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Do antidepressants regulate how cortisol affects the brain?

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Abstract

Although the effects of antidepressants on glucocorticoid hormones and their receptors are relevant for the therapeutic action of these drugs, the molecular mechanisms underlying these effects are unclear. Studies in depressed patients, animals and cellular models have demonstrated that antidepressants increase glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) expression and function; this, in turn, is associated with enhanced negative feedback by endogenous glucocorticoids, and thus with reduced resting and stimulated hypothalamic–pituitary–adrenal (HPA) axis activity. In a series of studies conducted over the last few years, we have shown that antidepressants modulate GR function in vitro by inhibiting membrane steroid transporters that regulate the intracellular concentration of glucocorticoids. In this paper, we will review the effects of membrane steroid transporters and antidepressants on corticosteroid receptors. We will then present our unpublished data on GR live microscopy in vitro, showing that ligand-induced translocation of the GR starts within 30 seconds and is completed within minutes. Furthermore, we will present our new data using an in situ brain perfusion model in anaesthetised guinea-pigs, showing that entry of cortisol to the brain of these animals is limited at the blood–brain barrier (BBB). Finally, we will present a comprehensive discussion of our published findings on the effects of chemically unrelated antidepressants on membrane steroid transporters, in mouse fibroblasts and rat cortical neurones. We propose that antidepressants in humans could inhibit steroid transporters localised on the BBB and in neurones, like the multidrug resistance *p*-glycoprotein, and thus increase the access of cortisol to the brain and the glucocorticoid-mediated negative feedback on the HPA axis. Enhanced cortisol action in the brain might prove to be a successful approach to maximise therapeutic antidepressant effects.

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1. Introduction

Patients with major depression show hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis, which is thought to participate in the development of the depressive symptoms (Nemeroff, 1996; Holsboer, 2000; Pariante & Miller, 2001; Pariante, 2003). One explanation for the HPA axis hyperactivity is an impaired feedback inhibition by the endogenous glucocorticoid, cortisol. This feedback is mediated by the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR) in the brain (de Kloet et al., 1998; McEwen, 2000). Patients with major depression exhibit impaired HPA axis negative feedback in the context of elevated circulating levels of cortisol (Nemeroff, 1996), and the GR is important in the regulation of the HPA when endogenous levels of cortisol are high (de Kloet et al., 1998). Consistent with this, the function of GR is reduced in depressed patients (GR resistance) and antidepressants reverse these putative GR changes (Holsboer, 2000; McQuade & Young, 2000; Pariante & Miller, 2001).

We have shown that antidepressants modulate GR function in vitro by inhibiting membrane steroid transporters that regulate the intracellular concentration of glucocorticoids (Pariante et al., 1997, 2001a,b, 2003a,b). Moreover, we have proposed that antidepressants in humans could inhibit steroid transporters localised on the blood–brain barrier (BBB) and in neurones, like the multidrug resistance (MDR) *p*-glycoprotein (PGP), and thus increase the access of cortisol to the brain and the glucocorticoid-mediated negative feedback on the HPA axis (Pariante et al., 2001a, 2003a,b). In this paper, we will review the role of membrane steroid transporters in regulating GR and MR function and the access of glucocorticoids to the brain. Furthermore, we will present our unpublished data on GR live microscopy in vitro and on cortisol access to the brain in guinea-pigs. We will then summarise the clinical and experimental evidence showing that antidepressants regulate corticosteroid receptors and the HPA axis, and that membrane steroid transporters could mediate these effects. Finally, we will present a comprehensive discussion of our published findings on the effects of antidepressants on membrane steroid transporters.

2. Corticosteroid receptors and the access of glucocorticoids to the brain

2.1. The role of corticosteroid receptors in HPA axis functioning

HPA axis activity is governed by the secretion of corticotrophin hormone-releasing factor (CRF) and vasopressin (AVP) from the hypothalamus, which in turn activates

the secretion of corticotrophin (adrenocorticotrophic hormone, ACTH) from the pituitary, which finally stimulates the secretion of glucocorticoids (cortisol in humans and corticosterone in rodents) from the adrenal cortex (Nemeroff, 1996; de Kloet et al., 1998; Ebrecht et al., 2000). Glucocorticoids then interact with their receptors in multiple target tissues, including the HPA axis where they are responsible for feedback inhibition of the secretion of ACTH from the pituitary and CRF from the hypothalamus (Nemeroff, 1996; de Kloet et al., 1998; Young et al., 1998; McEwen, 2000). The MR has a high affinity for endogenous glucocorticoids, and is believed to play a role in the regulation of circadian fluctuations of these hormones. In contrast to the MR, the GR has a lower affinity for endogenous glucocorticoids, and is believed to be more important in the regulation of the response to stress, when endogenous levels of glucocorticoids are high (de Kloet et al., 1998).

These receptors are ligand-induced transcription factors belonging to the steroid/thyroid receptor superfamily (de Kloet et al., 1998; Reul et al., 2000; Pariante & Miller, 2001; Kalman & Spencer, 2002). According to the "nucleocytoplasmic traffic" model, these receptors reside predominantly in the cytoplasm in an inactive form until they bind their glucocorticoid ligands. Ligand binding results in receptor activation and translocation to the nucleus (Guiochon-Mantel et al., 1996; Nishi et al., 2001). Within the nucleus, corticosteroid receptors regulate gene expression via binding to specific DNA sequences (glucocorticoid response elements, GREs). Several recent studies have confirmed the hormone-dependent translocation of the cytoplasmic MR and GR into the nucleus using time-lapse video microscopy of live cells (Htun et al., 1996; Yang & DeFranco, 1996; Nishi et al., 1999, 2001). Fig. 1 presents the video microscopy of GR translocation in live cells using a new chimeric protein of GR and green fluorescent protein (GFP–GR) that we have recently developed (see figure legend for details of the method). In steroid-free conditions, CHO cells show a diffuse cytoplasmic and nuclear fluorescence; after treatment with dexamethasone, translocation of GFP–GR starts within 30 s and is completed within minutes.

2.2. Membrane steroid transporters and HPA axis functioning

Membrane steroid transporters regulate the function of the GR and the MR by modulating the intracellular access of steroid hormones (de Kloet et al., 1998). Some glucocorticoids, like cortisol and dexamethasone, are actively excreted from cells by membrane transporters belonging to the ATP-binding cassette family of transporters (Ueda et al., 1992; Kralli & Yamamoto, 1996; Medh et al., 1998). One of these transporters, the MDR PGP, has been extensively described to regulate intracellular concentrations of steroids, to secrete naturally occurring metabolites and toxic substances directly into the urinary or gastrointestinal tracts, and to confer treatment resistance to tumour cells by expelling anticancer agents (Ueda et al., 1992; Shabbits et al., 2001). The MDR PGP is a polypeptide chain consisting of two similar halves, each containing six putative transmembrane segments and an intracellular ATP-binding site. Hydrolysis of ATP provides the energy for drug export, which can occur against a large concentration gradient (Schinkel, 1999; Ferte, 2000). Contrary to most known transporters, PGP and related steroid transporters do not move substrates
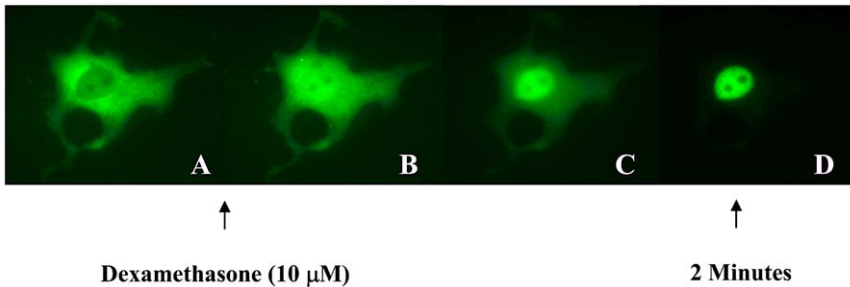
Fig. 1.   A new green fluorescent protein (GFP)–GR chimera. The GFP–GR is a chimeric protein of murine GR with green fluorescent protein (GFP), derived from the mouse wild-type GR cDNA vector pHWrec (a kind gift from M. Danielsen, Georgetown University School of Medicine, Washington, DC) (Danielson, Northrop & Ringold, 1986). After eliminating the stop codon in the 5′-untranslated region of the GR by polymerase chain reaction (PCR), the *Bgl*II–*Xba*I fragment (containing the GR cDNA) was cloned into the corresponding site of the pEGFP-C3 plasmid. The resulting GFP–GR protein contains the C-terminus of GFP fused in-frame to the N-terminus of the GR. We successfully transfected the GFP–GR in CHO, COS-7 and L929 cells, and in rat cortical and hippocampal neurones. Using live microscopy of CHO cells, in steroid-free conditions cells show a diffuse cytoplasmic and nuclear fluorescence (A). After treatment with dexamethasone, translocation of the GFP–GR starts within 30 s and is completed within minutes (B–D). For these experiments, CHO cells were cultured in coverslips and transiently transfected with GFP–GR. Cells were maintained at 37 °C on a heated stage during the experiment. Images were captured at baseline (A) and at time periods after dexamethasone (10 M) was added to the culture medium (B, 60 s; C, 90 s; D, 120 s).

across the cell membrane, but capture substrates from the cell membrane (while these substrates enter the cells by passive diffusion) and pump them out, thus preventing them from entering the cells (Goodsell, 1999).

The MDR PGP has been described in normal animal and human tissues. In the adrenal gland, MDR PGP regulates the secretion of glucocorticoids into the blood stream (Ueda et al., 1992); in lymphocytes, it regulates GR sensitivity to steroids (Bourgeois et al., 1993; Szabo et al., 1999); in the endothelial cells of the BBB, it limits the access of dexamethasone and cortisol to both mouse and human brain (de Kloet et al., 1998; Meijer et al., 1998; Karssen et al., 2001). Furthermore, recent studies have suggested that MDR PGP can regulate corticosterone access to the brain and HPA axis activity in rats and mice. In fact, rodents have two isoforms of PGP: the mdr1a and the mdr1b. The mdr1a PGP is predominantly expressed at the BBB (Regina et al., 1998) and does not expel corticosterone, the endogenous glucocorticoid, from the brain of rodents (de Kloet et al., 1998; Karssen et al., 2001). However, the mdr1b PGP transports corticosterone (Wolf & Horwitz, 1992; Uhr et al., 2002). This isoform is predominantly expressed in the adrenal and the ovaries (Lee et al., 2001), but is also expressed in the brain (Regina et al., 1998), particularly in the hippocampus (Kwan et al., 2002). Although mdr1b PGP has not been detected in brain capillaries (Regina et al., 1998), it is expressed in rat brain endothelial cells in vitro (Felix & Barrand, 2002). Consistent with the hypothesis that mdr1b PGP regulates the effects of corticosterone on the brain in rodents, mice that are knockout for mdr1a and mdr1b PGP genes show increased access of corticosterone to the brain

and increased negative feedback on the HPA axis by corticosterone (Muller et al., 2003). Therefore, there is some evidence suggesting that membrane transport of corticosterone across the BBB regulates HPA axis function in rodents.

2.3. The access of cortisol to the brain of guinea-pigs

Cortisol, the endogenous glucocorticoid in humans, is transported by MDR PGP at the BBB in mice, and by human MDR PGP in vitro (Ueda et al., 1992; Karssen et al., 2001; Uhr et al., 2002). Moreover, two postmortem studies have suggested that this mechanism can limit the access of cortisol to human brain (Brooksbank et al., 1973; Karssen et al., 2001). Recently, we have used an in situ brain perfusion model to study transport of radioactive cortisol across the BBB and the blood–cerebrospinal fluid (CSF) barrier in anaesthetised guinea-pigs (Thomas & Segal, 1997, 1998; Thomas et al., 2001; Gibbs & Thomas, 2002; Gibbs et al., 2003). Guinea-pigs are very similar to humans not only in the organization of the BBB, blood–CSF barrier and CSF flux, but also in the fact that cortisol is their main glucocorticoid (Keightley & Fuller, 1996). Therefore, it is conceivable that humans will be more similar to guinea-pigs than to mice in the regulation of the access of cortisol to the brain. In these experiments, guinea-pigs were anaesthetised, and the carotid arteries were perfused in situ with a warmed, oxygenated artificial plasma (see Table 1 and its legend for results and details of the method). After a 20-min pre-perfusion with artificial plasma (to eliminate endogenous cortisol from the brain), radiolabelled cortisol (2.5 nM) and sucrose (vascular marker) were infused into the inflowing plasma for 10 or 20 min. A CSF sample was taken and the animal was then decapitated. The results show that entry of cortisol to the brain of guinea-pigs is limited at the BBB (Table 1). In summary: (1) entry of radioactive cortisol into the brain ranges from 17% to 21.5% of plasma cortisol, and entry into the CSF ranges from 4% to 7.5% of plasma cortisol; (2) entry of radioactive cortisol into the pituitary, which does not have a BBB, is approximately 100% of plasma cortisol; and (3) in the presence of an excess of unlabelled cortisol (30 μM), the radioactive signal decreases in the hippocampus, but not in the rest of the brain. The discrepancy in cortisol concentration between plasma and brain, but not between plasma and pituitary, supports the presence of a functional efflux system for cortisol at the BBB. These preliminary findings are remarkably consistent with the results obtained by other laboratories when radioactive cortisol is administered subcutaneously in mice (Karssen et al., 2001; Uhr et al., 2002). Moreover, Karssen et al. (2001, 2002) also have demonstrated in mice that an excess of unlabelled glucocorticoid does not reduce the radioactive signal in the brain, except in the hippocampus (see below for the discussion of these data).

3. The effects of antidepressants on the HPA axis and on corticosteroid receptors

3.1. The HPA axis and corticosteroid receptors in major depression

Hyperactivity of the HPA axis in major depression is driven by the hypersecretion of CRF (and possibly AVP) in the hypothalamus (Owens & Nemeroff, 1993; Nemer-

Table 1
Brain and CSF uptake of radiolabelled cortisol in guinea-pigs

	^{3}H-cortisol perfusion (10 min)	^{3}H-cortisol perfusion (20 min)	Excess of unlabelled cortisol+[3]H-cortisol perfusion (10 min)
	3 Animals	3 Animals	2 Animals
CSF	4.0 ± 2	7.5 ± 2	6.9 ± 2
Pituitary	100.5 ± 44	150.4 ± 27	47.0 ± 3
Brain	17.0 ± 5	21.5 ± 5	16.0 ± 3
Hippocampus	14.1 ± 6	15.6 ± 9	8.7 ± 1
Capillary	3.3 ± 0.5	3.5 ± 0.5	1.7 ± 1

All experimental procedures were within the guidelines of the Animals (Scientific Procedures) Act, 1986. Adult Dunkin-Hartley guinea-pigs were anaesthetised and heparinised. The carotid arteries were perfused in situ with a warmed (37 °C), oxygenated (95% O_2; 5% CO_2) artificial plasma. With the start of perfusion the jugular veins were sectioned. After perfusion with plasma for 20 min, radiolabelled cortisol (2.5 nM) and sucrose (vascular marker) were infused into the inflowing plasma. After a further 10 or 20 min, a cisterna magna CSF sample was taken and the animal decapitated. Brain, CSF and plasma samples were taken for radioactive analysis. The uptake is expressed as mean ± SEM percentage ratio of tissue to plasma radioactivities, and corrected for vascular space. Using the capillary depletion analysis, which separates the level of radioactivity that has reached the brain from that in the cerebral endothelial cells ("Capillary"), there is no evidence that the measured signal represents cortisol in the endothelial cells rather than in the brain parenchyma. We also established that radioactive cortisol does not bind to the dextran/albumin present in the perfusion fluid (using ultrafiltration centrifugal dialysis and protein precipitation). The bilateral brain perfusion technique has several advantages, including: (1) the concentrations of radioactive glucocorticoids in the artificial plasma can be kept constant; (2) the movement of radioactive glucocorticoids into regions of the brain and into the CSF can be explored simultaneously; and (3) the levels of radioactivity in the endothelial cells of the BBB are separated from the levels in the brain tissue. The discrepancy between plasma and brain cortisol concentrations further supports the presence of a functional efflux system at the BBB, like MDR PGP.

off, 1996; Holsboer, 2000). These increased levels of CRF in the hypothalamus are related, at least in part, to altered feedback inhibition by endogenous glucocorticoids (de Kloet et al., 1998; Holsboer, 2000; McQuade & Young, 2000; Pariante & Miller, 2001; Pariante et al., 2002; Pariante, 2003). Consistent with the fact that patients with major depression exhibit impaired HPA negative feedback in the context of elevated circulating levels of cortisol, when the negative feedback is largely mediated by the GR, a multitude of studies have demonstrated that GR-mediated feedback inhibition is impaired in major depression. These patients have nonsuppression of cortisol secretion following dexamethasone (dexamethasone suppression test), non-suppression of ACTH secretion following hydrocortisone (fast-feedback test) and lack of inhibition of ACTH responses to CRF following dexamethasone pre-treatment (DEX/CRF test) (Young et al., 1991; Ribeiro et al., 1993; Heuser et al., 1994, 1996; Nemeroff, 1996; Holsboer, 2000). The only study that specifically looked at MR-mediated negative feedback in depression found that this pathway is intact (or possibly oversensitive) in these patients (Young et al., 2003).

In further support to the notion that patients with major depression exhibit

impaired GR-mediated HPA negative feedback, a number of studies have demonstrated that GR function is also reduced in other tissues of depressed patients, as shown by a decreased sensitivity to the effects of glucocorticoids on immune and metabolic functions in the absence of a reduction in GR expression (Pariante & Miller, 2001). In fact, there is no consistent evidence of reduced GR expression in blood mononuclear cells or fibroblasts from depressed patients (Pariante & Miller, 2001). However, studies that have examined postmortem brains have found reduced corticosteroid receptors expression: Lopez et al. (1998) found decreased MR (but not GR) mRNA levels in the hippocampus of six suicide victims with a history of depression; Webster et al. (2002) found decreased GR mRNA in the frontal cortex and hippocampus of patients with nonpsychotic depression, bipolar disorder and schizophrenia. These latter results suggest that the stress of having a psychiatric disorder may be more relevant to changes in brain GR or MR expression than depression per se (Cotter & Pariante, 2002).

3.2. The effects of antidepressants on the HPA axis and on corticosteroid receptors

The most striking support to the hypothesis that abnormalities in the corticosteroid receptors contribute to the pathophysiology of major depression derives from animals and in vitro studies demonstrating a direct effect of antidepressants on the GR and the MR, leading to increased receptor expression and function, and thus to increased negative feedback on the HPA axis. These studies support the clinical evidence that successful antidepressant treatment is associated with resolution of the impairment in the HPA axis negative feedback by glucocorticoids (Linkowski et al., 1987; Ribeiro et al., 1993; Heuser et al., 1996) and of glucocorticoid resistance in immune cells (Wodarz et al., 1992).

A number of studies have shown that long-term antidepressant treatment upregulates GR and MR in the brain, including in the hippocampus and in the hypothalamus, and decreases basal and stress-induced glucocorticoid secretion. The vast majority of studies using tricyclic antidepressants, like desipramine, amitriptyline and imipramine, or using electroconvulsive shock, has shown antidepressant-induced upregulation of brain GR, MR, or both (Kitayama et al., 1988; Young et al., 1990; Peiffer et al., 1991; Brady et al., 1991; Seckl and Fink, 1992; Pepin et al., 1992b; Reul et al., 1993; Przegalinski et al., 1993; Przegalinski and Budziszewska, 1993; Budziszewska et al., 1994; Peeters et al., 1994; Rossby et al., 1995; Yau et al., 1995; Eiring and Sulser, 1997; Johansson et al., 1998). Studies examining selective serotonin reuptake inhibitor (SSRI) antidepressants, like fluoxetine, citalopram and zimelidine, have found that chronic treatment with these antidepressants upregulates MR expression, while it has no effect on GR expression (Seckl and Fink, 1992; Brady et al., 1992; Budziszewska et al., 1994; Rossby et al., 1995; Lopez et al., 1998; Bjartmar et al., 2000; Yau et al., 2002), although one study found that a 2-day treatment with fluoxetine increases both MR and GR mRNAs in the hippocampus (Semont et al., 2000).

Interestingly, the few studies that have looked at shorter durations of treatment

have found that acutely (within 3–9 days) antidepressants induce a decrease of GR and MR expression. For example, Reul et al. (1993) showed a decreased GR and MR expression after 3–7 days of amitriptyline, and Yau et al. (2001) showed that fluoxetine and venlafaxine (a serotonin and noradrenaline reuptake inhibitor, SNRI) induce downregulation of GR and MR expression at 9 days. On the opposite end, studies looking at longer time points have indicated that GR expression returns to control levels after 6–9 weeks of treatment with antidepressants, while MR upregulation persists (Reul et al., 1993, 1994; Yau et al., 1995).

Several of these studies have also shown that treatment with antidepressants in rodents is associated with a reduction in basal and stress-induced HPA axis activity, and that—surprisingly—GR or MR upregulation is not a prerequisite for this reduction (Pariante & Miller, 2001). For example, Delbende et al. (1991) showed that a single injection of the antidepressant tianeptine, given 1–3 h before a tube restraint stress, significantly reduces the stress-induced ACTH and corticosterone release in rats: an acute effect unlikely to be related to GR or MR upregulation. Brady et al. (1992) found that a 2-week treatment with fluoxetine, idazoxan or phenelzine reduces basal corticosterone levels in the absence of GR or MR upregulation. Reul et al. (1993) showed a decrease in adrenal weight, likely representing a decrease in HPA axis function, in rats treated with amitriptyline for 5 days, together with a decrease in hippocampal GR binding. Montkowski et al. (1995) demonstrated that long-term antidepressant treatment with moclobemide (a monoamine-oxidase inhibitor) induces normalization of the HPA axis in the absence of any changes in GR binding. Finally, Yau et al. (2001) have found that a 9-day treatment with fluoxetine or venlafaxine induces a reduction in HPA axis activity together with the downregulation of GR and MR. As we will discuss below, our research also demonstrates that antidepressants increase GR function in vitro in the absence of GR upregulation.

A potent tool for clarifying the mechanisms underlying antidepressant-induced increase in corticosteroid receptor function and expression has been the study of the effects of antidepressants in in vitro cell culture systems. Antidepressants regulate GR translocation, GR function and GR expression in neuronal cell cultures (Pepin et al., 1989; Okugawa et al., 1999; Hery et al., 2000; Yau et al., 2001; Lai et al., 2003; Herr et al., 2003), fibroblasts (Pepin et al., 1992a; Pariante et al., 1997, 2001a, 2003a,b; Budziszewska et al., 2000; Miller et al., 2002) and human peripheral blood mononuclear cells (Vedder et al., 1999; Heiske et al., 2003; Okuyama-Tamura et al., 2003). These experimental systems do not contain noradrenaline or serotonin reuptake sites within synaptic connections (Okugawa et al., 1999; Hery et al., 2000; Lai et al., 2003). Therefore, these systems allow the study of molecular effects that are unrelated to the inhibition of noradrenaline or serotonin reuptake, the mechanism considered to be crucial in the therapeutic action of antidepressants (Nestler, 1998; Schafer, 1999). Indeed, antidepressant-induced GR upregulation in vitro is not blocked by antagonists of alpha or beta adrenergic receptors or of 5HT1a or 5HT2 serotonergic receptors (Okugawa et al., 1999; Lai et al., 2003). These intriguing in vitro findings confirm data in animals showing that inhibition of noradrenaline reuptake is not relevant for the antidepressant-induced changes in the GR or MR expression. In fact, desipramine has been shown to induce GR upregulation in rats

even following neurotoxic lesioning of noradrenergic neurones with DSP4 (Rossby et al., 1995). Moreover, the noradrenaline reuptake inhibitor, oxaprotiline, consistently has shown no effects on GR or MR expression in animals (Budziszewska et al., 1994; Eiring and Sulser, 1997).

Some evidence suggests a possible role of the membrane steroid transporters in the regulation of corticosteroid receptor function in major depression or during antidepressant treatment. In vitro expression of MDR PGP induces GR resistance in lymphocytes by limiting the intracellular access of glucocorticoids (Bourgeois et al., 1993), thus reproducing a condition similar to that described in lymphocytes of patients with major depression. Moreover, previous studies have suggested that antidepressants interact with the MDR PGP in animals and in vitro. For example, the tricyclic antidepressant clomipramine, at 10 mg/kg/day for 2 days, completely inhibits MDR activity in subcutaneous tumours in mice (Merry et al., 1991). Of note is that the dose used in this study is within the range (10–20 mg/kg/day) used in most animal studies showing GR or MR upregulation by tricyclic antidepressants (Pariante & Miller, 2001). In vitro, tricyclic antidepressants (at concentrations similar to those used in the studies on the GR) blocks the MDR PGP-mediated efflux of rhodamine 123 from human colon cancer cells (Varga et al., 1996), cells from a leukemia cell line (Szabo et al., 1999) and human peripheral blood mononuclear cells (Szabo et al., 1999). Finally, two recent papers have described that amitriptyline and citalopram are transported by the MDR PGP (Uhr et al., 2000; Uhr & Grauer, 2003). Our hypothesis—that antidepressants modulate corticosteroid receptors by inhibiting membrane steroid transporters—is a potential explanation of how chemically and pharmacologically unrelated antidepressants may have similar effects on the GR and the MR, without affecting the reuptake of serotonin or noradrenaline. In fact, inhibition of PGP and other membrane steroid transporters is not receptor-mediated and is related to the drugs physiochemical properties, that is, lipophilicity, electric charge and ability to accept hydrogen bonds (Ford, 1996; Castaing et al., 2000; Ekins et al., 2002).

4. Antidepressants enhance GR function in vitro by modulating membrane steroid transporters

4.1. Antidepressants potentiate GR function in vitro in the absence of GR upregulation

In their pivotal paper, Pepin et al. (1992a) used a fibroblast cell line to show that 24 h treatment with desipramine enhances GR function (GR-mediated gene transcription) as measured by increased activity of a transiently transfected reporter gene whose regulation is dependent on GREs. Desipramine was also found to induce upregulation of GR protein after 72 h of treatment. Based on these data, the authors hypothesised that antidepressants directly induce GR upregulation in vitro. Our work has originated from this seminal paper, and has led us to propose that the mechanism by which antidepressants regulate corticosteroid receptor in vitro, and possibly in

animals, is the inhibition of membrane steroid transporters, leading to increased intra-cellular levels of glucocorticoids. The results of our studies are summarised in Table 2.

We have used L929 mouse fibroblast cells, stably transfected with the MMTV–chloramphenicol acetyltransferase (MMTV–CAT) reporter gene (LMCAT cells). Expression of the CAT reporter gene (Sanchez et al., 1994) by these cells is under glucocorticoid control by virtue of several GREs residing within the MMTV pro-moter, which lies upstream of the CAT reporter gene. Work conducted in this and other laboratories has shown that these cells have a membrane steroid transporter that is virtually identical to MDR PGP in its substrates and modulators (Kralli & Yamamoto, 1996; Medh et al., 1998; Marsaud et al., 1998; Pariante et al., 2001a,b). More recently, Webster and Carlstedt-Duke (2002) have demonstrated MDR PGP mRNA in these cells. Therefore, L929/LMCAT cells have the unique advantage of expressing endogenous (not transfected) membrane steroid transporters, while also allowing the direct examination of GR function (Sanchez et al., 1994; Pariante et al., 1997, 1999, 2001a,b, 2003a,b; Budziszewska et al., 2000). These cells have been used in a variety of studies looking at molecular determinants of GR function.

Our first finding on the effects of antidepressants on the GR was that 24 h treatment with desipramine (at 1 and 10 μM concentrations) induces translocation of the GR from the cytoplasm to the nucleus in the absence of any glucocorticoids, and potentiates GR translocation induced by a low (10 nM) concentration of dexame-thasone (Pariante et al., 1997). These results were confirmed using immunocytoch-emistry as well as western blotting of the GR in the cytosolic and nuclear fractions. In a subsequent series of experiments, we found that co-incubation of cells for 24 h with dexamethasone and several, chemically unrelated antidepressants—the tricyclics clomipramine, desipramine and amitriptyline, and the SSRIs fluoxetine, paroxetine and citalopram—induces an increase in GR function (GR-mediated gene transcription) compared to cells treated with dexamethasone alone (Table 2, 1st col-umn; see table legend for details of the method). At a concentration of antidepressants of 10 μM, this potentiation is particularly intense in cells treated with dexamethasone and clomipramine (more than 10-fold) while is approximately +70% to +170% for the other antidepressants (Pariante et al., 1997, 2001a, 2003b). A smaller (approximately 15–25%) potentiation is evident at a lower (1 μM) concentration of antidepressants (Pariante et al., 1997, 2001a, 2003b). We did not elicit any effects of these antidepressants alone on GR-mediated gene transcription (Pariante et al., 1997, 2001a, 2003b). Finally, this potentiation is not related to an increase in GR expression, since we found that desipramine and clomipramine reduce, rather than increase, GR expression after 24 h of treatment (see below) (Pariante et al., 1997, 2003a).

4.2. Antidepressants potentiate GR-mediated gene transcription by inhibiting membrane steroid transporters

We hypothesised that the effects of antidepressants on GR-mediated gene tran-scription are mediated by the L929/LMCAT cells membrane steroid transporter. In

Table 2
Summary of our in vitro studies on antidepressants and GR

	GR-mediated gene transcription in LMCAT cells				Intracellular accumulation in LMCAT cells			Intracellular accumulation in rat neurones		Western blotting of the GR	
	Dex	Cortisol	Corticos.	Dex + verapamil	Cortisol	Corticos.	Cortisol + verapamil	Cortisol	Corticos.	LMCAT cells	Rat neurones
Clomipramine	↑	↑	↑	→	↑	=	=	↑	→	→	→
Fluoxetine	↑	↑	=	=	↑	→					
Desipramine	↑	↑	→								
Paroxetine	↑	↑	→								
Citalopram	↑										
Amitriptyline	↑										

Cells were treated for 24 h with clomipramine (10 μM), fluoxetine (10 μM), desipramine (10 μM), paroxetine (10 μM), citalopram (10 μM) or amitriptyline (10 μM). ↑ Indicates an antidepressant-induced increase; ↓ Indicates an antidepressant-induced decrease; = Indicates no effect of the antidepressant. GR-mediated gene transcription in LMCAT cells: LMCAT cells were treated with vehicle or the antidepressants in co-incubation with dexamethasone (Dex) (10 nM), cortisol (50 nM), corticosterone (corticos.) (50 nM), or dexamethasone (2.5 nM) + verapamil (100 μM). Measurement of CAT was performed using a colorimetric enzyme immunoassay (Roche Diagnostic, UK). Results were normalised with respect to cell number by measurement of metabolic activity by cleavage of the tetrazolium salt WST-1 (Roche Diagnostic). Intracellular accumulation of radioactive glucocorticoids in LMCAT cells or rat neurones: LMCAT cells or one-week old rat primary neurones were treated with vehicle or the antidepressants for 24 h, and then incubated for 1.5 h with ³H-cortisol (50 nM), or ³H-corticosterone (50 nM), or ³H - cortisol (50 nM) + verapamil (100 M), at 37 °C, in a CO₂ incubator. Cells were then scraped into lysis buffer and transferred to vials for liquid scintillation counting. The radioactive signal, as measured by scintillation counting, is proportional to the intracellular concentration of the radioactive glucocorticoid. Results were normalised with respect to cell number, as above. Western blotting of the GR in LMCAT cells or rat neurones: LMCAT cells and one-week old rat primary neurones were treated with vehicle or clomipramine for 24 h. Cells were lysed by scraping into hot (100 °C) 2X SDS PAGE sample buffer. GR present in the lysates was analysed by western blot using the anti-GR polyclonal antibody GR57, and quantified by densitometric analysis. Data from Pariante et al. (1997, 2001, 2003a, b)

particular, we hypothesised that antidepressants enhance GR-mediated gene transcription in the presence of dexamethasone by inhibiting the membrane steroid transporters, and therefore increasing dexamethasone intracellular concentrations. To test this hypothesis, we co-incubated the antidepressants with two glucocorticoids that are differentially affected by the transporter: cortisol and corticosterone. In fact, work by us and others in LMCAT cells demonstrated that steroid transporters inhibitors, like verapamil, H-89, and cyclosporin, increase GR-mediated gene transcription only in the presence of glucocorticoids that are expelled by the transporters, like dexamethasone and cortisol, but not in the presence of corticosterone. If the inhibition of the transporter were the mechanism by which antidepressants increase GR-mediated gene transcription, then we would see these effects also in the presence of cortisol, but not in the presence of corticosterone (Medh et al., 1998; Marsaud et al., 1998; Pariante et al., 2001a). We focused these experiments on four antidepressants: clomipramine, a tricyclic serotonin reuptake inhibitor; desipramine, a tricyclic noradrenaline reuptake inhibitor; paroxetine, an inhibitor of both noradrenaline and serotonin reuptake; and fluoxetine, an SSRI (Pariante et al., 2001a, 2003b). We used cortisol and corticosterone at a low (50 nM) concentration that, similar to dexamethasone (10 nM), induces only partial GR activation. As hypothesised, we found that all antidepressants strongly potentiate GR-mediated gene transcription in the presence of cortisol, but not in the presence of corticosterone (Table 2, 2nd and 3rd columns). In fact, we found that all antidepressants, except fluoxetine, induce less GR-mediated gene transcription in the presence of corticosterone compared to cells treated with corticosterone alone. Interestingly, clomipramine, which gives the strongest potentiation in the presence of dexamethasone, also gives the strongest potentiation in the presence of cortisol (approximately fourfold) and the largest inhibition in the presence of corticosterone (approximately 35% inhibition).

To corroborate these findings, we examined the effects of clomipramine or fluoxetine in the presence of dexamethasone and the steroid transporter inhibitor, verapamil. If inhibition of the steroid transporter were the mechanism by which antidepressants increase GR-mediated gene transcription in the presence of dexamethasone, this effect would disappear in the presence of verapamil. As hypothesised, we found that both clomipramine and fluoxetine do not potentiate GR-mediated gene transcription in the presence of dexamethasone after the membrane steroid transporter is blocked by verapamil (Table 2, 4th column). Moreover, consistent with the experiments in the presence of corticosterone, clomipramine reduces dexamethasone-induced GR-mediated gene transcription in the presence of verapamil, while fluoxetine has no effect.

4.3. Antidepressants increase intracellular accumulation of radioactive glucocorticoids in LMCAT cells and neurones

Because the increased GR-mediated gene transcription only provides indirect evidence of increased intracellular levels of glucocorticoids, we examined whether antidepressants directly increase the intracellular levels of radioactive glucocorticoids in

LMCAT cells. Moreover, we examined whether a similar, antidepressant-sensitive, membrane transport of glucocorticoids is present in cultured rat primary neurones.

We developed the assay to measure intracellular accumulation of radioactive glucocorticoids from Bourgeois et al. (1993) (Pariante et al., 2003a,b) (see legend of Table 2 for details of the method). We found that there is a linear relationship between ^{3}H-cortisol concentrations in the media (from 1 nM to 1 μM) and the levels of intracellular accumulation in LMCAT cells. Moreover, at all concentrations of ^{3}H-cortisol, verapamil increases the intracellular levels of the glucocorticoid. We also found that an excess of unlabelled cortisol induces an increase (+80%) of the intracellular accumulation of radioactive cortisol, while unlabelled corticosterone results in a reduction (−40%) of the intracellular accumulation of radioactive corticosterone. We interpreted these finding as showing that the unlabelled cortisol competes for the radioactive cortisol at the efflux system, thus increasing the intracellular accumulation of the radioactive cortisol. However, because corticosterone is not transported, the unlabelled corticosterone can compete with the radioactive corticosterone at GR binding sites or at uptake sites for corticosterone (Pariante et al., 2003a) (see below).

By using this experimental design in primary cultures of rat cortical neurones, we also showed that a membrane transport of cortisol is present in neurones. First, we found that unlabelled cortisol induces a small increase of the intracellular accumulation of radioactive cortisol in these cells (+7%), while unlabelled corticosterone induces a decrease (−15%) in the intracellular accumulation of radioactive corticosterone; second, we found that verapamil increases the intracellular accumulation of ^{3}H-cortisol (Pariante et al., 2003a).

We found that clomipramine and fluoxetine induce an increase in the intracellular accumulation of ^{3}H-cortisol in LMCAT cells: approximately +80% for clomipramine and +5% for fluoxetine (a small but statistically significant effect) (Table 2, 5th column) (Pariante et al., 2003a,b). In the presence of ^{3}H-corticosterone, clomipramine has no effect, while fluoxetine induces a small (−15%) reduction of the intracellular accumulation (Table 2, 6th column) (Pariante et al., 2003a,b). Because the effects of 24 h fluoxetine on the intracellular accumulation of ^{3}H-cortisol was relatively small, we also conducted another series of experiments in which cells were treated with fluoxetine for 72 h. We found that fluoxetine induces a larger increase of ^{3}H-cortisol intracellular accumulation after this longer incubation (+15%), while in the presence of ^{3}H-corticosterone has no effect (Pariante et al., 2003b). Consistent with our hypothesis and the data on GR-mediated gene transcription, treatment of LMCAT cells with clomipramine in the presence of verapamil induces no increase of intracellular accumulation of ^{3}H-cortisol (Table 2, 7th column).

Finally, we wanted to test whether the cortisol transporter in rat primary neurones can be inhibited by clomipramine. Consistent with the data in LMCAT cells, we found that clomipramine induces an increase (+20%) in the intracellular accumulation of ^{3}H-cortisol compared to cells treated with vehicle, while in the presence of ^{3}H-corticosterone clomipramine induces a small (−11%) reduction of the intracellular accumulation (Table 2, 8th and 9th columns) (Pariante et al., 2003a).

4.4. Antidepressants acutely reduce GR expression in vitro

As mentioned above, we found that some antidepressants *decrease*, rather than *increase*, GR function in the presence of corticosterone or in the presence of dexamethasone and verapamil (Pariante et al., 2001a). We also found that pre-incubation of cells with desipramine followed by treatment with dexamethasone reduces GR-mediated gene transcription (Pariante et al., 1997). This latter finding has been replicated by Budziszewska et al. (2000), who also found that pre-incubation of LMCAT cells with various antidepressants (including desipramine) reduces GR-mediated gene transcription induced by a subsequent treatment with corticosterone or dexamethasone. Finally, Miller et al. (2002) have recently found that incubation of LMCAT cells with desipramine alone induces a small decrease in the unstimulated GR-mediated gene transcription. Based on these results, we hypothesised that a reduction in GR-mediated gene transcription by antidepressants is present under experimental conditions that do not elicit the effects on the transporter, like in the presence of glucocorticoid that are not expelled by the transporters, in the presence of transporters inhibitors, or in the absence of any glucocorticoids. Moreover, we hypothesised that these inhibitory effects are due to a reduction in GR expression. Indeed, in our first paper, we found that 24–96 h of treatment with desipramine induces a 10–25% reduction in cytosolic GR binding in L929 cells; this reduction is still present after the cells have recovered for 24 h in media with no desipramine (Pariante et al., 1997). Since 24 h desipramine also induces activation and translocation of the GR from the cytoplasm to the nucleus, and this is associated with a decrease of GR in the cytosolic fraction, we had originally interpreted the desipramine-induced decrease in GR binding as representing a greater proportion of GR in the nucleus (Pariante et al., 1997). However, these results can also be interpreted as an overall decrease in the total number of GRs. Therefore, we examined the effects of clomipramine on GR expression, assessed by whole-cell western blot, in LMCAT cells and primary rat cortical neurones (see legend of Table 2 for details of the method) (Pariante et al., 2003a). We used clomipramine because it gives the largest inhibition of GR-mediated gene transcription in the presence of corticosterone, compared to the other antidepressants tested. In both cell types, treatment with clomipramine for 24 h results in a reduction of GR levels: 50% reduction in LMCAT cells and 80% reduction in neurones (Pariante et al., 2003a) (Table 2, 10th and 11th columns).

5. A model for brain sensitivity to glucocorticoids and the effects of antidepressants on the HPA axis

5.1. Physiological relevance of our findings

Our work, in vitro and in guinea-pigs, corroborates consistent lines of evidence from different laboratories showing that membrane steroid transporters localised on the BBB are crucial in the regulation of brain sensitivity to glucocorticoids (de Kloet et al., 1998; Meijer et al., 1998; Karssen et al., 2001, 2002; Uhr et al., 2002). First,

we have presented preliminary data suggesting that a functional efflux system for cortisol exists at the BBB of guinea-pigs, which have cortisol as their main endogenous glucocorticoid. Second, we have shown in vitro that the effect of steroid transport is present for a large range of concentrations of cortisol (1 nM–1 µM) that comprises the physiological levels of this hormone in humans (Orth & Kovacs, 1998). Finally, we have shown that neurones (rat cortical neurones) show a functional transport of cortisol.

Our data in guinea-pigs show that an excess of unlabelled cortisol does not reduce the levels of radioactive cortisol in the brain, except in the hippocampus. These results corroborate data by Karssen et al. (2001, 2002) in mdr1a PGP knockout mice, also showing that brain labelling by a radioactive glucocorticoid is not reduced by an excess of unlabelled glucocorticoid, except in the hippocampus. While these data indicate that most of the glucocorticoid in the brain is unbound to GR, the results in the hippocampus suggest that the unlabelled hormone can compete with the radioactive cortisol either at hippocampal MR binding sites or at an uptake system for glucocorticoids in this region (Thomas & Segal, 1997; Karssen et al., 2001, 2002; Gibbs et al., 2003). Similar to our work in guinea-pigs, our studies in vitro also demonstrate that most of the hormone in the cells is GR-unbound. In LMCAT cells, the 50% reduction in GR expression by clomipramine leads to no change in intracellular accumulation of radioactive corticosterone; in neurones, the 80% reduction of GR expression by clomipramine leads only to a 11% reduction in the intracellular accumulation of radioactive corticosterone (Pariante et al., 2003a). Our in vitro data also suggest that an uptake system for corticosterone is present in LMCAT cells (Pariante et al., 2003a,b). In fact, since most of the intracellular glucocorticoid is GR-unbound, the 40% reduction of ^{3}H-corticosterone intracellular levels by the excess of unlabelled corticosterone cannot be explained by competition at the GR binding sites (Pariante et al., 2003a,b). Membrane uptake systems for glucocorticoids have been described before (Bossuyt et al., 1996; Lackner et al., 1998), and co-existence of both efflux and uptake transporters for glucocorticoids has been described in hepatocytes (Bossuyt et al., 1996). We are now investigating these putative uptake systems in the hippocampus of guinea-pigs and in LMCAT cells.

To our knowledge, we have been the first to describe a functional membrane transport of glucocorticoids in neurones. Human neuroblastoma cells express MDR PGP (Kurowski & Berthold, 1998), and cells from the mouse hippocampal cell line HT22 express a membrane steroid transporter that is blocked by verapamil (Herr et al., 2000). However, no immunolabelling of PGP has been found in adult rat brain neurones (Matsuoka et al., 1999), although PGP and PGP-like transporters can be expressed at very low levels that are undetected by immunocytochemistry (Kralli & Yamamoto, 1996; Marsaud et al., 1998). Interestingly, immunoreactive and mRNA signals for another family of membrane transporters, the multidrug resistance-associated proteins (MRPs), have been found in rat neuronal cultures (Hirrlinger et al., 2002) and in dysplastic neurones from brain samples of epileptic patients (Sisodiya et al., 2002). The MRPs are organic anion transporters that participate to MDR (Leslie et al., 2001). The function and expression of MRPs are modulated by cortisol (Mulder et al., 1996) and MRP-overexpressing cells are resistant to the effects of

cortisol (Bergman et al., 2001). At present, however, the nature of the cortisol trans-
porters in rat neurones is unclear.

5.2. A model for the in vitro effects of antidepressants on the GR

We suggest the following model for the in vitro effects of antidepressants on the
GR. Treatment with antidepressants inhibits steroid transport, induces GR translo-
cation, and reduces GR expression (Pariante et al., 1997, 2001a, 2003a,b). These
three effects could be mediated by the same mechanism. For example, blocking of
steroid transport can increase the intracellular levels of steroids from the media, thus
leading to translocation of the GR. (Even if we "charcoal-strip" the serum used in
cell culture media to reduce the amount of endogenous steroids, this process cannot
be absolute.) Data showing that MDR PGP inhibitors induce partial GR translocation
in HeLa (human ovarian cancer) cells support this possibility (Prima et al., 2000),
although a recent study found no effect of verapamil on GR translocation in human
lymphocytes (Okuyama-Tamura et al., 2003). Antidepressants could also induce GR
translocation through their effect on cAMP-dependent protein kinases (Rangarajan
et al., 1992; Chen & Rasenick, 1995; Miller et al., 2002). Translocation of GR, in
turn, could lead to the reduction in GR expression. In fact, GR translocation by both
GR agonists and GR antagonists has been associated with GR downregulation. This
downregulation takes place over few hours and is due to a reduction in the protein
half-life and an inhibition of GR mRNA synthesis; it is temporary and can be fol-
lowed by a subsequent upregulation (Schmidt & Meyer, 1994). Therefore, it is poss-
ible that the inhibition of the transporters precedes the GR downregulation. Similar
to our study, treatment for 24–48 h with various antidepressants has been shown to
induce GR translocation or downregulation of GR mRNA in hippocampal cell cul-
tures (Okugawa et al., 1999; Yau et al., 2001) and human white blood cells (Heiske
et al., 2003; Okuyama-Tamura et al., 2003); this GR downregulation is followed by
an upregulation after 4–14 days of treatment (Okugawa et al., 1999; Lai et al., 2003).

If cells are co-incubated for 24 h with antidepressants and a glucocorticoid that
is expelled by the transporter, like dexamethasone or cortisol, an enhanced GR-
mediated gene transcription is evident (Pariante et al., 1997, 2001a, 2003b). This is
because the increase in the intracellular levels of the glucocorticoid overcomes the
GR downregulation, and possibly precedes the GR downregulation. However, if cells
are treated in experimental conditions that do not elicit the effects on the transporter,
the GR downregulation leads to a reduced GR-mediated gene transcription. This
would explain why antidepressants give a reduction in GR-mediated gene transcrip-
tion when cells are co-incubated with antidepressants and corticosterone (Pariante et
al., 2001a), or with dexamethasone and verapamil (Pariante et al., 2001a), or treated
with the antidepressants alone (Miller et al., 2002). Finally, pre-incubation of cells
with an antidepressant inhibits GR-mediated gene transcription induced by a sub-
sequent short treatment (1.5–2 h) with dexamethasone or corticosterone (Pariante et
al., 1997; Budziszewska et al., 2000). In this case, even if the inhibition of the
transporter increases the intracellular levels of dexamethasone (as suggested by our
experiments with ^{3}H-cortisol), these are unable to compensate for the GR downregul-

ation, possibly because of the short incubation, or possibly because the GR downregulation is present before the glucocorticoid is added (differently than in the co-incubation experiments).

It is of note that fluoxetine seems different from the other antidepressants tested in the fact that, after 24 h, it does not reduce GR-mediated gene transcription in the presence of corticosterone or in the presence of dexamethasone and verapamil (Pariante et al., 2003b). However, data from other laboratories show that fluoxetine reduces GR mRNA in vitro after longer incubation (Lai et al., 2003). Fluoxetine is different from the other antidepressants also because it is not transported by PGP, while tricyclic antidepressants as well as citalopram are transported (Uhr et al., 2000; Uhr & Grauer, 2003). Not all PGP inhibitors are transported by PGP; for example, progesterone is a potent inhibitor but it is not transported (Ford, 1996). It is possible that fluoxetine has a different mechanism for its effects on the transporter.

A recent important paper offers further support to our model. Herr et al. (2003) have confirmed, in the mouse hippocampal cell line HT22, that chemically unrelated antidepressants (including desipramine, clomipramine and fluoxetine) increase GR-mediated gene transcription in the presence of dexamethasone. Moreover, they have shown that treatment of cells with desipramine in the presence of verapamil (20 μM) reduces (although does not abolish) these enhancing effects of desipramine on GR-mediated gene transcription. It is of note that the concentration of verapamil used in this paper (20 μM) is lower than the concentration used in our work (100 μM), and this could explain the residual effects of desipramine. In fact, previous studies have shown, at least in LMCAT cells, that verapamil induces only partial inhibition of the cells steroid tranporter at concentrations of 10 μM and 50 μM (Marsaud et al., 1998; Medh et al., 1998); moreover, our original dose-finding experiments in these cells have shown that verapamil (100 μM) induces a larger potentiation of GR translocation in the presence of dexamethasone than verapamil (50 μM) (Pariante et al., 2001b). Therefore, we have consistently used verapamil (100 μM) in our work (Pariante et al., 2001a, 2001b, 2003a, 2003b). However, we cannot exclude that the residual effects of desipramine on GR function in these hippocampal cells are due to activation of other intracellular pathways leading to enhanced GR function.

5.3. A model for the effects of antidepressants in animals

As we discussed above, mice that are knockout for mdr1a and mdr1b PGP show increased access of corticosterone to the brain and increased negative feedback on the HPA axis by corticosterone (Uhr et al., 2002; Muller et al., 2003). By inhibiting membrane steroid transporters, antidepressants could directly increase the access of corticosterone, or of other steroids that are transported by PGP like aldosterone (Ueda et al., 1992), to the brain of mice, thus enhancing GR and MR activation. Although in the brain the highest expression of PGP has been found at the BBB (Lee et al., 2001), our data show that this effect could also occur directly at neuronal level (Pariante et al., 2003a). In turn, the increased access of glucocorticoids to the brain could lead to the decrease in HPA axis activity and the GR and MR downregulation described in rats after 3–9 days of treatment with antidepressants (Reul et al., 1993;

Yau et al., 2001). This receptor downregulation is consistent with our data in vitro and, theoretically, could explain part of the lag time seen with antidepressants for the onset of their therapeutic action. After 14 days of more of treatment, antidepressants induce GR and MR upregulation in the brain, as a compensatory mechanism following the initial downregulation or as a consequence of the reduced HPA axis activity. Consistent with our model, pre-treatment with nifedipine, an MDR PGP inhibitor, prevents the hippocampal GR upregulation induced by antidepressants (Przegalinski et al., 1993). This antidepressant-induced reduction in circulating corticosterone levels could also participate to the neuroprotective effects of these drugs (Duman et al., 2001).

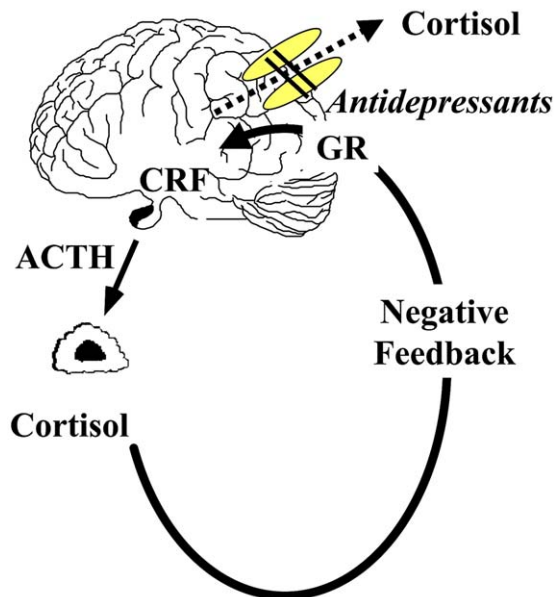## 5.4. In conclusion, a model for the effects of antidepressants in humans

Fig. 2. A model of the effects of antidepressants on MDR PGP, cortisol access to the brain and regulation of HPA axis function in humans The CRF in the hypothalamus stimulates the secretion of ACTH from the pituitary, which stimulates the secretion of cortisol from the adrenal gland. In turn, the circulating cortisol binds to the GR in the brain to exert its negative feedback on the HPA axis. Plasma cortisol cannot freely enter the brain by passive diffusion (continuous arrow), because its access is limited by MDR PGP and other membrane steroid transporters localised at the BBB and possibly in neurones. These transporters capture cortisol from the apical membrane of the endothelial cells of the BBB, while it is entering the cells by passive diffusion, and expel the hormone back into the plasma (dotted arrow). Therefore, membrane steroid transporters seem to participate in the regulation of GR-mediated negative feedback and HPA axis activity. Antidepressants may inhibit membrane steroid transporters at the BBB and in neurones, so that more cortisol is able to enter the brain. This leads to increased activation of brain GR (and MR), increased negative feedback on the HPA axis and, finally, normalisation of HPA axis hyperactivity in depressed patients.

We have demonstrated that antidepressants regulate the intracellular levels of cortisol by inhibiting membrane steroid transporters, in LMCAT cells and neurones, that are similar to MDR PGP in their substrates and modulators. Cortisol, the endogenous glucocorticoid in humans, is transported by the PGP at the BBB (Karssen et al., 2001). We propose that antidepressants in humans inhibit the steroid transporters localised on the BBB and in neurones, and thus increase the access of cortisol to the brain. In turn, facilitation of GR and MR activation by antidepressants may lead to an increased negative feedback by circulating glucocorticoids on the HPA axis, and then to resolution of glucocorticoid hypersecretion (see model in Fig. 2). This model is supported by studies showing that HPA hyperactivity in depressed patients begins to normalise after 7–9 days of antidepressant treatment, and that this precedes the therapeutic effects on depressive symptoms (Heuser et al., 1996; Holsboer, 2000). Moreover, this model is consistent with clinical studies showing that treatment with GR and MR agonists, including cortisol, has antidepressant effects in humans (Dinan et al., 1997; Bouwer et al., 2000; DeBattista et al., 2000). Hypothetically, the increased access of cortisol to the brain could balance the decreased function and expression of corticosteroid receptors in the brain of depressed patients. Or, perhaps, this effect could compensate for a "reduction of cortisol levels" in the brain of depressed patients, as described by Brooksbank et al. (1973). We believe that enhanced cortisol action in the brain might prove to be a successful approach to maximise therapeutic antidepressant effects (Pariante, 2003).

Acknowledgements

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