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Antidepressants, but not antipsychotics, modulate GR function in human whole blood: An insight into molecular mechanisms

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Abstract

Clinical studies have demonstrated an impairment of glucocorticoid receptor (GR)-mediated negative feedback on the hypothalamic–pituitary–adrenal (HPA) axis in patients with major depression (GR resistance), and its resolution by antidepressant treatment. Recently, we showed that this impairment is indeed due to a dysfunction of GR in depressed patients (Carvalho et al., 2009), and that the ability of the antidepressant clomipramine to decrease GR function in peripheral blood cells is impaired in patients with major depression who are clinically resistant to treatment (Carvalho et al. 2008). To further investigate the effect of antidepressants on GR function in humans, we have compared the effect of the antidepressants clomipramine, amitriptyline, sertraline, paroxetine and venlafaxine, and of the antipsychotics, haloperidol and risperidone, on GR function in peripheral blood cells from healthy volunteers ($n=33$). GR function was measured by glucocorticoid inhibition of lipopolysaccharide (LPS)-stimulated interleukin-6 (IL-6) levels. Compared to vehicle-treated cells, all antidepressants inhibited dexamethasone (DEX, 10–100 nM) inhibition of LPS-stimulated IL-6 levels (p values ranging from 0.007 to 0.1). This effect was specific to antidepressants, as antipsychotics had no effect on DEX-inhibition of LPS-stimulated IL-6 levels. The phosphodiesterase (PDE) type 4 inhibitor, rolipram, potentiated the effect of antidepressants on GR function, while the GR antagonist, RU-486, inhibited the effect of antidepressants on GR function. These findings indicate that the effect of antidepressants on GR function are specific for this class of psychotropic drugs, and involve second messenger pathways relevant to GR function and inflammation. Furthermore, it also points towards a possible mechanism by which one may be able to overcome treatment-resistant depression. Research in this field will lead to new insights into the pathophysiology and treatment of affective disorders.

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Contributors

Carvalho LA planned and conducted the experiments, analyzed the data and wrote the paper. Garner conducted the experiments and analyzed the data on antipsychotics. Dew conducted all the IL-6 ELISA measurements. Fazakerley conducted the experiments on paroxetine and antipsychotics. Pariante revised the paper before submission.

Conflict of interest

The authors have no relevant financial interest to disclose.

Keywords

Hypothalamic–pituitary–adrenal axis; Mood disorders; cAMP; Corticosteroids; Corticosteroid sensitivity; Protein-kinase A; Schizophrenia; Neuroinflammation; TNF

1. Introduction

Major depression is often associated with alterations in hypothalamic–pituitary–adrenal (HPA) regulation, including increased plasma cortisol levels, and enlarged anterior pituitary and adrenals (Carvalho and Pariante, 2008; Gold et al. 1988; Pariante et al. 2008). A dysregulation of the HPA axis in patients with major depression is seen after the dexamethasone suppression test (DST) (Pariante 2004) and the DEX/corticotrophin releasing hormone (CRH) (Holsboer 2000; Nemeroff 1996) test, indicating in depressed patients a relative impairment of glucocorticoid receptor (GR)-mediated negative feedback (glucocorticoid resistance). In further support to the notion of relative glucocorticoid resistance in depressed patients is the fact that the increased cortisol levels are not accompanied by physical signs of Cushing's Syndrome (Holsboer et al. 1992; Murphy 1991). Glucocorticoid resistance has also been described in the peripheral blood immune cells from depressed patients (Holsboer 2000; Pariante et al., 2001). Peripheral glucocorticoid resistance might lead to the decreased immunosuppressive effect of glucocorticoids and the increased levels of inflammation seen in these patients (Carvalho et al., 2008; Dantzer et al., 2008; Pariante et al. 2001). Interestingly, some studies indicate effective antidepressant treatment is associated with resolution of the HPA axis negative feedback disturbance (Linkowski et al. 1987)(Aihara et al. 2007; Binder et al. 2004; Hennings et al. 2009; Ising et al. 2005). In accordance, we have shown that citalopram increases HPA axis negative feedback by glucocorticoids (measured as cortisol suppression by prednisolone) after as little as four days of administration in healthy subjects (Pariante et al. 2004).

Recent studies show that antidepressants may reverse glucocorticoid receptor changes in depression by direct effects on the GR (Holsboer 2000). In laboratory animals, antidepressants increase GR receptor expression and increase negative feedback on the HPA axis (Carvalho and Pariante, 2008). Our own work over the last few years, in cellular and animal models, has contributed to elucidate the mechanisms by which antidepressants directly influence GR function. First, we demonstrated that antidepressants increase GR translocation in fibroblasts (Pariante et al. 1997), a finding later replicated by Funato et al. (2006), Heiske et al. (2003) and Okuyama-Tamura et al. (2003). Second, we showed that this GR translocation is associated with GR downregulation (Pariante et al. 2003a). Third, we described that antidepressants increase the intracellular concentrations of some glucocorticoids by inhibiting membrane transporters that actively expel glucocorticoid from cells, indirectly compensating for the GR downregulation and ultimately increasing GR function in cells where these transporters are present (Pariante et al. 2001; Pariante et al. 2003a; Mason and Pariante, 2006). In contrast, in the absence of functional membrane transporters, or in the presence of glucocorticoids that are not substrates for these transporters, the antidepressants-induced GR activation and downregulation leads to a reduced GR function (Carvalho and Pariante, 2008; Mason and Pariante, 2006; Pariante et al. 1997; Pariante et al., 2001). Again, these findings have been later replicated by Augustyn et al. (2005), Budziszewska (2002) and Budziszewska et al. (2005). Fourth, we have recently found that the antidepressant clomipramine decreases GR function in human peripheral blood cells – that is, in cells that do not express functional glucocorticoid transporters (Park et al., 2003). These findings are consistent with the notion that antidepressants induce GR translocation, GR downregulation, and hence reduce GR function

in cells that do not express membrane transporters. Finally, we have recently found that the ability of clomipramine to decrease GR function in peripheral blood cells is impaired in patients with major depression who are clinically resistant to treatment, thus suggesting that the ability of antidepressants to regulate GR function is related to their therapeutic action (Carvalho et al., 2008). These patients also had reduced GR function in peripheral blood cells (Carvalho et al. 2009) and increased levels of interleukin-6 possibly reflecting a pro-inflammatory state of monocytes (Carvalho et al., 2008). Taking together we hypothesize antidepressants correct the glucocorticoid resistance and pro-inflammatory state of monocytes in major depressive disorders. Clinical non-responsiveness to antidepressants is possibly reflected by an inability of antidepressants to exert these corrective functions.

As mentioned above, we have used clomipramine in our previous work in human peripheral blood cells, a tricyclic antidepressant with predominantly serotonergic, but also noradrenergic, reuptake inhibition ability (Carvalho et al., 2008). To further understand the effect of antidepressants on GR function in humans, we have evaluated the effect of other antidepressants with different mechanisms of action on GR function in peripheral whole blood cells of healthy volunteers, and compared to those of clomipramine. Moreover, we have investigated whether the GR regulation is specific to antidepressants by comparing them to antipsychotics. Finally, we have examined a possible molecular mechanisms underlying these effects, with particular reference to a pathway that has been described as relevant for GR regulation by antidepressants and by inflammation, cAMP (Chen and Rasenick, 1995a, 1995b; Nestler et al. 1989; Nibuya et al. 1996).

2. Experimental procedures

The study protocol was approved by the Research Ethics Committee of the Institute of Psychiatry, King's College London and Maudsley Hospital (London). All subjects gave their written and informed consent.

2.1. Healthy subjects

Thirty three healthy subjects (18 female, 15 male) participated in this study. They were recruited through department staff, students and members of the local community. Subjects had (average $\pm$ SEM) age of 33.3 ± 2.3 years and BMI of 22.9 ± 0.9 . Subjects were recruited using Mindsearch database (<http://www.mindsearch.iop.kcl.ac.uk/>). Mindsearch is a service which connects academic researchers at the Institute of Psychiatry (IoP) with healthy volunteers from the general public to participate in their research projects. Mindsearch contains lists of healthy volunteers who have previously participated in research studies at King's College London, UK. Complete history was taken before the study as assessed by open interview. Subjects with psychiatric, immune or endocrine disorders were excluded from the study. Healthy subjects were free from acute infections or allergic reactions, as well as from any psychotropic medication or drugs known to modify immune and endocrine functions for at least one month before blood sampling. We have successfully used this protocol before (Carvalho et al., 2008) although none of the subjects in the present study had participated in our previous work.

2.2. Blood collection

Subjects abstained from food, caffeine, tea, alcohol and cigarettes during the night before the study. On the study day, subjects were admitted to a research suite and blood was collected at 10:00 AM (± 30 min). A heparinised vacutainer tube was collected and used immediately as described below for glucocorticoid function assay.

2.3. Glucocorticoid function assay reagents and drugs

PBS Saline Gibco, 500 mL, ref. 2012-019, sterile, Invitrogen; RPMI-1640 Medium Sigma, 500 mL, sterile, R8758; Dexamethasone Sigma, D4902; Clomipramine Sigma, C7291; Penicillin/Streptomycin Sigma, 500 mL, sterile, P4458; Lypopolysaccharide (LPS) Gibco, cat. 20012-019, Lot L-2880, Amitriptyline Sigma A8404, Rolipram Sigma R6520-10MG, RU-486 Sigma M8046-100MG, Sertraline Sigma S6319, Venlafaxine Sigma V7264-10MG, Paroxetine Sigma P9623-10MG, Haloperidol Sigma H1512-5G, Risperidone MP Biomedicals Europe 0219371410.

2.4. Glucocorticoid function assay protocol

The protocol has been previously published (Carvalho et al., 2008), and it is based on the work by Rohleder et al. (2001), (2003). Five different antidepressants were used in the experiments: two tricyclics (clomipramine and amitriptyline), two selective serotonin reuptake inhibitors (SSRI; sertraline and paroxetine), and one serotonin and noradrenaline reuptake inhibitor (SNRI; venlafaxine). Furthermore, two antipsychotics (haloperidol and risperidone) were also tested.

Glucocorticoid function was measured by dexamethasone (DEX) inhibition of LPS-stimulated IL-6 levels. Whole blood was diluted tenfold with RPMI-0640 medium. All solutions were prepared in pyrogene-free sterile saline (NaCl 0.9%) in order to achieve final concentrations in the cultures of: 20 ng/mg for LPS; 10 μ M for all antidepressants, antipsychotics, and for rolipram; 10 nM and 100 nM for DEX; 40 μ M for RU-486. A total of 510 μ L of diluted blood (in RPMI-1640 medium with L-glutamine supplemented with 100 IU/mL penicillin and 100 mg/mL streptomycin) was added onto 48-well cell culture plates (Falcon, No 3078). LPS, antidepressants and DEX, or other drugs, were subsequently added to the wells. Samples were incubated for 24 h in a humidified atmosphere containing 5% CO₂. After the incubation, plates were centrifuged (1000 g, 10min, 4°C) and the supernatant carefully collected and kept at -20 °C until analysis. Determinations of IL-6 in the supernatant were performed in duplicates and always by the same researcher.

The concentration of DEX used in this study was based on the average IC₅₀=4.5 nM found in this study. This value is within the normal values of cortisol found in the plasma of healthy subjects from 11 AM to midnight (Conceptual Framework of Adrenal Stress Index, 2010). It is of note that this concentration of antidepressants and antipsychotics is somewhat in the upper range of the values found in the plasma. However, brain concentrations of tricyclics in humans – largely derived from post-mortem studies after overdose, have described brain-to-plasma concentration ratios ranging from 8 to 125 fold at lower or higher plasma concentrations respectively (Avella et al. 2004; Sunshine and Baeumler, 1963). Therefore, considering that the plasma concentrations of tricyclics in patients taking therapeutic doses range 100–250 ng/mL (that is, approximately 0.3–0.8 micromolar for clomipramine), even a conservative estimate of a brain-to-plasma concentration of 10 fold would lead to micromolar concentrations of antidepressants in the brain of patients.

2.5. Sample measurements

IL-6 analysis was carried out using a commercially available ELISA kit (Quantikine Adiponectin ELISA kit distributed by R & D Systems Europe). The coefficient of variation (CV) for IL-6 analysis was 4.7% within run and 6.5% in between runs, and the detection limit was 0.7 pg/mL.

2.6. Statistical analysis and data presentation

Data were expressed as mean $\pm$ standard error of the mean (SEM). We used Student's *t*-test or one-way analysis of variance (ANOVA) followed by Dunnet Comparison to examine

differences between two or more groups respectively and as appropriate. In each experiment data was compared to its appropriate control.

LPS-stimulated levels are expressed as mean $\pm$ SEM of raw IL-6 levels. Glucocorticoid (GC) suppression was calculated by normalising all data to LPS-stimulated IL-6 levels in the absence of glucocorticoids expressed as 100%. Specifically, the calculation of the percentage inhibition in glucocorticoids condition was as follows:

$$\text{IL - 6 levels \% (GConly)} = \frac{\text{mean rawIL - 6 levels (GC)}}{\text{mean rawIL - 6 levels (0nMGC)}} \times 100.$$

In the presence of the antidepressants (AD) or antipsychotics, the calculation of glucocorticoid inhibition was corrected by the presence of clomipramine as follows:

$$\text{IL - 6 levels \% (GC+AD)} = \frac{\text{mean rawIL - 6 levels (GC)}}{\text{mean rawIL - 6 levels (0nMGC+AD)}} \times 100.$$

The significance level was set to $p < 0.05$ (two-tailed) and computer statistical packages (Graph-Pad 4.1 Software Inc., San Diego, California, USA and SPSS 15.0 Inc. Software, Chicago, Illinois, USA) were used for this study.

3. Results

In the absence of LPS (unstimulated), cells showed IL-6 levels values in the lower limit of detection or undetectable. As expected, LPS stimulated a large increase of IL-6 production (around 2500 fold-induction). Tricyclic antidepressants like clomipramine and amitriptyline inhibited LPS-stimulated IL-6 levels when compared to vehicle-treated cells in the absence of glucocorticoids (Fig. 1). On the other hand, this effect was not shown in the presence of sertraline, venlafaxine or paroxetine ($F_{(1,3)}=0.093$, $p=0.91$, Fig. 2).

The synthetic glucocorticoid DEX (1–1000 nM) suppressed in a concentration dependent manner LPS-stimulated IL-6 levels (Fig. 3). The IC_{50} of DEX suppression of LPS-stimulated IL-6 levels was 4.5 nM.

In order to investigate the effect of antidepressants on GR function, we evaluated the effect of antidepressants on DEX suppression of LPS-stimulated IL-6 levels. As shown in Fig. 4, tricyclic antidepressants – clomipramine and amitriptyline – decreased DEX suppression of LPS-stimulated IL-6 levels (DEX 100 nM and clomipramine, $p=0.04$; DEX 10 nM and clomipramine, $p=0.03$; DEX 10 nM and amitriptyline, $p=0.02$). Thus, tricyclic antidepressants decreased GR function in human whole blood.

To examine whether this effect could also be seen with non-tricyclic antidepressants, we tested two selective serotonin reuptake inhibitors – sertraline and paroxetine – and one dual serotonin and noradrenaline reuptake inhibitor venlafaxine. As shown in Fig. 5, sertraline, venlafaxine and paroxetine, as the tricyclics above, decreased glucocorticoid function, that is, decreased DEX suppression of LPS-stimulated IL-6 levels (DEX 100 nM and sertraline, $p=0.02$; DEX 10 nM and sertraline, $p=0.007$; DEX 10 nM and venlafaxine, $p=0.05$). Paroxetine had a similar effect of decreasing DEX inhibition of LPS-stimulated IL-6 levels, but the results reached statistical significance only for the highest concentration of dexamethasone (DEX 100 nM and paroxetine, $p=0.01$) and only trend significance for the lowest concentration (DEX 10 nM and paroxetine, $p=0.075$).

To examine whether this effect was specific to antidepressants, we then investigated the effect of two antipsychotics on GR function. The typical antipsychotic, haloperidol, and the atypical, risperidone, were used. In the absence of glucocorticoids antipsychotics did not change LPS-stimulated IL-6 levels when compared to control-treated cells (LPS alone 2381 ± 372 , risperidone 2889 ± 342 , haloperidol 3199 ± 361 , $F_{(1,3)}=1.3$, $p=0.28$). In the presence of dexamethasone (10–100 nM), haloperidol and risperidone did not show an effect on the DEX suppression of LPS-stimulated IL-6 levels (DEX 100 nM and risperidone, $p=0.19$; DEX 10 nM and risperidone, $p=0.60$; DEX 100 nM and haloperidol, $p=0.31$; DEX 10 nM and haloperidol, $p=0.91$, Fig. 6).

To examine receptor specificity of the effects of antidepressants on DEX suppression of LPS-stimulated IL-6, the effect of clomipramine plus DEX was measured in the presence and absence of the glucocorticoid receptor antagonist RU-486 (40 μ M). RU-486 in the absence of glucocorticoids did not inhibit LPS-stimulated IL-6 levels (LPS 1819 ± 87 , LPS + RU-486 1768 ± 197 , $p=0.68$). In the presence of RU-486, clomipramine still inhibited LPS-stimulated IL-6 levels (LPS+clomipramine 1684 ± 169 , LPS+clomipramine+RU-486 1461 ± 189 , $p=0.09$). As shown in Fig. 7, drug treatment (clomipramine and RU-486) had a significant main effect on the DEX inhibition of LPS-stimulated IL-6 levels ($F_{1,4}=39.51$, $p=0.0001$). RU-486 significantly reduced the effects of DEX suppression of LPS-stimulated IL-6 levels (DEX 34.42 ± 3.58 , DEX + RU-486 101.9 ± 4.49 , $p=0.0001$). Relevant to the role of clomipramine-mediated IL-6 effects, in the presence of RU-486, clomipramine was unable to modify GR function (DEX+RU-486 101.9 ± 4.49 , DEX+RU-486+clomipramine 96.24 ± 7.75 , $p=0.54$).

Because the effect of antidepressants on GR function in laboratory animals have been shown to involve the cAMP pathway (Miller et al. 2002), we investigated whether antidepressant inhibition of GR function in humans involved activation of cAMP and downstream cAMP dependent protein kinases. The effect of rolipram, a phosphodiesterase (PDE) type 4 inhibitor that hinder cAMP breakdown, was used to investigate the effects of clomipramine on GR. Specifically, whole blood cells were incubated with rolipram (10 μ M) alone or in combination with DEX (10 nM) and clomipramine (10 μ M). As shown in Fig. 8, rolipram in the absence of DEX did not change IL-6 levels. Drug treatment had a significant main effect on the DEX inhibition of LPS-stimulated levels ($F_{1,3}=7.613$, $p=0.001$). Rolipram, in the presence of DEX, inhibited DEX inhibition of LPS-stimulated IL-6 levels (DEX 34.42 ± 3.58 , DEX and rolipram 56.75 ± 10.79 , $p=0.02$). More-over, rolipram had a synergistic effect with clomipramine, leading to further inhibition of GR function (DEX 34.42 ± 3.58 , DEX, rolipram and clomipramine 66.90 ± 8.70 , $p=0.0005$).

4. Discussion

In this study, we show that antidepressants of different mechanisms of action all inhibit glucocorticoid receptor function in whole blood cells of healthy subjects. This effect appears to be specific to antidepressants, as antipsychotics did not have the same effect. Furthermore, we have shown that increasing cAMP by the inhibition of phosphodiesterase with rolipram exert a similar effect as that of antidepressants — that of inhibiting GR function — and together rolipram and clomipramine lead to an even larger inhibition of GR function.

Antidepressants decrease GR function in whole blood of healthy subjects. We have previously described the effects of clomipramine on GR function (Carvalho et al., 2008), and this is a further replication in a new set of subjects. Our study is also in agreement with previous studies showing that antidepressants decrease GR function in cells/conditions associated with lower expression of membrane transporters (Carvalho et al., 2008; Carvalho

and Pariante, 2008; Miller et al. 2002; Pariante et al. 1997; Pariante and Miller, 2001; Yau et al. 2001). Our results are in line with the above mentioned evidence, as peripheral blood cells express low levels of the transporter (Park et al. 2003). Short-term in vitro incubation with antidepressants leads to activation of GR translocation, which in turn leads to acute downregulation of GR expression, which can then be measured functionally as *reduced* GR function (Pariante et al. 1997; Pariante et al. 2003b; Pariante et al. 2003a). Previous work on the effect of antidepressant on GR expression in rats has shown that GR downregulation is transient, and is followed by GR upregulation upon chronic treatment (Lai et al. 2003; Yau et al. 2001). It is thus possible that also in the periphery short-term antidepressant treatment leads to GR down-regulation and reduces GR function. On the other hand, chronic antidepressant treatment may induce GR upregulation and a subsequent increase in GR function. Of note, in cells or conditions associated with high levels of steroid membrane transporters, antidepressants “in vitro” have been shown to increase GR function (Pariante et al. 1997; Pariante et al. 2003b). Thus, the net effect of antidepressants on GR function “in vitro” is likely to be mediated by the balance between intracellular concentration of glucocorticoids, following inhibition by antidepressants of steroid transporters that expel some glucocorticoids from cells, and changes in GR expression (for review please refer to Carvalho and Pariante (2008).

Apart from interacting with membrane steroid transporters, antidepressants may also exert some of their therapeutic efficacy by altering a component of the membrane or cytoskeleton that is associated with lipid rafts (Allen et al. 2007). Lipid rafts are specialized structures on the plasma membrane that have an altered lipid composition as well as links to the cytoskeleton. Although chronic antidepressant treatment does not alter membrane cholesterol content, treatment with chemically distinct antidepressants results in the movement of G α s (stimulatory subunit of Protein G) out of lipid rafts and into a closer association with adenylyl cyclase (Donati and Rasenick, 2005; Menkes et al. 1983; Toki et al. 1999). This might contribute to the increased cAMP tone and synaptic changes that are observed subsequent to chronic antidepressant treatment (Donati and Rasenick, 2003). cAMP/protein kinase A (PKA) signal transduction pathways are reportedly involved in enhancing GR function (Pace et al. 2007). Several studies have demonstrated that PKA agonists, including forskolin and 8-Br-cAMP, can increase GR mRNA stability and GR mRNA levels, and enhance GR transcription and function (Dong et al. 1989; Penuelas et al. 1998).

Indeed, the data herein provides evidence for the involvement of cAMP as a potential molecular mechanism by which antidepressants decrease GR function in whole blood. Rolipram a prototypic phosphodiesterase (PDE4) inhibitor reduces cAMP catabolism (Meyers et al. 2007). The effects of rolipram on GR function is in the same direction and synergistic to that of antidepressants. This data, however, is inconsistent to the literature demonstrating that downstream elements of the cAMP cascade enhance GR function (Edgar et al. 1999; Rangarajan et al. 1992; Thome et al. 2000). Only one other work has evaluated the effect of antidepressants and rolipram on GR function in the same experimental condition (Miller et al., 2002). Interestingly, albeit in the opposite direction to what was found here, they have also found synergistic effect of antidepressants and rolipram in relation to GR function (Miller et al. 2002). As mentioned above, the discrepancy is likely to be due to the antidepressant- and rolipram-induced GR activation leading to different effects on GR expression and thus GR function in different experimental models. Unfortunately the paper by (Miller et al. 2002) did not measure GR expression.

There is now evidence that antidepressants decrease inflammation, and that normalization of components of the innate immune system altered in depressed patients might constitute a prerequisite to accomplish remission (Hernandez et al. 2008; Piletz et al. 2008). Indeed,

antidepressants decrease synthesis and release of the pro-inflammatory cytokine IL-1B (Castanon et al. 2002) and attenuates LPS-induced cFOS expression (Castanon et al. 2003). Furthermore, cyclooxygenase 2 (COX-2) has shown therapeutic benefits in major depression (Muller et al. 2006). Consistent with this, we have shown that treatment-resistant depressed patients have increased inflammation as shown by elevated plasma interleukin-6 levels in the context of hypercortisolemia and glucocorticoid resistance in peripheral blood cells (Carvalho et al., 2008; Carvalho and Pariante, 2008). Moreover, we also show a lack of effect of clomipramine on GR function in vitro (Carvalho et al. 2009). Based on the capacity of antidepressants to modulate GR function and inflammation, one might wonder whether antidepressants' effect on inflammation is in part via GR. A few studies support this possibility. First, the GR antagonist RU-486 reverses the inhibitory effect of rolipram on TNF-alpha induction (Laemontetal. 1999). Second, metyrapone — a glucocorticoid synthesis inhibitor attenuated rolipram-induced inhibition of eosinophilia (Kung et al. 2000). These results are strengthened by others that show complementary effect of glucocorticoid and antidepressants on immunologic effects like decreasing cytokine tumor necrosis factor alpha release (Klemm et al. 1995; Roumestan et al. 2007; Taler et al. 2007). Our study, however, did not support this possibility. The GR antagonist RU-486 did not inhibit LPS-stimulated levels in the absence of DEX, nor reversed the clomipramine inhibition of LPS-stimulated IL-6 levels.

In conclusion, our data shows that antidepressants modulate GR function in human blood cells, via glucocorticoid receptors and possibly involving cAMP signalling transduction pathway. Our study further indicates that indeed GR function might be involved in the therapeutic action of antidepressants. In vitro studies in humans are particularly relevant to understand the molecular mechanisms underlying GR abnormalities and its regulation by antidepressant treatment.

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References

- Aihara M, Ida I, Yuuki N, Oshima A, Kumano H, Takahashi K, Fukuda M, Oriuchi N, Endo K, Matsuda H, Mikuni M. HPA axis dysfunction in unmedicated major depressive disorder and its normalization by pharmacotherapy correlates with alteration of neural activity in prefrontal cortex and limbic/paralimbic regions. *Psychiatry Res.* 2007; 155:245–256. [PubMed: 17587554]
- Allen JA, Halverson-Tamboli RA, Rasenick MM. Lipid raft microdomains and neurotransmitter signalling. *Nat. Rev. Neurosci.* 2007; 8:128–140. [PubMed: 17195035]
- Augustyn M, Otczyk M, Budziszewska B, Jagla G, Nowak W, Basta-Kaim A, Jaworska-Feil L, Kubera M, Tetich M, Leskiewicz M, Lason W. Effects of some new antidepressant drugs on the glucocorticoid receptor-mediated gene transcription in fibroblast cells. *Pharmacol. Rep.* 2005; 57:766–773. [PubMed: 16382195]
- Avella J, Lehrer M, Katz M, Minden E. Two cases involving clomipramine intoxication. *J. Anal. Toxicol.* 2004; 28:504–508. [PubMed: 15516304]

- Binder EB, Salyakina D, Lichtner P, Wozniak GM, Ising M, Putz B, Papiol S, Seaman S, Lucae S, Kohli MA, Nickel T, Kunzel HE, Fuchs B, Majer M, Pfennig A, Kern N, Brunner J, Modell S, Baghai T, Deiml T, Zill P, Bondy B, Rupprecht R, Messer T, Kohnlein O, Dabitz H, Bruckl T, Muller N, Pfister H, Lieb R, Mueller JC, Lohmussaar E, Strom TM, Bettecken T, Meitinger T, Uhr M, Rein T, Holsboer F, Muller-Myhsok B. Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. *Nat. Genet.* 2004; 36:1319–1325. [PubMed: 15565110]
- Budziszewska B. Effect of antidepressant drugs on the hypothalamic–pituitary–adrenal axis activity and glucocorticoid receptor function. *Pol. J. Pharmacol.* 2002; 54:343–349. [PubMed: 12523487]
- Budziszewska B, Basta-Kaim A, Kubera M, Jaworska L, Leskiewicz M, Tetich M, Otczyk M, Zajicova A, Holan V, Lason W. Effect of lipopolysaccharide and antidepressant drugs on glucocorticoid receptor-mediated gene transcription. *Pharmacol. Rep.* 2005; 57:540–544. [PubMed: 16129923]
- Carvalho LA, Pariante CM. In vitro modulation of the glucocorticoid receptor by antidepressants. *Stress.* 2008; 11:411–424. [PubMed: 19065455]
- Carvalho LA, Juruena MF, Papadopoulos A, Poon L, Kerwin RW, Cleare A, Pariante CM. Clomipramine in vitro reduces glucocorticoid receptor function in healthy subjects but not in patients with major depression. *Neuropsychopharmacology.* 2008; 33:3182–3189. [PubMed: 18368033]
- Carvalho LA, Juruena MF, Papadopoulos A, Poon L, Cleare A, Pariante CM. Clomipramine and glucocorticoid receptor function. *Neuropsychopharmacology.* 2009; 34:2194–2195.
- Castanon N, Konsman JP, Medina C, Chauvet N, Dantzer R. Chronic treatment with the antidepressant tianeptine attenuates lipopolysaccharide-induced Fos expression in the rat paraventricular nucleus and HPA axis activation. *Psychoneuroendocrinology.* 2003; 28:19–34. [PubMed: 12445834]
- Castanon N, Leonard BE, Neveu PJ, Yirmiya R. Effects of antidepressants on cytokine production and actions. *Brain Behav. Immun.* 2002; 16:569–574. [PubMed: 12401470]
- Chen J, Rasenick MM. Chronic antidepressant treatment facilitates G protein activation of adenylyl cyclase without altering G protein content. *J. Pharmacol. Exp. Ther.* 1995a; 275:509–517. [PubMed: 7562593]
- Chen J, Rasenick MM. Chronic treatment of C6 glioma cells with antidepressant drugs increases functional coupling between a G protein (Gs) and adenylyl cyclase. *J. Neurochem.* 1995b; 64:724–732. [PubMed: 7830066]
- Conceptual Framework of Adrenal Stress Index. 2010Index. Diagnos-Techs Web site. <http://www.diagnostechs.com/main.htm>
- Dantzer R, O'Connor JC, Freund JJ, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat. Rev. Neurosci.* 2008; 9:46–57. [PubMed: 18073775]
- Donati RJ, Rasenick MM. G protein signaling and the molecular basis of antidepressant action. *Life Sci.* 2003; 73:1–17. [PubMed: 12726882]
- Donati RJ, Rasenick MM. Chronic antidepressant treatment prevents accumulation of α 5 in cholesterol-rich, cytoskeletal-associated, plasma membrane domains (lipid rafts). *Neuropsychopharmacology.* 2005; 30:1238–1245. [PubMed: 15726116]
- Dong Y, Aronsson M, Gustafsson JA, Okret S. The mechanism of cAMP-induced glucocorticoid receptor expression. Correlation to cellular glucocorticoid response. *J. Biol. Chem.* 1989; 264:13679–13683. [PubMed: 2547771]
- Edgar VA, Sterin-Borda L, Cremaschi GA, Genaro AM. Role of protein kinase C and cAMP in fluoxetine effects on human T-cell proliferation. *Eur. J. Pharmacol.* 1999; 372:65–73. [PubMed: 10374716]
- Funato H, Kobayashi A, Watanabe Y. Differential effects of antidepressants on dexamethasone-induced nuclear translocation and expression of glucocorticoid receptor. *Brain Res.* 2006; 1117:125–134. [PubMed: 16956592]
- Gold PW, Goodwin FK, Chrousos GP. Clinical and biochemical manifestations of depression. Relation to the neurobiology of stress (1). *N. Engl. J. Med.* 1988; 319:348–353. [PubMed: 3292920]

- Heiske A, Jesberg J, Krieg JC, Vedder H. Differential effects of antidepressants on glucocorticoid receptors in human primary blood cells and human monocytic u-937 cells. *Neuropsychopharmacology*. 2003; 28:807–817. [PubMed: 12655328]
- Hennings JM, Owashi T, Binder EB, Horstmann S, Menke A, Kloiber S, Dose T, Wollweber B, Spieler D, Messer T, Lutz R, Kunzel H, Bierner T, Pollmacher T, Pfister H, Nickel T, Sonntag A, Uhr M, Ising M, Holsboer F, Lucae S. Clinical characteristics and treatment outcome in a representative sample of depressed inpatients – findings from the Munich Antidepressant Response Signature (MARS) project. *J. Psychiatr. Res.* 2009; 43:215–229. [PubMed: 18586274]
- Hernandez ME, Mendieta D, Martinez-Fong D, Loria F, Moreno J, Estrada I, Bojalil R, Pavon L. Variations in circulating cytokine levels during 52 week course of treatment with SSRI for major depressive disorder. *Eur. Neuropsychopharmacol.* 2008; 18:917–924. [PubMed: 18805677]
- Holsboer F. The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology*. 2000; 23:477–501. [PubMed: 11027914]
- Holsboer F, Spengler D, Heuser I. The role of corticotropin-releasing hormone in the pathogenesis of Cushing's disease, anorexia nervosa, alcoholism, affective disorders and dementia. *Prog. Brain Res.* 1992; 93:385–417. [PubMed: 1336204]
- Ising M, Kunzel HE, Binder EB, Nickel T, Modell S, Holsboer F. The combined dexamethasone/CRH test as a potential surrogate marker in depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry*. 2005; 29:1085–1093. [PubMed: 15950349]
- Klemm P, Harris HJ, Perretti M. Effect of rolipram in a murine model of acute inflammation: comparison with the corticoid dexamethasone. *Eur. J. Pharmacol.* 1995; 281:69–74. [PubMed: 8566119]
- Kung TT, Crawley Y, Luo B, Young S, Kreutner W, Chapman RW. Inhibition of pulmonary eosinophilia and airway hyperresponsiveness in allergic mice by rolipram: involvement of endogenously released corticosterone and catecholamines. *Br. J. Pharmacol.* 2000; 130:457–463. [PubMed: 10807686]
- Laemont KD, Schaefer CJ, Juneau PL, Schrier DJ. Effects of the phosphodiesterase inhibitor rolipram on streptococcal cell wall-induced arthritis in rats. *Int. J. Immunopharmacol.* 1999; 21:711–725. [PubMed: 10576617]
- Lai M, McCormick JA, Chapman KE, Kelly PAT, Seckl JR, Yau JLW. Differential regulation of corticosteroid receptors by monoamine neurotransmitters and antidepressant drugs in primary hippocampal culture. *Neuroscience*. 2003; 118:975–984. [PubMed: 12732243]
- Linkowski P, Mendlewicz J, Kerkhofs M, Leclercq R, Golstein J, Brasseur M, Copinschi G, Van Cauter E. 24-hour profiles of adrenocorticotropin, cortisol, and growth hormone in major depressive illness: effect of antidepressant treatment. *J. Clin. Endocrinol. Metab.* 1987; 65:141–152. [PubMed: 3034952]
- Mason BL, Pariante CM. The effects of antidepressants on the hypothalamic–pituitary–adrenal axis. *Drug News Perspect.* 2006; 19:603–608. [PubMed: 17299602]
- Menkes DB, Rasenick MM, Wheeler MA, Bitensky MW. Guanosine triphosphate activation of brain adenylate cyclase: enhancement by long-term antidepressant treatment. *Science*. 1983; 219:65–67. [PubMed: 6849117]
- Meyers JA, Taverna J, Chaves J, Makkinje A, Lerner A. Phosphodiesterase 4 inhibitors augment levels of glucocorticoid receptor in B cell chronic lymphocytic leukemia but not in normal circulating hematopoietic cells. *Clin. Cancer Res.* 2007; 13:4920–4927. [PubMed: 17699872]
- Miller AH, Vogt GJ, Pearce BD. The phosphodiesterase type 4 inhibitor, rolipram, enhances glucocorticoid receptor function. *Neuropsychopharmacology*. 2002; 27:939–948. [PubMed: 12464451]
- Muller N, Schwarz MJ, Dehning S, Douhe A, Cerovecki A, Goldstein-Muller B, Spellmann I, Hetzel G, Maino K, Kleindienst N, Moller HJ, Arolt V, Riedel M. The cyclooxygenase-2 inhibitor celecoxib has therapeutic effects in major depression: results of a double-blind, randomized, placebo controlled, add-on pilot study to reboxetine. *Mol. Psychiatry*. 2006; 11:680–684. [PubMed: 16491133]
- Murphy BE. Steroids and depression. *J. Steroid Biochem. Mol. Biol.* 1991; 38:537–559. [PubMed: 1645586]

- Nemeroff CB. The corticotropin-releasing factor (CRF) hypothesis of depression: new findings and new directions. *Mol. Psychiatry*. 1996; 1:336–342. [PubMed: 9118360]
- Nestler EJ, Terwilliger RZ, Duman RS. Chronic antidepressant administration alters the subcellular distribution of cyclic AMP-dependent protein kinase in rat frontal cortex. *J. Neurochem*. 1989; 53:1644–1647. [PubMed: 2795022]
- Nibuya M, Nestler EJ, Duman RS. Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. *J. Neurosci*. 1996; 16:2365–2372. [PubMed: 8601816]
- Okuyama-Tamura M, Mikuni M, Kojima I. Modulation of the human glucocorticoid receptor function by antidepressive compounds. *Neurosci. Lett*. 2003; 342:206–210. [PubMed: 12757900]
- Pace TW, Hu F, Miller AH. Cytokine-effects on glucocorticoid receptor function: relevance to glucocorticoid resistance and the pathophysiology and treatment of major depression. *Brain Behav. Immun*. 2007; 21:9–19. [PubMed: 17070667]
- Pariante CM. Glucocorticoid receptor function in vitro in patients with major depression. *Stress*. 2004; 7:209–219. [PubMed: 16019586]
- Pariante CM, Miller AH. Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. *Biol. Psychiatry*. 2001; 49:391–404. [PubMed: 11274650]
- Pariante CM, Pearce BD, Pisell TL, Owens MJ, Miller AH. Steroid-independent translocation of the glucocorticoid receptor by the antidepressant desipramine. *Mol. Pharmacol*. 1997; 52:571–581. [PubMed: 9380019]
- Pariante CM, Makoff A, Lovestone S, Feroli S, Heyden A, Miller AH, Kerwin RW. Antidepressants enhance glucocorticoid receptor function in vitro by modulating the membrane steroid transporters. *Br. J. Pharmacol*. 2001; 134:1335–1343. [PubMed: 11704655]
- Pariante CM, Hye A, Williamson R, Makoff A, Lovestone S, Kerwin RW. The antidepressant clomipramine regulates cortisol intracellular concentrations and glucocorticoid receptor expression in fibroblasts and rat primary neurones. *Neuropsychopharmacology*. 2003a; 28:1553–1561. [PubMed: 12784111]
- Pariante CM, Kim RB, Makoff A, Kerwin RW. Antidepressant fluoxetine enhances glucocorticoid receptor function in vitro by modulating membrane steroid transporters. *Br. J. Pharmacol*. 2003b; 139:1111–1118. [PubMed: 12871829]
- Pariante CM, Papadopoulos AS, Poon L, Cleare AJ, Checkley SA, English J, Kerwin RW, Lightman S. Four days of citalopram increase suppression of cortisol secretion by prednisolone in healthy volunteers. *Psychopharmacology (Berl)*. 2004; 177:200–206. [PubMed: 15179544]
- Park SW, Lomri N, Simeoni LA, Fruehauf JP, Mechetner E. Analysis of P-glycoprotein-mediated membrane transport in human peripheral blood lymphocytes using the UIC2 shift assay. *Cytometry A*. 2003; 53:67–78. [PubMed: 12766968]
- Penuelas I, Encio IJ, Lopez-Moratalla N, Santiago E. cAMP activates transcription of the human glucocorticoid receptor gene promoter. *J. Steroid Biochem. Mol. Biol*. 1998; 67:89–94. [PubMed: 9877208]
- Piletz JE, Halaris A, Iqbal O, Hoppensteadt D, Fareed J, Zhu H, Sinacore J, Lindsay DC. Pro-inflammatory biomarkers in depression: treatment with venlafaxine. *World J. Biol. Psychiatry*. 2008; 1–11. [PubMed: 19117270]
- Rangarajan PN, Umesono K, Evans RM. Modulation of glucocorticoid receptor function by protein kinase A. *Mol. Endocrinol*. 1992; 6:1451–1457. [PubMed: 1435789]
- Rohleder N, Schommer NC, Hellhammer DH, Engel R, Kirschbaum C. Sex differences in glucocorticoid sensitivity of proinflammatory cytokine production after psychosocial stress. *Psychosom. Med*. 2001; 63:966–972. [PubMed: 11719636]
- Rohleder N, Wolf JM, Kirschbaum C. Glucocorticoid sensitivity in humans-interindividual differences and acute stress effects. *Stress*. 2003; 6:207–222. [PubMed: 13129814]
- Roumestan C, Michel A, Bichon F, Portet K, Detoc M, Henriquet C, Jaffuel D, Mathieu M. Anti-inflammatory properties of desipramine and fluoxetine. *Respir. Res*. 2007; 8:35. [PubMed: 17477857]
- Sunshine I, Baeumler J. A fatal case of poisoning with amitriptyline. *Nature*. 1963; 199:1103–1104. [PubMed: 14066956]

- Taler M, Gil-Ad I, Lomnitski L, Korov I, Baharav E, Bar M, Zolokov A, Weizman A. Immunomodulatory effect of selective serotonin reuptake inhibitors (SSRIs) on human T lymphocyte function and gene expression. *Eur. Neuropsychopharmacol.* 2007; 17:774–780. [PubMed: 17499975]
- Thome J, Sakai N, Shin K, Steffen C, Zhang YJ, Impey S, Storm D, Duman RS. cAMP response element-mediated gene transcription is upregulated by chronic antidepressant treatment. *J. Neurosci.* 2000; 20:4030–4036. [PubMed: 10818138]
- Toki S, Donati RJ, Rasenick MM. Treatment of C6 glioma cells and rats with antidepressant drugs increases the detergent extraction of G(s alpha) from plasma membrane. *J. Neurochem.* 1999; 73:1114–1120. [PubMed: 10461902]
- Yau JL, Noble J, Hibberd C, Seckl JR. Short-term administration of fluoxetine and venlafaxine decreases corticosteroid receptor mRNA expression in the rat hippocampus. *Neurosci. Lett.* 2001; 306:161–164. [PubMed: 11406320]

Figure 1. Tricyclic antidepressants clomipramine ($n=16$) and Amitryptiline ($n=8$) inhibited LPS-stimulated IL-6 levels. Results are expressed by MEAN $\pm$ SEM of IL-6 levels (ng/mL). * $p<0.05$.

Figure 2. SSRI/SNRI sertraline ($n=10$, venlafaxine $n=9$, and paroxetine $n=14$) do not inhibit LPS-stimulated IL-6 levels. Results are expressed by MEAN $\pm$ SEM of IL-6 levels (ng/mL). * $p<0.05$.

Figure 3. Dexamethasone (1–1000 nM) suppression of LPS-stimulated IL-6 levels in whole blood from healthy subjects ($n=5-20$) $IC_{50}DEX=4.5nM$.

Figure 4.

Dexamethasone (100 and 10 nM) suppression of LPS-stimulated IL-6 levels in healthy subjects ($n = 9$) with (hashed columns) or without (white columns) clomipramine (10 μM) and amitryptiline (10 μM). Results are expressed by MEAN $\pm$ SEM of the % dexamethasone suppression (LPS-stimulated IL-6 levels with glucocorticoid divided by LPS-stimulated IL-6 levels without glucocorticoids). * $p < 0.05$.

Figure 5.

Dexamethasone (100 and 10 nM) inhibition of LPS-stimulated IL-6 levels in healthy subjects with (hashed columns) or without (white columns) A) Sertraline (10 μ M, $n = 10$), B) Venlafaxine (10 μ M, $n = 9$, and C) Paroxetine (10 μ M=14). Results are expressed by MEAN $\pm$ SEM of the % dexamethasone suppression (LPS-stimulated IL-6 levels with glucocorticoid divided by LPS-stimulated IL-6 levels without glucocorticoids). * $p < 0.05$.

Figure 6.

A) Dexamethasone (100 and 1 nM) inhibition of LPS-stimulated IL-6 levels in whole blood from healthy subjects ($n = 17-19$) with (hashed/dark columns) or without (white columns) haloperidol 10 μ M or risperidone (10 μ M). Results are expressed by MEAN $\pm$ SEM of the % dexamethasone inhibition (LPS-stimulated IL-6 levels with glucocorticoid divided by LPS-stimulated IL-6 levels without dexamethasone).

Figure 7.

A) LPS-stimulated IL-6 levels in whole blood of healthy subjects ($n = 8-14$) in the presence or absence of Dexamethasone (100 nM), RU-486 (40 μ M) and clomipramine 10 μ M. Results are expressed by MEAN $\pm$ SEM of the % glucocorticoid inhibition (LPS-stimulated IL-6 levels with glucocorticoid divided by LPS-stimulated IL-6 levels without glucocorticoids). * $p < 0.05$.

Figure 8.

A) LPS-stimulated IL-6 levels in whole blood of healthy subjects ($n = 5-20$) in the presence or absence of dexamethasone (10 nM), clomipramine 10 μ M or rolipram (10 μ M). Results are expressed by MEAN $\pm$ SEM of the % dexamethasone inhibition (LPS-stimulated IL-6 levels with glucocorticoid divided by LPS-stimulated IL-6 levels without dexamethasone).

* $p < 0.05$.