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The Antidepressant Desipramine Requires the ABCB1 (Mdr1)-Type p-Glycoprotein to Upregulate the Glucocorticoid Receptor in Mice

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The mechanisms by which antidepressants regulate the hypothalamic-pituitary-adrenal (HPA) axis are still unknown. The ABCB1-type multiple drug resistance (MDR) p-glycoprotein (PGP) regulates the HPA axis by limiting the access of glucocorticoids to the brain in mice and humans. Previous work in cell cultures has found that antidepressants enhance glucocorticoid receptor (GR) function *in vitro* by inhibiting MDR PGP, and therefore by increasing the intracellular concentration of glucocorticoids—but this model has never been tested directly in animals. Here, the tricyclic antidepressant, desipramine (20 mg/kg/day, i.p., for seven days), was administered to *abcb1ab* MDR PGP knockout mice (congenic on the FVB/N background strain) and to FVB/N controls. The hippocampal mRNA expression of GR, mineralocorticoid receptor (MR), MDR (Mdr1a) PGP, and 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) were measured, together with plasma corticosterone levels. In FVB/N controls, desipramine induced a significant upregulation of GR mRNA in the CA1 region (+31%; $p=0.045$); in contrast, in *abcb1ab* (−/−) mice, desipramine induced a significant downregulation of GR mRNA in the CA1 region (−45%; $p=0.004$). MR mRNA expression was unaltered. Desipramine decreased corticosterone levels in both FVB/N controls and in *abcb1ab* (−/−) mice, but in *abcb1ab* (−/−) mice the effects were smaller. Specifically, in FVB/N controls (but not in *abcb1ab* (−/−) mice), desipramine reduced corticosterone levels not only compared with saline-treated mice but also compared with the 'physiological' levels of untreated mice (−39%; $p=0.05$). Finally, desipramine reduced Mdr1a mRNA expression across all hippocampus areas (−9 to −23%), but had no effect on 11β-HSD1 mRNA expression. These data support the notion that the MDR PGP is one of the molecular targets through which antidepressants regulate the HPA axis.
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INTRODUCTION

Antidepressants normalize the hormonal stress response in patients with major depression, but the molecular mechanisms underlying this effect are still unknown (Holsboer, 2000; Pariante *et al*, 2004b; Neigh and Nemeroff, 2006; Pariante, 2006). Patients with major depression have a hyperactivity of the main hormonal stress response system, the hypothalamic-pituitary-adrenal (HPA) axis, as shown by increased levels of corticotropin-releasing factor (CRF) in the brain, and of the endogenous glucocorticoid hormone, cortisol, in the plasma (Holsboer, 2000; Pariante *et al*, 2004b; Neigh and Nemeroff, 2006; Pariante, 2006). Moreover, several lines of evidence indicate that these HPA axis abnormalities contribute to the development of the depressive symptoms. First, treatment with antidepressants reduces HPA axis activity, and this reduction is associated with the clinical response in depressed patients (Kunzel *et al*, 2003). Second, genes that regulate the HPA axis also influence the likelihood of developing depression (van West *et al*, 2006). Third, drugs that directly regulate HPA axis activity have therapeutic effects in affective disorders (Nemeroff and Owens, 2002; Young *et al*, 2004; Flores *et al*, 2006). Finally, and of particular relevance to this

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paper, antidepressants can directly regulate the function and the expression of the corticosteroid receptors (Pariante, 2004, 2006).

Antidepressants reduce HPA axis activity by increasing the negative feedback on the HPA axis by the endogenous glucocorticoids, cortisol in humans and corticosterone in rodents (Pariante, 2004, 2006). This feedback is mediated by intracellular corticosteroid receptors in the brain: the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR) (de Kloet *et al*, 1998). Although there is consensus that GR function is reduced in depressed patients (Pariante, 2006), MR function seems to remain intact (Young *et al*, 2003; Juruena *et al*, 2006). Indeed, antidepressants increase GR expression, GR function, and GR nuclear translocation in cellular and animal experimental systems; in turn, this is associated with enhanced negative feedback and thus with reduced HPA axis activity (Pariante, 2004, 2006). Moreover, in humans, we have shown that an increase in GR-mediated negative feedback is already present after as little as 4 days of antidepressant treatment (Pariante *et al*, 2004a). However, considering that the first paper describing this effect in cells was published in 1989 (Pepin *et al*, 1989), and the first papers in animals shortly afterward (Peiffer *et al*, 1991b; Seckl and Fink, 1992), it is perhaps surprising that we do not yet know *how* antidepressants increase GR expression and function. According to the classical model of antidepressant action, these drugs work by increasing the monoaminergic neurotransmission in the brain (Nemeroff and Owens, 2002), and in fact there is a direct cross talk between monoaminergic neurotransmission, glucocorticoid hormones, and corticosteroid receptors (Yau *et al*, 1997; Lai *et al*, 2003). However, the effects of antidepressants on GR can occur independently from this mechanism. For example, desipramine, a tricyclic antidepressant that increases noradrenaline neurotransmission, induces GR upregulation in rats even following neurotoxic lesioning of noradrenergic neurons with DSP4 (Rossby *et al*, 1995). Moreover, antidepressant-induced GR upregulation in cell cultures is not blocked by antagonists of α or β adrenergic receptors, or of 5HT1a or 5HT2 serotonergic receptors (Okugawa *et al*, 1999; Lai *et al*, 2003).

Recently, we have described in cell cultures that antidepressants control GR function by increasing the intracellular concentration of glucocorticoids (Pariante *et al*, 2001, 2003a, b, 2004b). Glucocorticoids are excreted from fibroblasts, leukocytes, and epithelial cells by the ABCB1-type multiple drug resistance (MDR) p-glycoprotein (PGP) present in two isoforms in rodents (*abcb1a* and *abcb1b*) and one isoform only in humans (ABCB1). Interestingly, the MDR PGP is also localized at the luminal membrane of the endothelial cells of the blood–brain barrier (BBB), and limits the access of endogenous glucocorticoids to the mouse and human brain (de Kloet *et al*, 1998; Meijer *et al*, 1998; Karssen *et al*, 2001, 2002; Uhr *et al*, 2002; Muller *et al*, 2003). We have found that antidepressants enhance GR function in mouse fibroblast cells by inhibiting the MDR PGP, and thus increasing intracellular concentrations of glucocorticoids (Pariante *et al*, 2001, 2003a, b); a similar effect is also present in rat cortical neurones (Pariante *et al*, 2003a). Consistent with this finding, pretreatment of cells with an MDR PGP inhibitor, or coincubation with a glucocorticoid that is not transported by MDR PGP *in vitro*, prevents these effects of antidepressants (Pariante *et al*, 1997, 2001, 2003a, b). Although other researchers have independently replicated these *in vitro* findings (Budziszewska *et al*, 2000; Miller *et al*, 2002; Herr *et al*, 2003), this model has never been directly tested in animals.

Here, we examine the effects of the tricyclic antidepressant, desipramine, in mice that are knockout (−/−) for both the *abcb1a* and the *abcb1b* MDR PGP, and in FVB/N controls. The *abcb1ab* (−/−) mice have been previously shown to have increased access of corticosterone to the brain and a more effective HPA axis negative feedback because of the facilitated entry of endogenous and exogenous glucocorticoids to the brain (Uhr *et al*, 2002; Muller *et al*, 2003). Our hypothesis is that antidepressants increase GR expression and decrease HPA axis activity by modulating MDR PGP; and therefore that these effects of antidepressants would *not* be present in the *abcb1ab* (−/−) mice. To investigate further, the mechanism by which antidepressants regulate the HPA axis, we also examine the effects of desipramine on the expression of MDR PGP itself (Mdr1a, more expressed in the brain), and also on the expression of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). 11β-HSD1 is an intracellular enzyme which catalyzes the regeneration of active glucocorticoids from circulating inert 11-keto steroids, and therefore effectively amplifies glucocorticoid action in the brain (Seckl and Walker, 2001).

MATERIALS AND METHODS

Animals

Male FVB/N controls and *abcb1ab* (−/−) mice, originally created by Schinkel *et al* (1997), backcrossed 12 generations to the FVB/N background strain, 8–9 months of age, were obtained from Taconic (Germantown, NY, USA), housed individually and maintained on a 12:12 h light/dark cycle (lights on 0700 h) with standard chow (Special Diet Services, Essex, UK) and water available *ad libitum*. For the initial antidepressant dose–response and time–course study (see below), the male FVB/N mice (4 months of age) were from Harlan UK. All mice were killed by decapitation, in the morning, 16 h after the last injection. Brains were removed, snap frozen on soft dry ice, and stored at −80°C. Trunk blood was collected for corticosterone measurements. All studies were performed to the highest standard of animal care under the aegis of the UK Animals (Scientific Procedures) Act, 1986.

Antidepressant Administration

For the main experiments presented in this paper, the *abcb1ab* (−/−) mice and FVB/N controls were administered desipramine (Sigma, UK), 10 mg/kg, i.p., twice daily (total dose: 20 mg/kg/d) for 7 days ($n = 5$–7 per group). This dose and length of treatment was chosen because we were interested in examining the shortest period of time needed by the antidepressant for inducing GR changes. In a preliminary time–course and dose–response study, we compared FVB/N controls treated with desipramine

20 mg/kg/day for 1 week, 20 mg/kg/day for 2 weeks, or 10 mg/kg/day for 3 weeks (see below for the methods). We found the greatest effects after 1 week of 20 mg/kg/day, with a significant upregulation of the GR in the CA1 area (+ 32%; $p = 0.011$). After 2 weeks of 20 mg/kg/day, there were smaller, nonsignificant increases of GR in the CA1 (+ 10%), whereas after 3 weeks of 10/mg/kg/day, there was no evidence of GR upregulation. Therefore, we used the regime of 1 week of desipramine 20 mg/kg/day for our subsequent main experiments. The 20 mg/kg/day dose was administered in 2 separate i.p. injections, as described by Uhr *et al* (2000) in these mice. Desipramine was dissolved in sterile 0.9% saline at 1 mg/ml, and controls received saline, 10 ml/kg, i.p., twice daily. Untreated (ie naive mice that have not received any treatments) *abcb1ab* (−/−) and FVB/N ($n = 4$ per group) were also included in the study, for comparison.

mRNA *In Situ* Hybridization Histochemistry in the Hippocampus

Brain sections (10 μm) at the level of the hippocampus (Bregma − 2.3 mm, plate 50 from Franklin and Paxinos, The Mouse Brain in Stereotaxic Coordinates) were mounted on silane-coated slides and postfixed in 4% paraformaldehyde followed by acetylation (0.25% acetic anhydride in 0.1 M triethanolamine, pH 8.0); the sections were then washed in phosphate-buffered saline, dehydrated through graded alcohols, and air-dried. The medial amygdaloid nucleus and retrosplenial agranular cortex were also analyzed on the same sections. Hybridization was carried out as described previously (Yau *et al*, 1997) using [^{35}S]UTP-labeled cRNA antisense probes transcribed *in vitro* from cDNA clones encoding: rat MR and GR (kindly supplied by Drs R Evans, J Arriza, and R Miesfeld); the mouse Mdr1a (889 bp fragment excised from the original full length clone from Alfred Schinkel and subcloned into pBluescript, kindly supplied by Dr Onno Meijer) (Not1 cut plasmid and T3 RNA polymerase for antisense probes; *Eco*R1 cut plasmid and T7 RNA polymerase for sense probes); and the mouse 11β-HSD1 (160–615 bp PCR product inserted into a pCR™11 vector) (Not1 cut plasmid and SP6 RNA polymerase for antisense probes; *kpn*1 cut plasmid and T7 RNA polymerase for sense probes). Following hybridization, sections were treated with ribonuclease A (30 μg/ml, 45 min, 37°C) and washed to a final stringency of 0.1 × SSC at 60°C. Slides were dehydrated, dipped in photographic emulsion (NTB-2, Kodak, UK), and exposed at 4°C for 21 days before developing and counterstaining with 1% pyronine. Hybridization signal within hippocampal subregions was assessed by computer-assisted grain counting using an image analysis system (Imaging Associates Ltd, UK). Silver grains were counted in a fixed circular area, under bright-field illumination, over individual hippocampal cells within each hippocampal subfields (see Figure 1), except for the dentate gyrus, where it was difficult to define cell boundaries, and therefore the fixed circular area covered one cell and a fraction of neighboring cells). The analysis was carried out blind to drug treatment. For each animal, 15–18 cells per subregion were assessed (over three hippocampal sections per animal), and background, counted over areas of white matter, was subtracted.

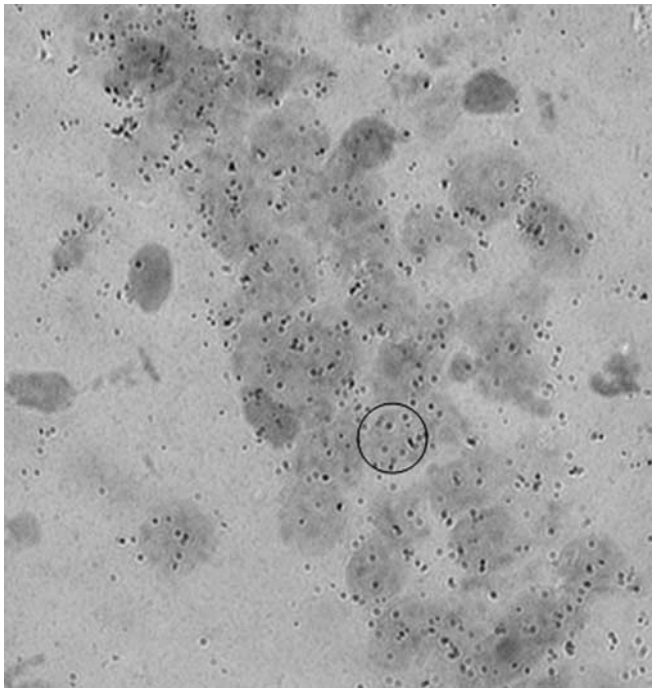

Figure 1 Photomicrograph showing GR mRNA expression in CA1 pyramidal cells from FVB/N controls mice treated with saline, under × 40 light microscope objective. Silver grains, which appear black under bright field, represent GR mRNA expression. The black circle shows the size of the fixed area used on the image analysis program to measure the grain density per cell.

Plasma Corticosterone Levels

Plasma corticosterone levels were measured by radioimmunoassay modified for microtitre plate scintillation proximity assay (Amersham Int., UK) with a highly specific antiserum (Dr C Kenyon, Centre for Cardiovascular Science, Queen's Medical Research Institute, Edinburgh, UK) and [^{3}H]corticosterone (Amersham Int., UK). The detection limit of the RIA was 0.02 pmol corticosterone in 20 μl, or 0.03 μg/dl.

Statistical Analysis

Data are presented as mean and standard error of the mean (SEM). Data were analyzed using two-way analysis of variance (ANOVA) (multivariate when appropriate) with genotype (FVB/N controls *vs abcb1ab* (−/−) mice) and treatment (untreated *vs* saline *vs* desipramine) as main factors; this was followed by between-group Student's *t*-test comparisons when the ANOVA indicated a significant effect.

RESULTS

Effects of Desipramine on GR mRNA Expression in the Hippocampus, Amygdala, and Cortex

GR mRNA was measured in the hippocampus, amygdala, and cortex, as detailed in the methods. The analysis of hippocampal GR showed a significant interaction between

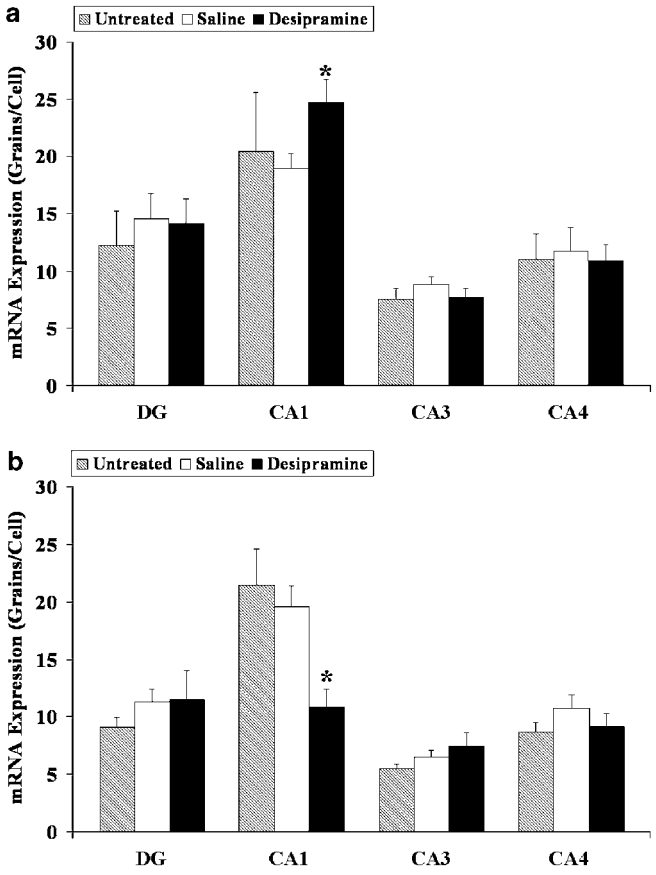

Figure 2 Expression of GR mRNA in the hippocampus of FVB/N controls (a) and *abcb1ab* (−/−) mice (b), measured by *in situ* hybridization histochemistry. Data are presented as mean ± SEM of grains/cell. Compared to saline, desipramine induced a significant upregulation of the GR mRNA expression in the CA1 region of FVB/N controls (Panel a, black *vs* white column), whereas it induced a significant *downregulation* of GR mRNA expression in the CA1 region of the *abcb1ab* (−/−) mice (Panel b, black *vs* white column). There were no differences in GR expression between saline-treated and-untreated mice, for both strains (gray *vs* white columns in a and b). *$p < 0.05$ *vs* saline in the same strain.

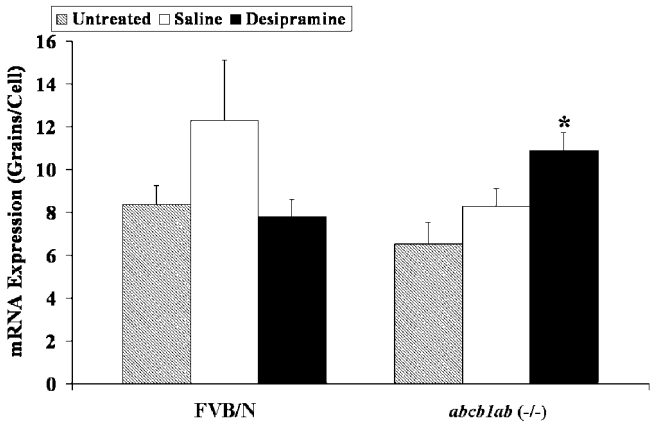

Figure 3 Expression of GR mRNA in the amygdala of FVB/N controls and *abcb1ab* (−/−) mice measured by *in situ* hybridization histochemistry. Data are presented as mean ± SEM of grains/cell. Compared to saline, desipramine induced a significant upregulation of the GR mRNA expression in the CA1 region of *abcb1ab* (−/−) mice (black *vs* white column on the right). There were no differences in GR expression between saline-treated and untreated mice, for both strains (gray *vs* white columns). *$p < 0.05$ *vs* saline in the same strain.

hippocampal subregion, genotype, and treatment (two-way (multivariate) ANOVA; $F_{6,52} = 2.4$; $p = 0.04$; see Figure 2, panel a for FVB/N controls and panel b for *abcb1ab* (−/−) mice). *Post hoc* analysis showed that, compared to saline, desipramine induced a significant upregulation of GR mRNA in the CA1 region of FVB/N controls (+31%; $p = 0.045$; see Figure 2, panel a, black *vs* white column), whereas it induced a significant *downregulation* of GR mRNA in CA1 of *abcb1ab* (−/−) mice (−45%; $p = 0.004$; see Figure 2, panel b, black *vs* white column). The analysis of amygdala GR also showed a significant interaction between genotype and treatment (two-way ANOVA; $F_{5,27} = 4.4$; $p = 0.022$; see Figure 3). *Post hoc* analysis, however, showed opposite results than in the hippocampus: compared with saline, desipramine induced a significant *upregulation* of GR mRNA in *abcb1ab* (−/−) mice (+31%; black *vs* white columns on the right; $p = 0.04$), whereas inducing a (nonsignificant) downregulation of GR mRNA in FVB/N controls mice (−27%; black *vs* white columns on the left; $p = 0.12$). Finally, the analysis of cortical GR showed no interaction between genotype and treatment (two-way ANOVA; $F_{5,27} = 1.4$; $p = 0.3$; see Figure 4), nor any main effects of genotype ($F_{1,27} = 1.7$; $p = 0.2$) or treatment ($F_{2,27} = 1.2$; $p = 0.3$).

It is of note that there were no differences in GR mRNA levels between saline-treated and untreated mice, for all three brain areas and for both strains; this indicates that the i.p. injections had no effects on GR expression *per se* (gray *vs* white columns in Figures 2–4).

Effects of Desipramine on MR mRNA Expression in the Hippocampus

The analysis of MR mRNA revealed no interaction between the hippocampal subregion, genotype, and treatment (two-way ANOVA; $F_{8,50} = 0.7$; $p = 0.7$; see Figure 5, panel a for FVB/N controls and panel b for *abcb1ab* (−/−) mice), nor any main effects of genotype ($F_{1,27} = 0.3$; $p = 0.6$) or treatment ($F_{2,27} = 0.1$; $p = 0.9$).

Effects of Desipramine on Plasma Corticosterone Levels

The analysis of corticosterone levels in the two strains of mice showed a significant interaction between genotype and treatment (two-way ANOVA; $F_{5,27} = 3.1$; $p = 0.025$; see Figure 6). *Post hoc* analyses showed that untreated *abcb1ab* (−/−) mice had lower corticosterone levels than untreated FVB/N mice (gray column on the right *vs* gray column on the left; $p = 0.02$); this replicates the findings by Muller *et al* (2003). It is also of note that there was an increase of corticosterone levels in saline-treated *abcb1ab* (−/−) mice compared with untreated *abcb1ab* (−/−) mice (gray *vs* white columns on the right; $p = 0.053$), whereas the same phenomenon did not occur in FVB/N controls (gray *vs* white columns on the left; $p = 0.7$).

Desipramine reduced corticosterone levels in both mice groups, but in *abcb1ab* (−/−) mice the effects were smaller. In FVB/N controls, desipramine decreased corticosterone levels not only compared with saline-treated mice (black

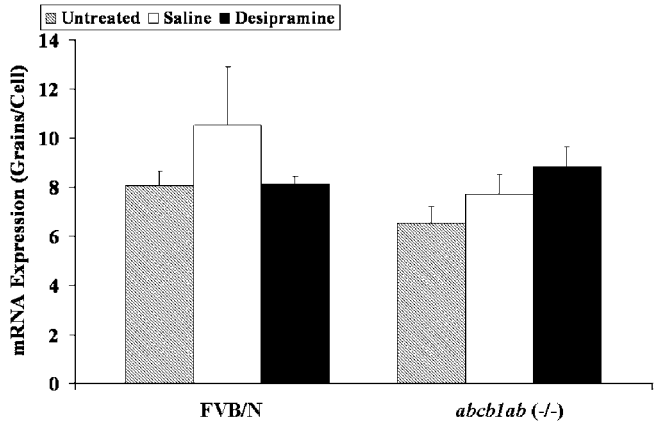

Figure 4 Expression of GR mRNA in the cortex of FVB/N controls and *abcb1ab* (−/−) mice measured by *in situ* hybridization histochemistry. Data are presented as mean ± SEM of grains/cell. There were no effects of desipramine and no differences between strains.

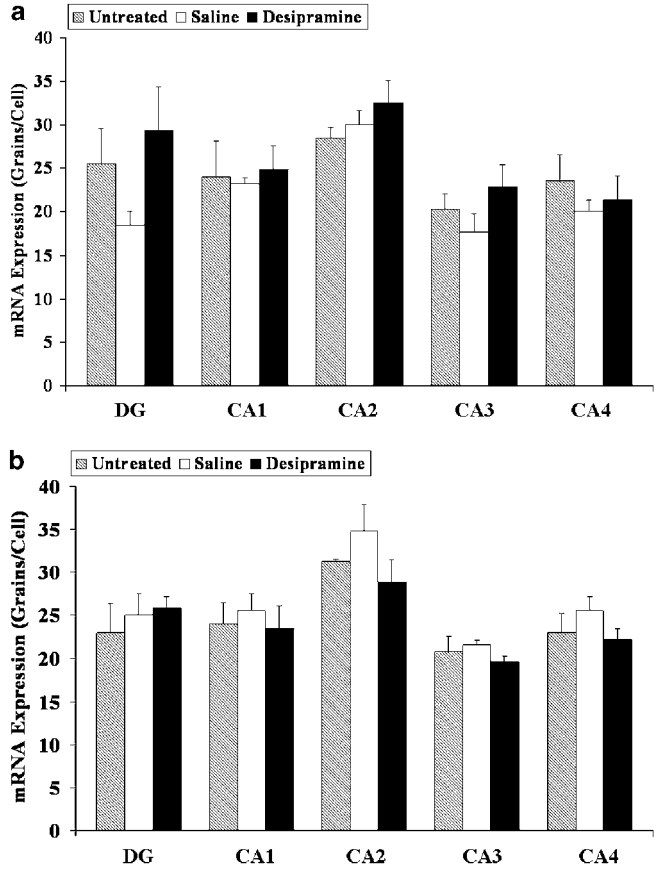

Figure 5 Expression of MR mRNA in the hippocampus of FVB/N controls (a) and *abcb1ab* (−/−) mice (b) measured by *in situ* hybridization histochemistry. Data are presented as mean ± SEM of grains/cell. There were no effects of desipramine and no differences between strains.

vs white columns on the left; $p = 0.05$), but also compared with naive mice (black *vs* gray columns on the left; $p = 0.05$): ie after desipramine, corticosterone in FVB/N controls was lower (−39%) than the 'physiological' levels of naive mice. In contrast, in *abcb1ab* (−/−) mice, desipramine only reduced corticosterone levels compared with saline treatment (black *vs* white columns on the right; $p = 0.05$) to levels that were equal to those of naive mice (black *vs* gray columns on the right; $p = 0.9$).

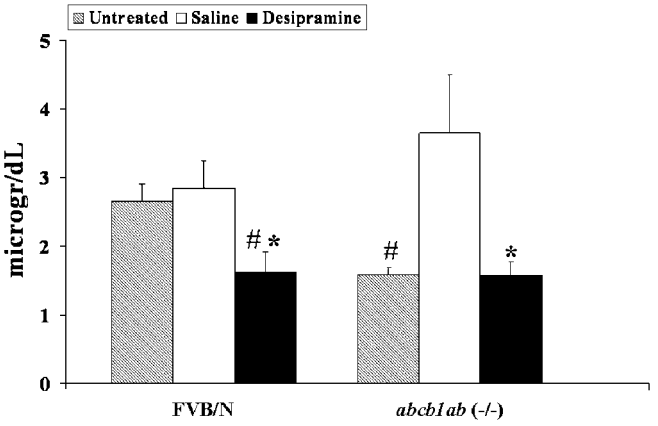

Figure 6 Corticosterone levels measured in the plasma of FVB/N controls (left) and *abcb1ab* (−/−) mice (right). Untreated *abcb1ab* (−/−) mice had lower corticosterone levels than untreated FVB/N mice (gray column on the right *vs* gray column on the left). There was an increase of corticosterone levels in saline-treated *abcb1ab* (−/−) mice compared with untreated *abcb1ab* (−/−) mice (gray *vs* white columns on the right; $p = 0.053$). In FVB/N controls, desipramine decreased corticosterone levels not only compared with saline-treated mice (black *vs* white columns on the left), but also compared with untreated mice (black *vs* gray columns on the left). In contrast, in *abcb1ab* (−/−) mice, desipramine only reduced corticosterone levels compared with saline treatment (black *vs* white columns on the right), to levels that were equal to those of untreated mice (black *vs* gray columns on the right). $^{\#}p \leqslant 0.05$ *vs* untreated FVB/N controls; $^{*}p \leqslant 0.05$ *vs* saline in the same strain.

Effects of Desipramine on MDR PGP and 11β-HSD1 mRNAs

The analysis of Mdr1a in the hippocampus (of FVB/N controls only) showed a significant interaction between subregion and the treatment (multivariate ANOVA; $F_{8,20} = 2.7$; $p = 0.03$; see Figure 7). Specifically, compared to saline, desipramine induced a significant downregulation of Mdr1a mRNA across all five subregions (−9 to −23%; multivariate ANOVA, $F_{1,9} = 5.7$; $p = 0.04$; black *vs* white columns). It is of note that there were no differences between saline-treated and untreated mice, showing that the i.p. injections had no effects of Mdr1a (gray *vs* white columns).

The analysis of hippocampal 11β-HSD1 revealed no interaction between hippocampal subregion, genotype, and treatment (two-way ANOVA; $F_{8,54} = 1.5$; $p = 0.2$; see Figure 8, panel a for FVB/N controls and panel b for *abcb1ab* (−/−) mice), nor any main effects of genotype ($F_{1,29} = 1.9$; $p = 0.2$) or treatment ($F_{1,29} = 1.0$; $p = 0.4$).

DISCUSSION

This is the first study to identify a mechanism through which antidepressants can induce GR upregulation and reduce HPA axis activity *in vivo*. We find that lack of the glucocorticoid transporters, MDR PGP *abcb1a* and *1b*,

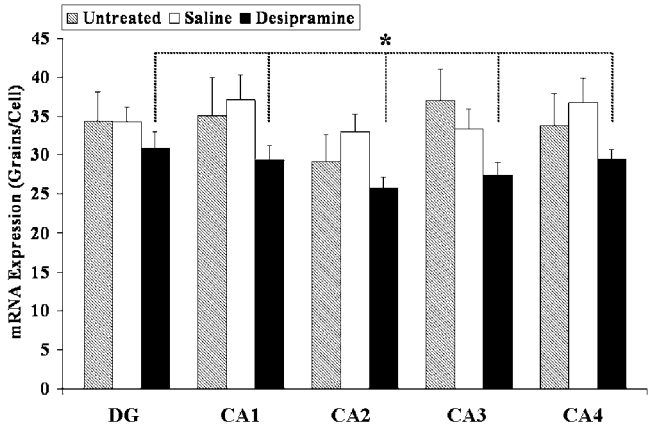

Figure 7 Expression of MDR PGP mRNA in the hippocampus of FVB/N controls measured by *in situ* hybridization histochemistry. Data are presented as mean ± SEM of grains/cell. Compared to saline, desipramine induced a significant downregulation of MDR PGP mRNA expression across all five subregions (black *vs* white columns). There were no differences between saline-treated and untreated mice (gray *vs* white columns). The dotted line marked by * indicates the significant ($p = 0.04$) main difference between desipramine- and saline-treated mice.

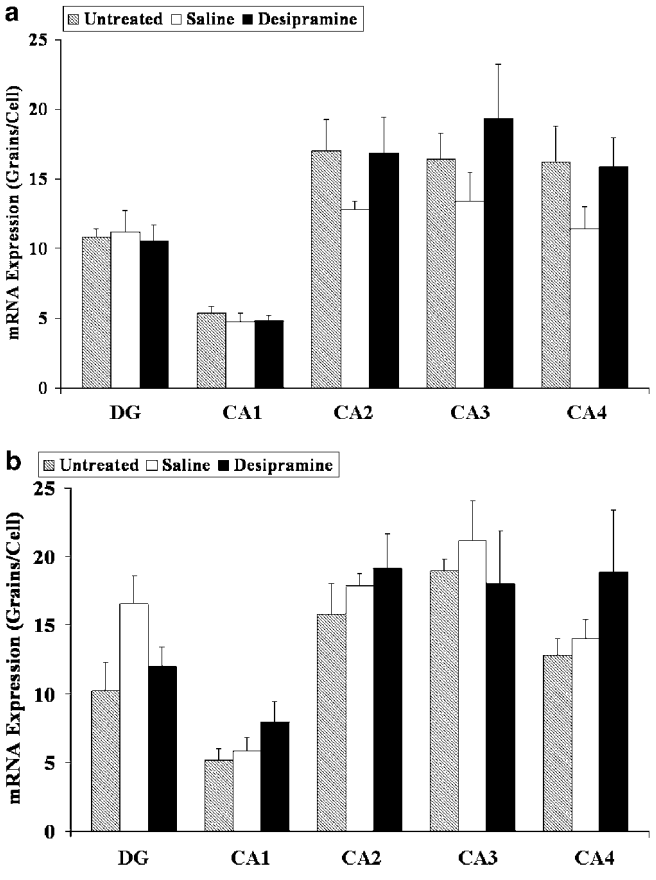

Figure 8 Expression of 11β-HSD1 mRNA in the hippocampus of FVB/N controls (a) and *abcb1ab* (−/−) mice (b) measured by *in situ* hybridization histochemistry. Data are presented as mean ± SEM of grains/cell. There were no effects of desipramine and no differences between strains.

prevents GR upregulation in the hippocampus induced by desipramine, and reduces the ability of desipramine to decrease plasma corticosterone levels. These findings are remarkably consistent with previous work in cell lines, by us and others, showing that antidepressants are unable to potentiate GR-mediated gene transcription in the presence of an MDR PGP inhibitor or of a glucocorticoid that is not transported by MDR PGP (Pariante *et al*, 1997, 2001, 2003a, b, 2004b; Budziszewska *et al*, 2000; Miller *et al*, 2002; Herr *et al*, 2003). Moreover, we find that desipramine reduces MDR PGP (Mdr1a) expression in the hippocampus of FVB/N control controls, again consistent with previous work in cell lines (Varga *et al*, 1996; Szabo *et al*, 1999; Weiss *et al*, 2003; Pariante *et al*, 2003b; Weber *et al*, 2005). Finally, we replicate the previous finding by Muller *et al* (2003) showing that *abcb1ab* (−/−) mice have lower corticosterone levels than FVB/N controls. Indeed, desipramine treatment (in our model, a pharmacological 'knockdown' of MDR PGP) also lowers plasma corticosterone levels in controls, to levels indistinguishable from *abcb1ab* (−/−) mice. This confirms the notion that MDR PGP is indeed a barrier to corticosterone access to the brain, and that its absence leads to more corticosterone entering the brain, and thus to an increased negative feedback on the HPA axis. Taken together with previous *in vitro* work, the present study strongly supports our proposed model that one mechanism through which antidepressants regulate the HPA axis is by reducing the action of glucocorticoid transporters like MDR PGP on the endothelial cells of the BBB (and possibly in neurons), thus leading to enhanced entry of glucocorticoids into the brain and so to facilitated negative feedback (Pariante *et al*, 2001, 2003a, b, 2004b; Pariante, 2006).

It is interesting that, in FVB/N controls, there were no effects of desipramine on GR expression in the cortex, and there was a tendency (although not significant) for a GR downregulation in the amygdala. Very few studies have examined the effects of tricyclic antidepressants on GR expression in these areas, and, consistent with our results, have found that GR is decreased (not significantly) in the amygdala (Peiffer *et al*, 1991b), and unchanged in the cortex (Seckl and Fink, 1992), in the presence of GR upregulation in the hippocampus (Peiffer *et al*, 1991b; Seckl and Fink, 1992). In contrast, one study in transgenic mice with decreased GR expression has found that desipramine increases GR expression in the cortex of these animals (Pepin *et al*, 1992). The desipramine-induced GR *upregulation* in the amygdala of *abcb1ab* (−/−) mice (as opposed to the tendency for a downregulation in the amygdala of FVB/N controls) is also puzzling, although is consistent with the notion that, as for the hippocampal GR, desipramine have opposite effects on the GR in these two mice groups. GR expression is differently regulated in the hippocampus, cortex, and amygdala, and powerful GR manipulations such as adrenalectomy, glucocorticoid treatment, and programming induce different effects on GR expression in these areas (Meaney *et al*, 1985; Sapolsky and McEwen, 1985; Pepin *et al*, 1990; Peiffer *et al*, 1991a; Welberg *et al*, 2001), although the molecular mechanisms underlying these localized differences are still unclear.

The second important finding of this study is that desipramine decreases corticosterone levels in both FVB/N controls and in *abcb1ab* (−/−) mice, but in *abcb1ab* (−/−) mice the effects are smaller. Specifically, in FVB/N controls (but not in *abcb1ab* (−/−) mice), desipramine reduces

corticosterone levels not only compared with saline-treated mice, but also compared with the physiological levels of untreated mice. Interestingly, saline treatment seems to activate the HPA axis in the *abcb1ab* (−/−) mice, but not in FVB/N controls. This may be due to the fact that *abcb1ab* (−/−) mice suffer from spontaneous colitis (Banner *et al*, 2004) and, therefore, could be more sensitive to the manipulation associated with two daily i.p. injections for 1 week. Taken together, these results suggest that desipramine in *abcb1ab* (−/−) mice can reduce an activated HPA axis, but cannot reduce basal HPA axis activity, whereas in FVB/N controls, desipramine can also reduce basal HPA activity. This, of course, also emphasizes that MDR PGP is not the only mechanisms involved in the antidepressant-induced reduction in HPA axis activity (see below).

How can we reconcile that desipramine decreases corticosterone levels in both mice groups, but in the presence of hippocampal GR *upregulation* in FVB/N controls and hippocampal GR *downregulation* in *abcb1ab* (−/−) mice? Our preferred model is that desipramine *initially* increases hippocampal GR translocation and function in both mice groups; the increased GR translocation and function lead to both the reduced corticosterone levels (following enhanced negative feedback) and the GR downregulation (following GR internalization into cellular nuclei). This model is supported by studies in rats, where antidepressants decrease HPA axis activity and induce GR downregulation within the first few days of treatment (Reul *et al*, 1993; Yau *et al*, 2001), and by studies *in vitro*, where antidepressants induce GR translocation and GR downregulation within one or 2 days of treatment (Pariante *et al*, 1997, 2003a; Okugawa *et al*, 1999; Yau *et al*, 2001; Heiske *et al*, 2003). Indeed, Mukherjee *et al* (2004) found that one single dose of imipramine in mice induces GR translocation in the CA1 subregion, the same area where we find the GR downregulation. GR translocation and internalization into cellular nuclei may lead to GR downregulation via reduced GR protein half-life and inhibition of GR mRNA synthesis (Schmidt and Meyer, 1994). Of course, this model implies that GR activation by antidepressants is independent from inhibition of MDR PGP, as it seems to be present in the *abcb1ab* (−/−) mice. It is possible that *abcb1ab* (−/−) mice express other transporters that regulate the access of glucocorticoids to neuronal cells and that could be inhibited by antidepressants, like the MDR-associated protein (MRP or ABCC1) (Herr *et al*, 2000; Hirrlinger *et al*, 2002; Sisodiya *et al*, 2002). Indeed, we have recently described that the tricyclic antidepressant, clomipramine, increases the intracellular concentration of glucocorticoids in rat cortical neurons, where MDR PGP has not been described (Pariante *et al*, 2003a). Alternatively, or additionally, antidepressants can activate the GR by regulating a variety of second-messenger mechanisms (Maes *et al*, 1999; Budziszewska *et al*, 2000; Basta-Kaim *et al*, 2002, 2004, 2006; Budziszewska *et al*, 2004; Yehuda *et al*, 2004, 2006; Wang *et al*, 2005). Incidentally, we show here that one important pathway regulating glucocorticoid action in the brain, the enzyme 11β-HSD1 (Seckl and Walker, 2001), is *not* regulated by desipramine. Why then would the GR upregulation develop only in FVB/N mice, but not in the *abcb1ab* (−/−) mice? We propose that, in FVB/N mice only, the additional effects of desipramine on MDR PGP increase corticosterone access to the brain and thus further enhance the negative feedback, thus reducing plasma corticosterone beyond physiological levels and inducing GR upregulation as a *consequence* of the peripheral changes in HPA axis. Indeed, in rats, GR upregulation by antidepressants tends to occur after several days or weeks of treatment in the presence of reduced corticosterone levels and following the initial GR downregulation (reviewed in Pariante *et al*, 2004b).

In two recent papers, Weber *et al* (2005, 2006) have directly challenged our hypothesis that inhibition of MDR PGP function is relevant to the effects of antidepressants on the HPA axis. In the first paper, in mice, the authors have found that the tricyclic antidepressant, amitriptyline, had no effects on plasma or brain corticosterone levels (Weber *et al*, 2006). However, measuring endogenous corticosterone in the brain may not be appropriate to address this issue, because antidepressant-induced changes in plasma corticosterone would alter the levels in the brain and, hence, confound any inference on BBB permeability (Carroll *et al*, 1975). Measuring the access to the brain of radioactive glucocorticoids administered in the periphery (Meijer *et al*, 1998; Karssen *et al*, 2001 2002; Muller *et al*, 2003), or through brain perfusion (Pariante *et al*, 2004b), are more appropriate techniques to answer this kind of question. Moreover, these authors administered amitriptyline 10 mg/kg/d, a dose that may be too low to affect the HPA axis in mice. In the present study, we administered desipramine 20 mg/kg/day, and in our dose–response study, we found no effect of 10 mg/kg/day of desipramine on GR or MR in the hippocampus. Two other studies using tricyclic antidepressants in mice have also found that 20 mg/kg/day increase brain GR levels and reduce HPA axis activity (Pepin *et al*, 1992; Mukherjee *et al*, 2004). In the second paper, *in vitro*, the authors have confirmed previous work by us and others showing that the inhibiting effects of antidepressants on MDR PGP function occur at micromolar concentrations (Varga *et al*, 1996; Szabo *et al*, 1999; Weiss *et al*, 2003; Pariante *et al*, 2003b), but have concluded that these concentrations are 'above therapeutically relevant plasma levels' (Weber *et al*, 2005). However, micromolar concentration of antidepressants are achieved in the brain of animals treated with the doses used in this and similar studies showing HPA axis changes by antidepressants (Glotzbach and Preskorn, 1982), and, most importantly, are achieved in the brain of patients taking therapeutic doses of antidepressants. In fact, *in vivo* neuroimaging studies using spectroscopy, which are only possible with antidepressants containing fluorine atoms such as fluoxetine and fluvoxamine, have consistently described steady-state brain concentrations of these drugs in the micromolar range (Bolo *et al*, 2000). Moreover, brain concentrations of tricyclics in humans, largely derived from postmortem studies following overdoses, have described brain-to-plasma concentration ratios ranging from eightfold, at higher plasma concentrations, to 125-fold, at lower plasma concentrations (Sunshine and Baeumler, 1963; Bickel *et al*, 1967; Avella *et al*, 2004). Therefore, considering that the plasma concentrations of tricyclics in patients taking therapeutic doses range 100–250 ng/ml (ie approximately 0.4–0.9 μM for desipramine and amitryptiline, and 0.3–0.8 μM for clomipramine), even a conservative estimate of a brain-to-plasma concentration of 10 would lead to

micromolar concentrations of tricyclic antidepressants in the brain of patients.

One limitation of our study is that we have not been successful in measuring the levels of desipramine in the brain (using high-performance liquid chromatography; data not shown): desipramine levels were below the limit of detection (ranging 150–300 ng/g in different samples). These would have been interesting data, as previous studies (Uhr *et al*, 2000, 2003; Uhr and Grauer, 2003) have shown, also using *abcb1ab* (−/−) mice, that MDR PGP limits the access to the brain of the tricyclic antidepressants, doxepine, amitriptyline, nortriptyline and trimipramine, after a single injection (although no data are available on desipramine). However, our study protocol was aimed at detecting persisting changes in the HPA axis, rather than the steady-state brain concentrations of antidepressants, and, therefore, the mice were killed 16 h after the last injection, whereas in previous studies measuring brain antidepressants levels, the animals were killed 15–60 min after single injections (Glotzbach and Preskorn, 1982; Uhr *et al*, 2000, 2003; Uhr and Grauer, 2003) or 4 h after the last injections in chronic-treatment studies (Grauer and Uhr, 2004); this could explain our negative findings. Theoretically, it is possible that *abcb1ab* (−/−) mice in our study have higher brain concentrations of desipramine, and that this might explain the differences in the GR expression. However, it is important to note that chronic treatment is different from a single injection; indeed, these same authors have found that after 10 days of administration (20 mg/kg/d in two i.p. injections, as in our study) only nortriptyline brain levels were elevated in *abcb1ab* (−/−) mice (approximately threefold), whereas amitriptyline brain levels were identical to those of FVB/N controls (Grauer and Uhr, 2004). Nevertheless, even if brain levels of desipramine were increased in *abcb1ab* (−/−) mice, there is no evidence that higher doses of tricyclic could lead to hippocampal GR *downregulation* rather than *upregulation*. In fact, one study in rats treated with amitriptyline (for 5 weeks) have found no evidence of hippocampal GR downregulation even using a dose that was 15-fold larger than the minimal dose able to induce GR upregulation (Reul *et al*, 1993).

In conclusion, we have found that desipramine requires MDR PGP to induce GR upregulation in mice, and that this effect is associated with downregulation of MDR PGP. Although some effects on the HPA axis are present in *abcb1ab* (−/−) mice treated with desipramine, our study clearly indicates that decreasing MDR PGP function by downregulation (and possibly direct pharmacological inhibition) is at least one of the molecular mechanisms involved in the HPA axis regulation by antidepressants. We suggest that MDR PGP could be a novel pharmacological target for developing new drugs that can treat depression by normalizing HPA axis activity in depressed patients (Nemeroff and Owens, 2002).

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This paper is dedicated to the memory of Professor Robert Kerwin. He was highly regarded by all of his colleagues, and he will be much missed.

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