiving hormonal replacement.

Thirteen out of 29 patients were followed up and treated according to clinical judgement with drugs of the physician's choice. The 16 remaining patients were

excluded from the study for different technical reasons (change of address, refusal to visit the clinic or complications after drug administration). Different kinds of antidepressant drugs were administered in those patients, either as mono-therapy or in combination with other psychotropic drugs during four weeks, namely tricyclic antidepressants, selective 5-HT reuptake inhibitors (SSRIs), MAO inhibitors (iMAOs), associated or not with mood stabilizers. The 13 patients, who were followed up, were observed in the clinic for monitoring of drug treatment and administration of the psychopathometric rating instruments. The monitoring of the drug treatment did not reveal any adverse effects. These patients underwent a drug washout period under close clinical supervision before the second blood sample collection. The length of the washout period corresponded to 4–5 cycles of half-time elimination of the parent drugs and their active metabolites (e.g., 1 day for moclobemide and 30 days for fluoxetine were the shortest and the longest periods, respectively). The clinical status of each patient was monitored daily. Healthy laboratory personnel served as control subjects and matching was done by age criteria. All patients and control subjects gave their written informed consent for the study, and an internal ethical committee approved a protocol made in accordance with the Declaration of Helsinki.

Sixteen of the initial untreated patients were also assayed for cell growth inhibition assays matched with ten healthy controls. Demographic and clinical features of patients are summarised in Table 1.

2.2. Sample collection and PBMCs isolation

Peripheral blood (sample volume, 50 ml) was obtained by venipuncture and collected to EDTA-containing siliconized glass tubes at 0800 h. The samples were immediately transported to the laboratory and processed. Blood was diluted with 2 volumes of phosphate-buffered saline. PBMCs were prepared from anti-coagulated venous blood by Histopaque®-1077 (Sigma Diagnostics Inc, St. Louis, MO, USA) density gradient centrifugation. Cells were extensively washed with PBS and sus-

Table 1
Demographic characteristic and HDRS[b] scores of patients and controls

	Healthy controls	**Untreated**	**Treated**
n	16	16	13
Age (years)	39±2[a]	40±2[a]	39±2[a]
Sex (female/male)	4;12	12;4	9;4
HDRS (total score)	–	26.9±0.9[a]	17.9±0.5[a]

[a] Means±SEM.
[b] Hamilton Depression Rating Scale (21-item).

pended in RPMI-1640 medium supplemented with 10% heat inactivated fetal calf serum (FCS), sodium piruvate, L-glutamine and gentamicine.

Cells were counted using a hemocytometer and their viability was found to be more than 95% as determined by Trypan blue exclusion.

2.3. Glucocorticoid receptor assays

Freshly isolated PBMCs were suspended in RPMI 1640 medium at a concentration ranging from $5–10\times10^6$ cells/ml. GR density was determined as previously reported by Kontula et al. (1980) with slight modifications. Briefly, 450 μl of the cell suspension was added to the tubes containing 50 μl RPMI-1640 medium and 30 nM of [6,7-^{3}H]-dexamethasone (35 Ci/mmol, NEN Life Science Products, Inc). Non-specific binding was estimated in another set of tubes with a 1000-fold excess of unlabelled DEX. Specific binding to GR was calculated by subtracting the amount of binding displaced by unlabelled DEX from the amount of total binding. Incubation was performed at 37°C with gentle shaking for 1 h. Three millilitres of cold PBS were added to each tube, and PBMCs were separated by centrifugation (1500 rpm, 4°C, 10 min). The cell pellets were further washed and centrifuged twice, and finally mixed with a fluid for liquid scintillation counting (30% mean efficiency, Tricarb 2100 TR, Packard). All determinations were performed in duplicate.

The single DEX concentration used was selected from previously performed saturation binding assays, using five different increasing concentrations of radioligand. Specific binding was expressed as number of sites per cell.

2.4. Plasma cortisol determination

For the determination of plasma cortisol concentration, a commercially available radioimmunoassay (RIA) kit (ICN Biomedicals, Carson, CA) was used. The detection limit was 0.3 μg/ 100 ml plasma; intra- and interassay coefficients of variation were less than 7.5%.

2.5. Dexamethasone suppression test (DST)

Patients were pretreated with 1 mg of DEX (PO) at 2300 h. The next day peripheral venous blood was taken at 1600 h. Plasma cortisol concentration at this time point, reflects the suppressive effects of the DEX administered the day before. A cut-off value for normal post-DEX plasma cortisol levels is usually 5 μg/dl (Gormley et al., 1985). Patients underwent DST before and after antidepressant treatment.

2.6. Cell growth inhibition assay

To examine cells proliferation, PBMCs were cultured in 96-well microtiter plates (Corning, NY) at 1×10^6 cells/well in 200 μl of complete medium: RPMI 1640 plus 10 mM HEPES (Sigma Chemical), 2 mM L-glutamine (Life Technologies, UK), 50 μM 2-mercaptoethanol, and 100 μg/ml gentamicin, supplemented with 10% heat-

inactivated FCS (Life Technologies, UK) in the absence or in the presence of PHA 10 μg/ml. To evaluate the susceptibility of lymphocytes to GCs, cells were simultaneously exposed to PHA and cortisol 10 μM, a concentration selected after a dose-response curve. Cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air. After 72 h, the cultures were pulsed 1 μCi/well [methyl-^{3}H] thymidine ([^{3}H]TdR sp act: 20.00 Ci/ mmol) for an additional 18 h. Cells were then harvested and [^{3}H]TdR uptake was monitored by using a liquid scintillation counter.

Percentages of cell proliferation for each patient were calculated as follows: (cpm "x" - cpm medium) / (cpm PHA-cpm medium) x 100, where "*x*" represents cells incubated with PHA plus cortisol. Results are expressed as percentage of cell proliferation of triplicate determinations for each patient. Cell viability was assessed by means of the trypan blue exclusion test.

2.7. Statistical analysis

Comparisons were made among groups and significant differences were determined by one or two-way analysis of variance (ANOVA). Cortisol data were subjected to one-way analysis of covariance (ANCOVA) with basal cortisol values as covariates. Post-hoc comparisons were carried out with Newman–Keuls test. *P* values less than 0.05 were considered to be statistically significant. When analyzing data for cell growth inhibition in response to cortisol a logarithmic transformation of the percentages of proliferation data was used to obtain homogeneity of error variance and normality of within-condition distribution. Chi Square test was used to ascertain the goodness of fit between the empirical observations and the theoretical model for normal distribution of data. The percentage of proliferation data from untreated patients were split into two groups by means of a non-hierarchical clustering method, namely *k* means. By this method the groups with the minimal inner variance were obtained. After that, these data were classified by the method of minimal spanning tree in order to obtain the value of the variable used to separate the population in two groups. After applying Bonferroni *t*-test, the results were considered significant only at the 0.01 level.

3. Results

In order to study the modulation of GR number in PBMCs by antidepressant treatment, we first examined GR density in untreated patients with a diagnostic of major depression**.** Results of GR density in treated or untreated patients and healthy individuals are depicted in Fig. 1 as individual values (a) or as average ± SEM (b). Statistical analysis using a one-way ANOVA revealed a significant effect [$F(2,42)=29.8$, $p<0.0001$]. Subsequent post-hoc analysis indicated a diminished number of GR on lymphocytes from untreated patients in comparison with healthy normal individuals. When patients received antidepressant treatment for four weeks, a clear increase in GR sites/cell was evidenced as compared with the groups of untreated patients and controls. This effect was common to all the antidepressant

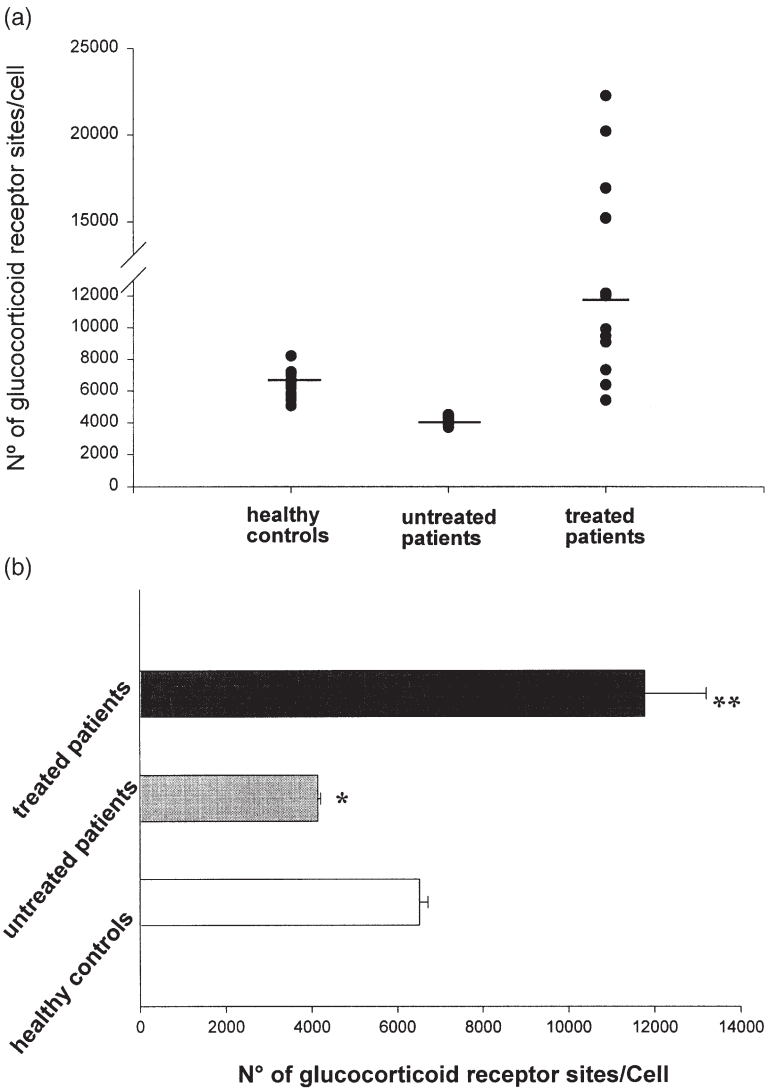

Fig. 1. Characterization of GR density in patients with major depression before or after four weeks of treatment with antidepressants.
a Individual GR values for healthy controls, treated and untreated patients
b Mean+SEM of GR number
$*p<0.05$ vs healthy controls, $**p<0.01$ vs healthy controls and untreated patients.

treatments, independently of their pharmacological mechanism of action. Interestingly, GR levels in patients receiving antidepressants were even higher than those found in healthy individuals.

Both treated and untreated patients showed high cortisol levels in the upper normal

range (Normal Values: 5–25 μg/dl) (Fig. 2). An initial one-way ANOVA determined significant group differences in basal cortisol levels [F (2, 42): 24.99, $p<0.0001$]; therefore these values were used as covariates in analyzing the cortisol response to 1 mg of DEX. Nonetheless, after correcting for this contribution to the cortisol response to DEX, ANCOVA revealed significant main effect for group [F (2, 41): 39.4 $p<0.0001$]. Post-hoc testing showed, as previously reported, a strong inhibition in cortisol secretion after DEX challenge in healthy subjects. On the other hand, both groups of patients showed a similar response to DST. In fact, treated and untreated patients were less sensitive to the effect of DEX in suppressing plasma cortisol concentration.

To gain insight into the functional significance of GR changes in blood mononuclear cells between depressed and healthy subjects, we investigated the inhibitory effects of cortisol on PHA-activated lymphocytes from these patients. When analyzing the data for control subjects samples it was observed that they suited to a normal distribution (χ^2=0.849; df: 0; p=1.000). Whereas on the contrary, data belonging to untreated patients were not distributed in a symmetrical bell-shaped fashion (χ^2= 6.522; df: 2; p=0.038) and therefore, could fit better to a bimodal distribution. Thus, individual lymphocyte percentages of proliferation from untreated patients were split into two groups using the k means clustering method. After that, the data were classi-

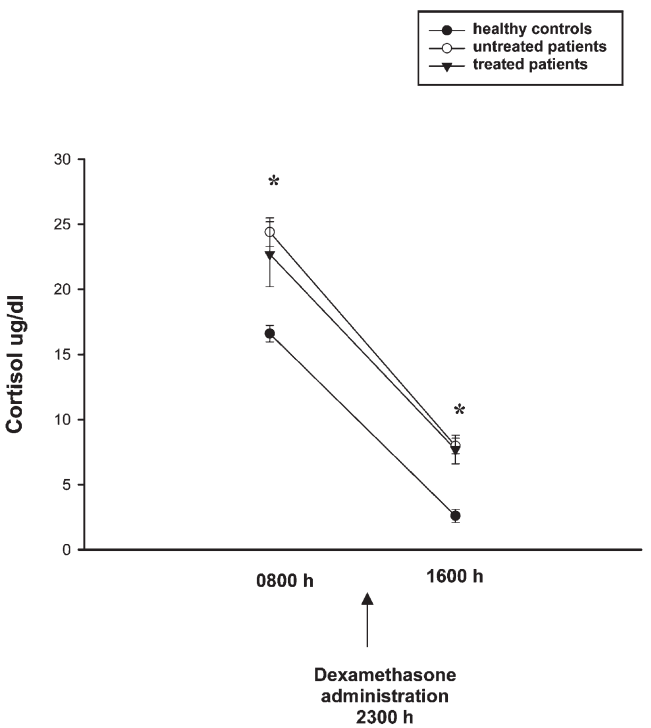

Fig. 2. Plasma cortisol levels (mean±SEM) before and after a single dexamethasone dose (1 mg) in treated and untreated depressed patients and in healthy controls. $*p<0.01$ vs healthy controls

fied by the method of minimal spanning tree, being the separation point 34.65%. Individual percentages of proliferation for untreated patients are shown in Fig. 3. Here, the two subgroups of untreated patients, namely untreated I (*n*:9) and untreated II (*n*:7) are depicted. The first included 56% of the cases, while the second group was represented by the remaining 44%. Bonferroni *t*-test validated statistically significant differences among the two groups ($p < 0.001$).

A two-way ANOVA test revealed a significant effect of diagnosis [$F(2;69)=36.1$, $p<0.0001$], medium conditions [$F(2;69)=141.1$, $p<0.0001$] and a significant interaction diagnosis×medium conditions [$F(4;69)=26.2$, $p<0.0001$]. In healthy individuals, approximately 25% of proliferation was found in PHA-stimulated lymphocytes exposed to an optimal dose of cortisol. Subsequent post-hoc analysis revealed that in agreement with the reduced number of GR sites in untreated patients, the untreated I group showed an increased mitogen-induced proliferation (average 58%) in response to cortisol (Fig. 4). This result indicates a correlation between the number and functional response of GR sites in this group of patients. In contrast, the untreated II group evidenced a normal response to cortisol (15% proliferation) in spite of the diminished GR density. Thus, low GR density in depressed patients does not always correlate with an impaired functional response to cortisol.

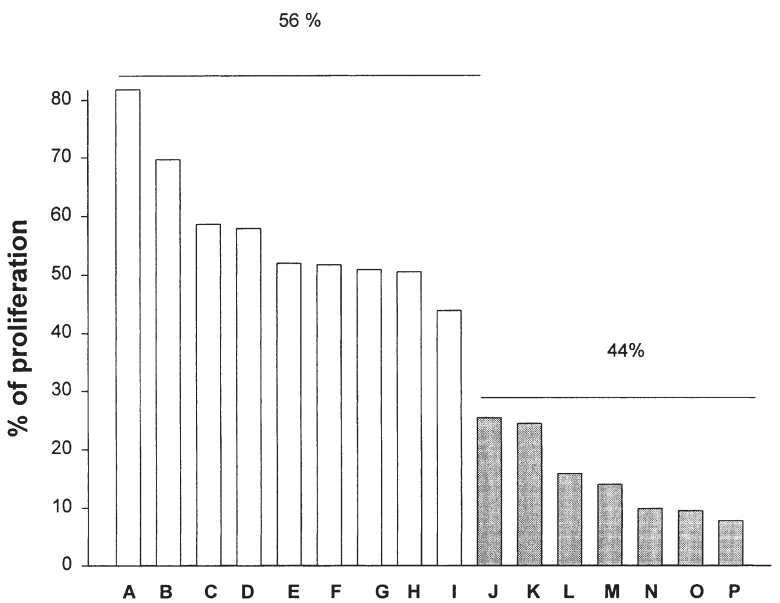

Fig. 3. Individual percentages of cortisol-induced inhibition of PHA-induced proliferation of PBMCs obtained from untreated patients. Capital letters stand for each individual case. Untreated I and Untreated II groups can be distinguished (for details see Results).

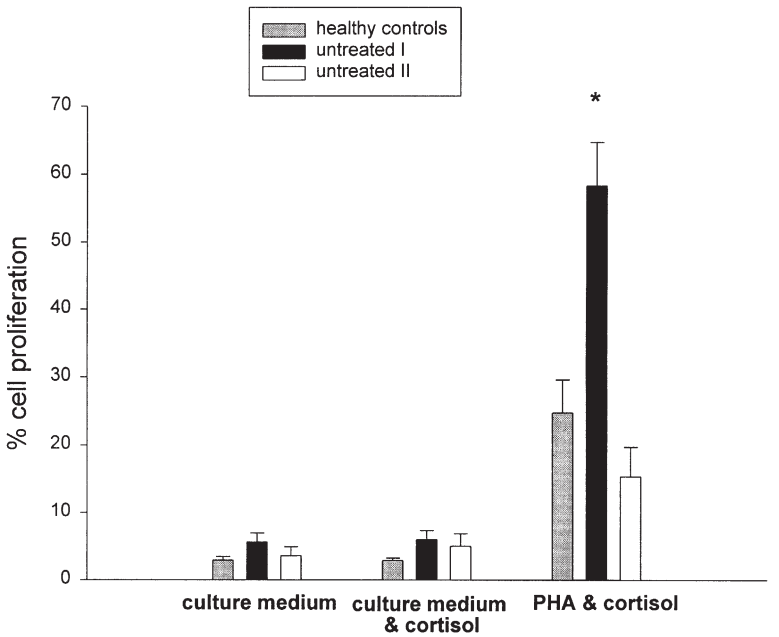

Fig. 4. Susceptibility of PHA-stimulated lymphocytes to glucocorticoid effects. Percentage of proliferation of PBMCs (mean±SEM) of healthy controls and untreated patients with or without cortisol inhibition. $*p<0.05$ vs healthy controls and untreated II.

4. Discussion

Results presented in this study show that the GR number is significantly lower in PBMCs from depressed patients who had not been treated with antidepressant drugs, in comparison to control subjects. These findings are in agreement with those reported by other authors, who assessed available GR in cytosolic fractions (Gormley et al., 1985; Yehuda et al., 1993) or using whole-cell assays (Whalley et al., 1986). However, some studies showed no significant changes in GR binding assays in depressed patients (Schlechte and Sherman, 1985; Wassef et al, 1990; Rupprecht et al., 1991a; Maguire et al., 1997). These controversial findings may be due to a number of factors, such as patient heterogeneity, cell populations studied, and the methods used to determine receptor number. It is important to highlight that our results are strengthened by the use of a highly selective criteria of patients' inclusion. Only individuals with a score of 20 or greater in HRDS, high cortisol levels in the morning, non-suppressors following DST and those who had never been treated with antidepressants were included in this study**.** Besides, a strict washout period of two weeks was applied to avoid a possible influence of different medications on the HPA axis.

In severe mood disorders, the HPA axis hyperactivity is reflected by increased levels of cortisol in plasma, urine and cerebrospinal fluid; increased cortisol

responses to ACTH, enlarged pituitary and adrenal glands and flattening of cortisol circadian rhythm (Owens and Nemeroff, 1993; Holsboer and Barden, 1996). Evidence strongly indicates that hypercortisolaemia is a result of CRH hypersecretion. In fact, in patients with depression an increased concentration of CRH in cerebrospinal fluid, a blunted ACTH response to a CRH challenge and downregulation of CRH receptors were found in post-mortem frontal cortices of suicide victims (Nemeroff et al., 1984; Plotsky et al., 1995; Holsboer and Barden, 1996; Deuschle et al., 1997).

In addition, an impaired feedback inhibition by endogenous corticosteroids — mediated by GR at different levels of the HPA axis — might also contribute to hypersecretion of CRH and the resultant hypercortisolaemia (Sapolsky et al., 1986). The DST is a measure of the functional integrity of the GR-mediated negative feedback mechanism at the pituitary level. The observation that some patients who received DEX do not adequately suppress cortisol, suggests the possibility that a primary GR abnormality might occur in major depression. An updated revision on GR expression and function in major depression has recently been published by Pariante and Miller (2001). Our findings presented herein are consistent with a subsensitivity of GR sites in major depression and their regulation by cortisol. In fact, high basal cortisol levels found in those patients might be associated with downregulation of GR sites in PBMCs. In agreement with the increased peripheral HPA activity in those patients, no suppression of cortisol was found in response to a low dose of DEX. Supporting evidence for GR abnormalities in severe depression, a recent and direct post-mortem demonstration of reduced GR mRNA was found in the hippocampus of individuals suffering from affective disorders (Webster et al., 2000). In addition, suicide victims with a history of depression showed alterations on the mineralocorticoid receptor (MR)/ GR mRNA ratio in the same brain region (López et al., 1998).

The indicated evidence suggests that the GR dysfunction could be due to a decreased receptor number. However, abnormalities downstream of the receptor could also underlie the impaired GR function seen in untreated patients (Holsboer, 2000).

Although a small number of studies have investigated the functional sensitivity of PBMCs from depressed patients to the inhibitory effects of cortisol, all of them showed an increased GCs resistance as determined by in vitro or in vivo challenge with DEX (Lowy et al., 1988; Rupprecht et al., 1991b; Miller et al., 1999). In agreement with these reports, in the present study a group of untreated patients showed an expected correlation between a reduced GR number and an attenuated effect of cortisol on PHA-induced lymphocyte proliferation. This result supports the hypothesis that GCs insensitivity is associated with no suppression of DST (Lowy et al., 1988).

In contrast, a second group of depressed patients evidenced a normal response to cortisol associated with a diminished GR density in PBMCs. Therefore, the sensitivity of mitogenically-activated lymphocytes to GCs does not always correlate with the number of GR. This indicates that, in spite of the reduced GR levels, in some patients the functionality in the PBMCs remains intact. Our observations, are in

agreement with the phenomenon already reported in the pioneering works of Munck (Munck and Náray-Fejes-Tóth, 1992). Thus, although a decreased receptor number often reduces sensitivity to the mediator, sometimes, sensitivity may be modulated by other changes at the molecular level with no significant alterations.

It is not clear whether the dichotomous and paradoxical effects of cortisol on proliferation of different subgroups of patients might be attributed to activation, clonal expansion or differentiation of certain subpopulations of lymphocytes or to a differential susceptibility of lymphocytes caused by disturbances in signal transduction pathways induced by different neuropeptides or cytokines in the course of the disease. One might speculate that differences in the Th1–Th2 response or in the pro-inflammatory/anti-inflammatory cytokine balance induced by different genetic backgrounds of individuals or previous acquired immune pathologies would also determine lymphocyte response to cortisol stimulation. The controversial effects of GCs towards activated T lymphocytes in depressed patients still remains to be elucidated.

The study of lymphocyte proliferation in the population of treated patients would provide new insights into the understanding of the correlation between GR number and function. Tricyclic antidepressants increase GR mRNA in primary neuronal cultures, as has been first reported by Pepin et al. (1989). Moreover, the ability of antidepressants to increase GR mRNA levels and binding activity has also been demonstrated by studies in vivo (Seckl and Fink, 1992; Reul et al., 1993). Interestingly, antidepressant treatment of non-neuronal cell lines, fibroblast or neuroblastoma cells, also increased GR mRNA and [^{3}H]dexamethasone binding, suggesting that a mechanism independent of monoaminergic neurotransmission is involved in GR regulation (Pepin et al., 1992). More recently, an upregulation of GR mRNA in normal human blood cells after treatment with amitriptyline, demonstrated a direct action of this antidepressant on GR-gene expression in vivo (Vedder et al., 1999).

In the present report, a clear increase in GR levels was observed, following only four weeks of antidepressant treatment in a group of patients, compared to untreated individuals. Interestingly, GR density in patients receiving antidepressants was even higher than that found in healthy individuals. The effect was common to all the antidepressant treatments — tricyclics, SSRIs, iMAOs — independently of their pharmacological mechanism of action. Modulation of GR by antidepressants was concurrent with a tendency to improve the HDRS scores (in Table 1) and clinical features (Vega, personal communication), although patients were still non-suppressors (Fig. 2). Therefore, we postulate that the effect of antidepressants on lymphocytes GR could be predictive of their therapeutic efficacy. Considering that the sample size was modest, further investigation would be necessary to address the effects of individual antidepressants on GR function using different immunological approaches.

Regulation of GR may be different in the periphery and at the pituitary level. For example, GR in lymphocytes may be more sensitive to circulating steroids, while receptors in the pituitary may be less responsive to steroids due to local inhibitory factors. Furthermore, time course of the effects of antidepressants on lymphocytes might precede the changes observed in pituitary GR and might consequently modu-

late responsiveness to DST. The follow-up of patients after a longer treatment will allow us to demonstrate this hypothesis.

Circulating mononuclear cells are frequently used to assess GR regulation under pathological conditions, when HPA abnormalities occur. It remains to be established whether these effects are representative of those observed in the CNS. In this sense, Lowy (1990) showed that changes in GR concentration and binding affinity in lymphocytes might be used as a model for a neuronal receptor change in disease. Moreover, Meaney et al., (1988) showed that GR in lymphocytes and neuronal tissues have the same steroid specificity and affinity. In a clinical setting, PBMCs proved to be useful for the evaluation of corticosteroid receptors in the brain (Armanini, 1994).

The results presented in this study have been analyzed and discussed as a group; however, it is evident that heterogeneity could exist in terms of the status of the HPA axis in depression. Particularly, age and gender may constitute a source of possible variance (von Bardeleben and Holsboer, 1991; Heuser et al., 1994; Seeman et al., 1995). Although patients and healthy controls were age-matched in our study, we did not make a direct comparison on the basis of gender because no significant differences were found between male and females regarding all the parameters studied (data not shown).

This study attempts to characterize GR expression and function in mononuclear cells from patients with major depression. Our results are consistent with the hypothesis that alterations in GR number underlie the HPA hyperactivity and might be associated with the increasing susceptibility to chronic infections and immunosuppression in patients with affective disorders. Finally, our findings point to a rational basis for understanding the non-neuronal effects of antidepressants.

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