Antiglucocorticoids, Neurogenesis and Depression

Carlos P. Fitzsimons[1], Lenneke W.A. van Hooijdonk[1], John A. Morrow[1], Bernard W.M.M. Peeters[3], Niall Hamilton[2], Mark Craighead[2] and Erno Vreugdenhil[1,*]

[1]*LACDR/Medical Pharmacology Dpt., Gorlaeus Laboratories, Einsteinweg 55, 2333CC, Leiden University, The Netherlands;* [2]*Department of Molecular Pharmacology, Organon Laboratories Ltd, part of Schering-Plough Corporation, Newhouse, Lanarkshire, ML1 5SH, United Kingdom;* [3]*Department of Medicinal Chemistry, N.V. Organon, part of Schering-Plough Corporation, Oss, PO Box 20, 5340BH, The Netherlands*

Abstract: Recent evidence suggests that antiglucocorticoids, like conventional antidepressants, may recover depressive symptoms by boosting hippocampal neurogenesis. Here, we explore several possible antiglucocorticoid-based antidepressive therapeutic strategies. Firstly, we review specific glucocorticoid receptor/antagonist interactions. Secondly, we discuss a potential new therapeutic target, doublecortin-like kinase, which regulates glucocorticoid signaling in neuronal progenitor cells.

Key Words: Stress, glucocorticoid receptor, dentate gyrus, hippocampus, neurogenesis, antidepressants, depression, doublecortin-like kinase, anti-glucocorticoids.

SECTION I: STRESS AND THE HPA AXIS

Introduction

Physiological and behavioral adaptations in response to (acute) stress are essential to animals for survival, coping and recovery. These adaptations, are mediated by the activation of a neuroendocrine cascade; the hypothalamo-pituitary-adrenal (HPA) axis (for review see [1, 2]). Activation of this essential system by a stressor ultimately leads to an increase in the secretion of glucocorticoids (GCs; cortisol in humans and corticosterone in rodents) from the adrenals in a diurnal rhythm [3]. In addition to modulation of immune and metabolic function, an important target for circulating GCs is the central nervous system, in particular the hippocampus. Here, steroids regulate brain physiology by modulating a broad range of neural functions. All these functions of GCs are mediated through binding to two similar, intracellular transcription factors termed the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR; [4, 5]). MRs display a high affinity for GCs (K_d~0.5nM) and show a restricted distribution within the brain, with high abundance in some limbic areas, like the hippocampus and in motor nuclei of the brain stem [2]. MRs are activated by low levels of GCs and accordingly it is thought that the MR mediates more 'tonic' actions of corticosteroids involving the maintenance of homeostasis, circadian fluctuations, the sensitivity of the stress response, and the organization of the behavioral response to stress [5-7].

GRs in contrast, have a tenfold lower affinity for GCs and are expressed in nearly all brain regions and in both neurons and glia. As a consequence, the majority of GRs become substantially occupied at elevated levels of hormone [6-8]. In this context, the GR is involved in the 'phasic' actions of the GCs, aimed at counteracting stress-induced disruption of homeostasis.

In cells where MRs and GRs are coexpressed, physiological fluctuations in GC level will range from a situation of predominant MR activation when the organism is at rest and at the circadian nadir, to concomitant MR and GR occupation after stress or at the circadian peak. This is particularly relevant in the hippocampus where GCs act in a dose and time-dependent fashion. Both MR and GR are co-expressed at high levels in the hippocampal CA1, CA2 and dentate gyrus subfields [7, 9-16]. Both receptors are involved in the negative feedback regulation of the HPA axis to prevent overproduction of GCs [17]. In addition, MR and GR work in a coordinated fashion to maintain hippocampal function, neuronal viability and integrity [1, 18-20]. Moreover, GCs and their receptors influence processes including neuronal excitability and plasticity, neuronal death, stress reactivity and cognitive, emotional and physiological responses to ultimately maintain or restore homeostasis [1, 21-23]. In turn, optimal function of the hippocampal formation is critical for modulation of the HPA axis and regulation of the stress response [24, 25].

Stress and Depression

There is strong evidence that in genetically predisposed or otherwise vulnerable individuals, chronic stress, HPA axis hyperactivity and ultimately aberrant negative feedback by e.g. GR resistance [26-28] is a primary, causal factor in the pathogenesis of stress-related neuropsychiatric disorders such as major depressive disorder (MDD; [21, 29-32]).

Depression is a serious multifactorial disorder with a complex clinical nature [33]. The diversity of symptoms suggests that multiple neuronal circuits are likely to be involved. Typically, a number of these behavioral and psychological symptoms have features similar to stress and elevated

*Address correspondence to this author at the LACDR/Medical Pharmacology Department, Leiden University, Gorlaeus Laboratory, Einsteinweg 55, 2333CC Leiden, The Netherlands, Tel: ++31-71-527-6230, Fax: ++31-71-5274715; E-mail: vreugden@lacdr.leidenuniv.nl

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GCs. Exogenous GCs, such as cortisol and prednisone, particularly when given at high doses for extended periods of time, produce symptoms that include depression, hypomania, insomnia, cognitive deficits and psychosis [34, 35]. Similar abnormalities in HPA function and depression-like behavior are observed in animal models of chronic stress [36-38].

Major stressful or traumatic events seem to precede or even trigger depressive episodes, and about 50% of the depressive patients display hypercortisolemia, which appears to exist prior to the onset of clinical symptoms of depression [21, 39, 40]. There also appears to be a direct correlation between the severity of symptoms and circulating cortisol levels [41, 42]. This conclusion is strengthened by observations in patients suffering from depression secondary to Cushing's disease. Cushing's disease is linked to HPA axis hyperactivity, and typically, patients often also suffer from anxiety and depression and in some cases from psychosis and suicidal thoughts [43].

Typical observations done in depressed patients with a hyperactive HPA axis are: reduced GR function as tested in the dexamethasone (DEX) suppression test, elevated amplitudes of cortisol secretory periods [44, 45], an increased frequency of adrenocorticotropic hormone (ACTH) secretory episodes [46], and several other aberrations at different levels of the neuroendocrine system [24, 47-49]. These symptoms of HPA hyperactivity can typically be reversed with antidepressant (AD) treatment in both humans and animal models [50].

Studies in animal models have shown that exposure to high GCs or chronic stress induce neuronal damage that selectively affects the hippocampus. However, the GCs neurotoxicity hypothesis emerging from these observations is not fully supported by all clinical observations in humans. In recent postmortem studies in patients treated with corticosteroids and patients who had been seriously and chronically depressed no indications of massive cell loss could be found, while the incidence of apoptosis was rare, therefore suggesting no major irreversible damage in the human hippocampus. In addition, various studies in animal models failed to find massive cell loss in the hippocampus following exposure to stress or steroids, but rather showed adaptive and reversible changes in structural parameters after stress (recently reviewed in [51]).

Thus, although a direct causality in between HPA axis hyperactivity and depression is still circumstantial, the GR function is altered in major depression. Moreover, some ADs have direct effects on the GR [52] and potential novel ADs, as galanin, modulate HPA axis activity and enhance GCs secretion, suggesting a tight interaction with the GR/GCs system [53, 54]. Altogether, a beneficial effect on depressive symptoms by intervening on the glucocorticoid system is suggested.

Antiglucocorticoids as Antidepressants

As outlined above, excessive glucocorticoid levels and consequently chronic GR activation is associated with the pathogenesis of depression. Therefore, a potentially innovative strategy for the treatment of depression is to identify novel classes of drugs that act on the stress system by blocking GR activation. To this end, several antiglucocorticoids have come under investigation. Additionally, modulators of the HPA-axis often approved for different clinical applications, can transiently block the synthesis of GCs in the adrenals or block the access of GCs to their receptors in the brain and are associated with beneficial effects in the treatment of depression. These include cortisol synthesis inhibitors such as ketoconazole [55-59], and metyrapone [60], corticotropin-releasing factor (CRF) antagonists [32, 61] and GR antagonists such as mifepristone [47, 62-64].

Interestingly, short-term treatment (4 days) with mifepristone has been successfully applied to treat/ameliorate depression in clinical trials. It was found that mifepristone reduced depressive symptoms in a subset of severely depressed patients with highly elevated GC levels. In this respect, there is evidence that mifepristone is especially helpful in treating MDD [63], Psychotic Major Depression (PMD; [62, 63, 65-67] and psychotic depression secondary to Cushing's disease [68, 69]. Given the fact that PMD patients tend to be the most resistant to the effects of traditional antidepressants, these findings seem even more promising [65, 70]. Therefore, patients who are unresponsive to antidepressants alone and only partially responsive to an antidepressant–antipsychotic combination may benefit from treatment with GR antagonists.

However, only high doses of mifepristone are effective [65, 71], and these doses are often associated with adverse drug effects, although not uniformly across patient populations. These adverse effects include fatigue, anorexia and nausea [72]. Even higher doses are associated with skin rash, endometrial hyperplasia and hot flashes in women [71]. Other inhibitors of HPA axis function are associated with severe side effects, as well [73]. Another concern is mifepristone's clinical efficacy [74], as highlighted by studies that compared treatment with mifepristone to placebo or other antidepressive and antipsychotic treatments [75].

SECTION II: RECENT ADVANCES IN GR ANTAGONISTS: STRUCTURE-FUNCTION RELATIONSHIPS

Introduction

Within this section we will review the current information available on drugs with antagonistic activity on the GR and their structure function relationships. Therefore, to avoid misunderstanding generated by inappropriate use of pharmacology terms, we will attach to the definitions suggested by the International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification [76].

While there are many marketed GR agonists [77] there is still no drug approved specifically as an antiglucocorticoid. Mifepristone (RU-486) (**1**) is a potent antiprogestagen that is marketed as an abortifacient and at higher doses it is also an antiglucocorticoid. It is currently in phase III clinical trials for the treatment of psychotic features of psychotic depression and recently received orphan drug designation for the treatment of Cushing's syndrome, but its interaction with the progesterone receptor (PR) severely limits its utility due to the adverse side effects [78].

Aside from mifepristone, the only GR antagonist being evaluated clinically for treatment of depression is Org 34517

(**2**). Currently in Phase II trials, this steroid is structurally similar to mifepristone with the only difference being the substitution pattern on the 11β-aryl group. The 11β-dimethylaniline of mifepristone is susceptible to oxidative N-demethylation and yields a potent active metabolite, a discovery that has recently been exploited through synthesis of compounds with bile acid or other conjugates in position 11, such as A-348441 (**3**) [79, 80]. This compound retains GR antagonist activity with the conjugated bile acid conferring liver selectivity.

Steroid Receptor Antagonist Selectivity: GR *vs.* PR

Establishing good selectivity for GR over PR through modification of substituents on the steroid nucleus has proved difficult, and with the exception of cyproterone acetate (**4**) all steroids retain the 11β-aryl function that confers antagonism at both GR and PR [81]. For binding to GR fairly marked changes to the steroid are tolerated. For instance RU-43044 (**5**) which has aryl substitution at C-19 rather than 11β, onapristone (**6**) which has unnatural stereochemistry at the 13-position and Org 34850 (**7**) which has a bulky 17α-aryl substituent all retain antiglucocorticoid activity, but selectivity for GR over PR remains an issue. To date no new steroids have yet progressed to the clinic despite some reports citing selective compounds [82-84].

The difficulty of realizing selectivity for GR over PR, coupled with the high doses that adversely impact on cost of goods and the lengthy synthetic routes which hamper introduction of chemical diversity, has led to research for new glucocorticoid antagonists moving away from steroids.

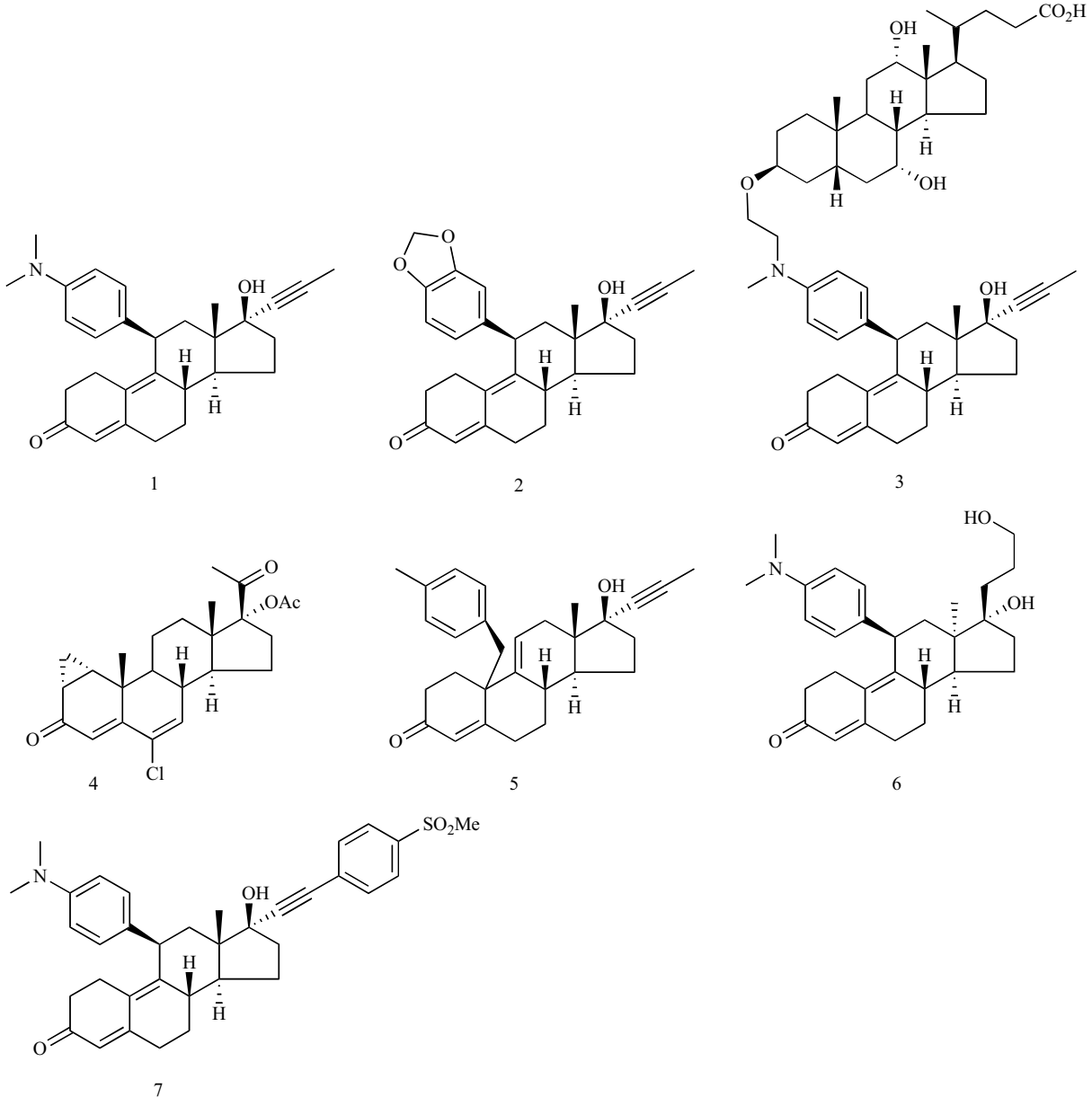

Fig. (1). Chemical structure of steroidal GR antagonists. Numbering corresponds with bold numbers in main text. For further details: see text.

Non-Steroidal GR Antagonists

A large number of different non-steroidal GR antagonists have been identified and progress in this area has been reviewed recently [85]. To derive back-ups to the current clinical compounds with improved selectivity for GR both Corcept (in collaboration with Argenta) [86-90] and Organon (in collaboration with Pharmacopeia) [91] have discovery programs and new GR antagonists/modulators such as the 6-aminopyrimidinediones (**8**), azadecalins (**9**) and arylsulfonamides (**10**) have been identified.

Other GR antagonists reported in the literature include α-methyltryptamine sulfonamides (**11**) [92], dihydroquinolines (**12**) [93], Trifluoromethyl derivatives (**13**) [94], and the weak antagonist **14** [95, 96].

Profiling of these and similar compounds showed that their modulatory effect on GR and their antagonism can differ markedly from the steroids. Improved selectivity for GR over PR was demonstrated for CP-409069 (**15**) and CP394531 (**16**) which were designed from pharmacophore models

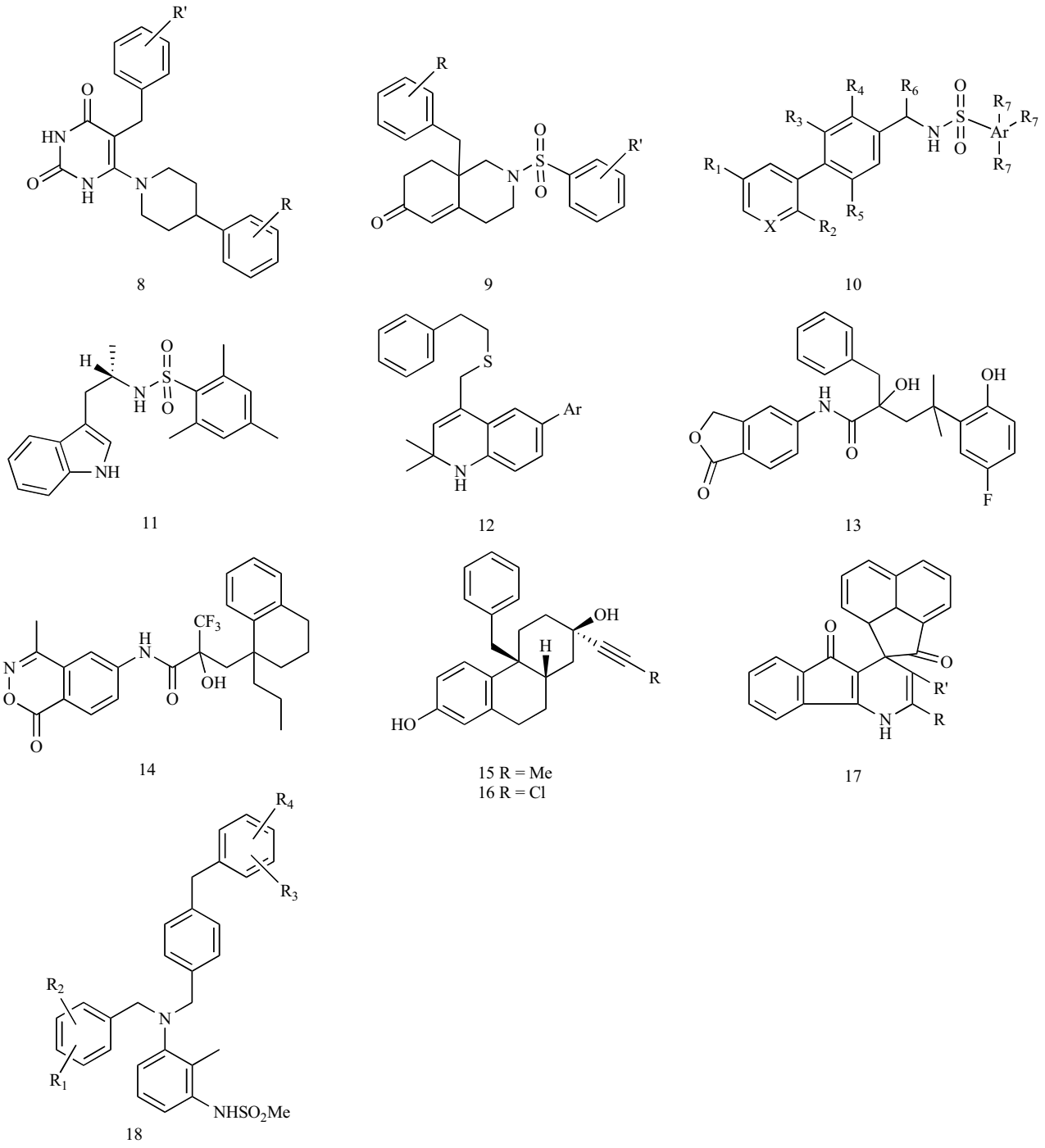

Fig. (2). Chemical structure of non-steroidal GR antagonists. Numbering corresponds with bold numbers in main text. For further details: see text.

based on steroid ligands [97]. The selective antagonism of ligands such as spirocyclic dihydropyridines (**17**) [98] and passive antagonism of sulphonamide (**18**) [99] mean such compounds have utility as treatments for diabetes – indeed the lack of acute activity on the HPA axis argues against utility as treatment for Cushing's syndrome or aspects of depression arising from hypercortisolemia [100, 101]. While a large number of non-steroidal GR antagonists have been discovered there are as yet no reports of clinical studies with these compounds. The furthest progressed appears to be A-348441 (**3**), which is being developed by Abbott and Karo Bio and was due to begin clinical trials for treatment of type II diabetes in 2007, following evaluation of new formulations [102].

Central Nervous System Activity, PET and Biomarkers

The need for proof of concept within the clinic through identification of biomarkers and demonstration of receptor occupancy at clinically relevant doses is challenging for central nervous system (CNS) disorders and more difficult when the target is a nuclear receptor like GR. A number of potential PET ligands have been synthesized in attempts to image GR within the CNS but progress so far has been hampered by non-specific binding [103-105].

Insights into the Mechanism of Action of Different GR Antagonists

A number of possible explanations could account for differential modulatory effects on GR activity produced by steroids like RU-486 and Org 34517, and many of the new non-steroidal compounds shown above [32, 106, 107]. Studies performed at Organon have focused upon elucidating the differences between the two steroidal antagonists, RU486 and Org 34517, being developed as treatments for major depression.

Receptor binding experiments have indicated that the potencies of both compounds are reasonably similar with EC_{50} values of $6.3x10^{-9}$ M for Org 34517 and $2.5x10^{-9}$ M for RU486 [108]. Studies to determine functional antagonism of GR in Chinese hamster ovary (CHO) cells co-transfected with recombinant human GR and a luciferase reporter gene (luc) under the control of the GR responsive mouse mammary tumour virus promoter demonstrated that both Org 34517 and RU-486 inhibited cortisol induced recombinant GR activity with similar potency (Fig. **3A**; [108]). However, RU-486 did not completely block the effect of cortisol, the maximum inhibition observed being less than 60%.

One potential mechanism by which an antagonist could operate is to block GR translocation to the nucleus. In AtT20 cells derived from mouse anterior pituitary which endogenously express GR, Org 34517 had no effect upon translocation while both corticosterone and RU-486 induced a significant translocation of GR from the cytosol to the nucleus. When given in conjunction with corticosterone Org 34517 was able to significantly reduce the translocation of GR (Fig. **3B** and **C**)[1]. These results indicate that Org 34517 and RU-486 have fundamentally different effects upon GR *in vitro* and potentially contribute to explaining the partial agonism that has been observed with the latter. For instance, Zhang and colleagues [109] have reported that increasing the level of recombinant GR in a transfected cell line system resulted in enhanced agonism being observed with RU-486.

Biostructural Approaches to Understanding the Action of GR Antagonists

The crystallization of the GR ligand binding domain (LBD) provides an insight into the basic mechanisms of GR action, receptor dimerization and coactivator recruitment together with transactivation and transrepression of genes. Furthermore, the determination of the binding mode of GR ligands will provide a further understanding of structure activity relationships and will be useful in the design of novel GR modulators, both agonists and antagonists, for therapeutic utility in the treatment of disease.

Like most nuclear hormone receptors, GR consists of three functional domains. The N-terminal activation function-1 (AF-1) domain has a constitutive transcriptional activation and protein-protein interaction role and is also the target for phosphorylation by kinases [110]. The DNA binding domain (DBD), highly conserved amongst all nuclear hormone receptors, is involved in the selective recognition of DNA response elements and receptor dimerization [111]. Finally, the C-terminal of GR contains the LBD, which also has important receptor dimerization and ligand-dependent activation (AF-2) functions.

Structure of the GR LBD and Agonist Binding

Although the first three-dimensional structure of a nuclear receptor was obtained when the structure of the GR DNA-binding domain was solved [112, 113] the structure of the GR LBD proved somewhat elusive because of purification issues relating to the solubility of the protein. It wasn't until investigators took advantage of mutations in the human GR LBD to improve expression levels in recombinant systems that the three dimensional structure of the GR LBD was finally solved [114, 115]. The study by Bledsoe *et al.* [114] described the structure of the GR LBD containing a F602S mutation in combination with DEX and a short peptide representing the transcriptional intermediary factor 2 (TIF2) accessory protein. Kauppi *et al.* [115], using a construct with the N517D, F602S and C638D mutations, subsequently published three different crystal structures of the GR LBD in complex with the antagonist RU-486 as well as the combination of Dex and TIF2.

The GR LBD structures, like those of several other steroid hormone receptor LBDs [116-120] shares a common structural motif with all members of the nuclear hormone superfamily determined to date. The GR LBD comprises 12 alpha-helices (H1-H12) arranged in a three-layer helical sandwich and 4 beta-strands that form two short beta-sheets. Fig. (**4**) shows the agonist conformation of the GR LBD in complex with DEX and the TIF2 peptide. The ligand binding

[1] Peeters, B. W. M. M., Ruigt, G. S. F., Craighead, M., and Kitchener, P. Differential effects of the new glucocorticoid receptor antagonist ORG 34517 and RU486 (mifepristone) on glucocorticoid receptor nuclear translocation in the AtT20 cell line. Ann.NY Acad.Sci. 2008. In Press

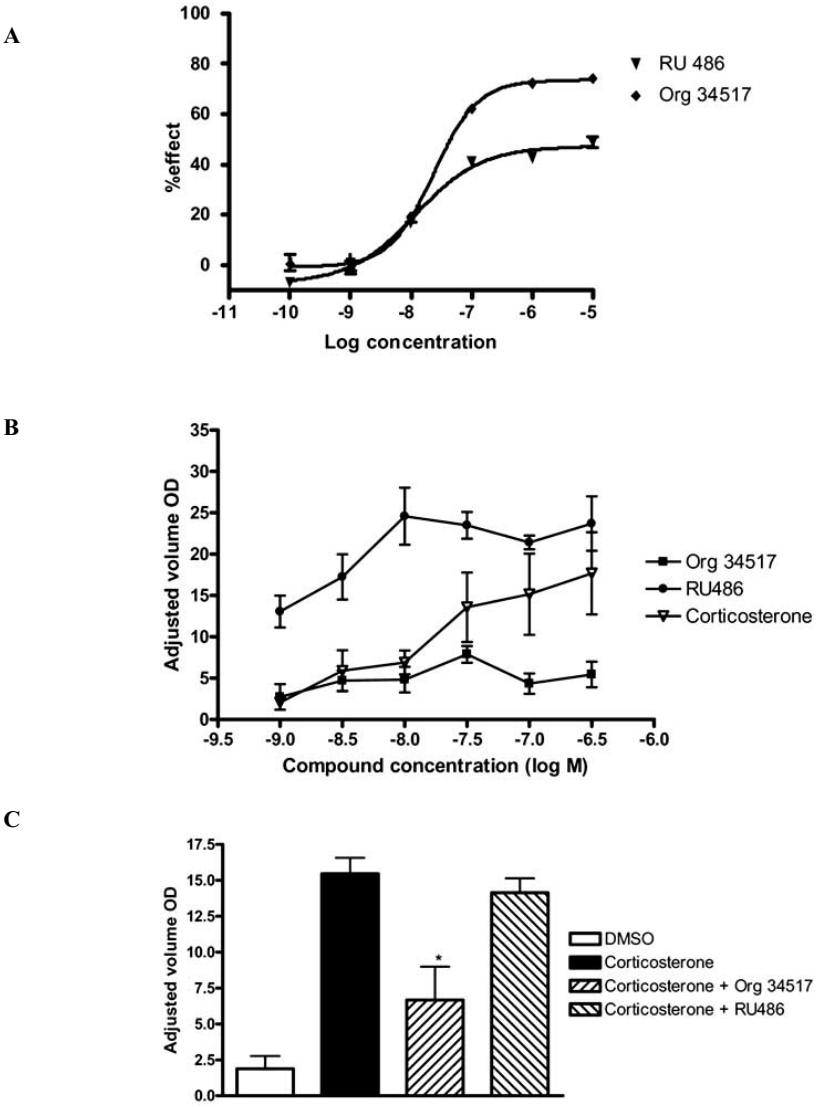

Fig. (3). A: Effect of antagonists in GR transactivation assay. CHO cells transfected with the human GR were incubated with increasing concentrations of Org 34517 and RU486 in the presence of 20nM cortisol (EC50 value) for 16 hours at 37°C. Agonist-induced luciferase expression was detected using a luclite luciferase luminescence kit (Perkin Elmer). The data shown is a typical experiment and each point was determined in triplicate. The experiment was carried out at least 3 times with essentially identical results. **B**: Effect of 3 GR ligands on GR nuclear translocation in the AtT20 cell line. AtT20 cells were treated with Org 34517, RU-486 and corticosterone at increasing doses for 1 hour. The extent of translocation was subsequently determined by sub-cellular fractionation and measurement of GR levels in the nucleus and the cytoplasm by western blotting and densitometric analysis. **C**: Effect of co-treatment with corticosterone and Org 34517 or RU-486. AtT20 cells were co-treated with $3x10^{-8}$M corticosterone and $3x10^{-7}$M Org 34517 or $3x10^{-7}$M RU-486 for 1 hour and then the extent of translocation of GR to the nucleus was determined by sub-cellular fractionation and measurement of GR levels in the nucleus and the cytoplasm by western blotting and densitometric analysis. Results in B and C are expressed as normalized optical density (NOD), used as a measure of GR present in the nucleus. In all cases, the data represents the mean ± SEM. * $p<0.01$ versus corticosterone alone (two tailed T-test).

pocket is predominantly hydrophobic in nature, though both polar and non-polar residues play specific roles in ligand recognition (reviewed in detail by Bledsoe *et al.* [121]). In contrast to other steroid hormone receptors, the GR LBD has a unique branched side-pocket beside the core steroid shaped pocket as a result of the presence of a proline residue (P637) in the linker between helices H6 and H7. The resulting shift in H6 and H7 to form the pocket allows Q642 of H7 to form a hydrogen bond with 17alpha-hydroxyl groups in GCs. The lack of a 17 alpha-hydroxyl group in mineralocorticoids provides an explanation as to why GR fails to bind these steroids. Futhermore, the observation that the MR pocket is more compact than the GR pocket provides an explanation as to why corticosterone and cortisol bind MR with higher affinity than GR [120].

A key feature of the binding of agonists to nuclear receptors is the induction of a conformational change in helix12 (also referred to as the activation function 2 (AF-2) helix), revealing a recognition site groove for interaction with coactivators [122]. Helix 12 forms the lid of the ligand binding pocket and in the GR/DEX structure it is positioned close to

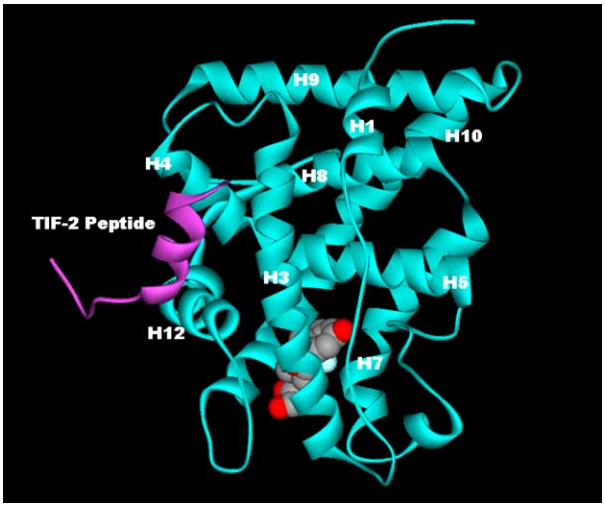

Fig. (4). Structure of the GR LBD in complex with dexamethasone. Coordinates 1M2Z.pdb from Bledsoe et al [114]. The TIF-2 peptide is colored purple. Helix number is based on historical numbering of previously published NR LBD structures. Consequently, this structure has no H2 and H11.

H3, H4 and H10. The ligand directly contributes to stabilization of this conformation through interaction with residues on helix 12 such as L753 and is considered crucial in maintaining the agonist conformation. The location of H12 (Fig. **4**) in this position completes an exposed hydrophobic channel consisting of residues on H3, H4 and H12 which serves as a co-factor binding site.

Active Antagonism of GR by RU-486

The GR LBD/RU-486 crystal structure [115] has demonstrated that the antagonistic action of RU-486 is not just mediated by simple blockade of agonist binding but is an active process involving displacement of H12 from its normal agonist conformation. In this structure (Fig. **5**) the RU-486 molecule is bound in the same general way as other (agonistic) steroid hormone molecules in nuclear receptors, however, the 11beta-dimethylaniline group of RU-486 protrudes into the space where H12 sits in the agonist structure. This steric interference results in the displacement of H12. A similar displacement is observed in a number of other nuclear receptor antagonist complexes such as Oestrogen receptor (ER) alpha/raloxifene, [117], ERbeta/raloxifene [118] and ERbeta/hydroxytamoxifen [123]. Not all GR antagonists are likely to act in such an active fashion however. Cyproterone acetate, for example, [81] lacks the bulky side chain at position C11beta in RU-486, required for displacement of H12, and most likely confers cyproterone acetate's effect *via* 'passive' antagonism as described with the R,R enantiomer of 5,11-cis-diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol (THC)-ERbeta complex [124]. Nevertheless, cyproterone acetate is able to induce nuclear translocation of the androgen receptor THC also lacks a bulky side chain and the crystal structure of THC-ERbeta did not reveal any steric displacement of H12. Thus, cyproterone acetate, like THC, has been proposed to antagonise ERbeta by stabilizing an inactive conformation of the receptor. Nevertheless, cyproterone acetate is able to induce nuclear translocation of the androgen receptor, which questions its "passive" antagonistic properties [125].

Fig. (5). Structure of the GR LBD in complex with RU486. Coordinates, 1NHZ.pdb from Kauppi et al [115]. H12 is clearly displaced outwards from the main body of the LBD by RU486 thereby eliminating the cofactor binding site.

The overall picture of the GR LBD is one of an extremely dynamic and adaptable structure that is able to bind many related but distinct ligands and perform a diverse array of signalling activities. Not only will these structures provide the basis for understanding structure-activity relationships in the design of novel anti-glucocorticoids, they also suggest alternate ways of modulating GR function such as blocking co-factor recruitment. Indeed, proof-of-principle of this has been provided by the demonstration that ERalpha and ERbeta are effectively antagonised by the small peptides containing the cofactor LXXLL motif critical for cofactor interaction with the nuclear receptor [126]. A similar strategy using a peptidomimetic drug may well provide an effective alternative means of antagonizing GR function.

SECTION III: STRESS, NEUROGENESIS AND ANTI-GLUCOCORTICOIDS

Introduction

During the last decade, it has become clear that adult neurogenesis occurs in the dentate gyrus of the hippocampus. The rate of neurogenesis is decreased by chronic stress-induced elevated levels of adrenal corticosteroids, a symptome observed in many depressed patients. Moreover, as mifepristone and other antidepressants increase neurogenesis, the rate of proliferating progenitor cells and the incorporation of newly formed neurons in existing networks might be involved in the pathogenesis of depression. Although increasing evidence points to a role of neurogenesis in hippocampal function, i.e. learning and memory formation, the existence of a causal relationship between neurogenesis and depression continues to be controversial (for a recent review see [127]). In the remaining part of this review, we will highlight the role of GCs and other factors on hippocampal neurogenesis and its possible relevance for depression and antidepressant action. Finally, we will propose alternatives to block maladaptive glucocorticoid signaling in neuronal progenitor cells as potential future therapy for neurogenesis-related mood disorders.

Stimulatory and Inhibitory Effects of GCs on Cell Proliferation and Adult Neurogenesis

GCs have been shown to be critically involved in the inhibition of proliferation and differentiation of neuronal progenitors, and also the survival of young neurons [128, 129]. This is in line with the general picture of systemic growth inhibition by GCs, hypothesized to be an adaptive strategy in times of stress. However, the mechanism(s) through which stress and GCs act on neurogenesis are still largely unknown.

The context, time course, duration, and level of GCs and stress are essential factors affecting neurogenesis. Removal of circulating GCs following adrenalectomy (ADX) increases cell proliferation and neurogenesis in young adult and aged rodents [130-133]. These increases can be reversed by treating ADX animals with a low replacement dose of corticosterone [134, 135].

In contrast, excess levels of GCs, due to stress or treatment with exogenous GCs, results in structural changes in the hippocampus and a decrease in cell proliferation and neurogenesis both *in vitro* and *in vivo* [2, 3, 136-141].

However, it is interesting to note that under certain circumstances such as learning [142], exposure to an enriched environment [143, 144], or voluntary physical exercise such as running [145-150], elevated GC levels are associated with enhanced neurogenesis [151-154].

Depression and Neurogenesis

According to the neurogenic and neuroplasticity hypothesis of depression, a decrease in hippocampal neurogenesis is related to the pathophysiology of depression while enhanced neurogenesis is necessary for the treatment of depression [155-161]. However, thus far there is no clear evidence that the reduction of neurogenesis is causally related to the etiology of depression [162, 163].

Nevertheless, decreased neurogenesis could affect neuronal function in the hippocampus in different ways [162] with secondary consequences for other brain structures involved in the pathophysiology of depression such as the prefrontal cortex, amygdala, and nucleus accumbens [2, 3, 6, 141, 164]. Moreover, all these structures are modulated by GCs [165]. One way in which impaired neurogenesis could lead to depression is by weakening the mossy fiber pathway connecting the dentate gyrus and the CA3 region in the hippocampus. As the mossy fiber synapses are involved in controlling the dynamics of excitation and inhibition within CA3 [166], a decreased dentate gyrus-CA3 connectivity could result in a downward spiral leading to impaired learning and decreased possibility of coping with a complex environment, further impairing neurogenesis. In fact this hypothesis is strikingly similar to what is observed in depressive patients: they show aversion to novelty and withdrawal from normal activities and challenges, which trap them in a vicious circle [157, 165, 167].

Antidepressants and Neurogenesis

Typically, both HPA hyperactivity and impaired neurogenesis can be normalized with antidepressant treatment. One plausible explanation is through the action of antidepressants on the serotonergic system [168, 169]. Although it is not clear how ADs influence the maturation of immature neurons, serotonin selective reuptake inhibitors (SSRIs) are known to induce expression of growth factors and neurotrophins [167, 170-172]. In addition, ADs may also function by facilitating GR-mediated feedback inhibition of the HPA axis [173]. However, there are probably also other mechanisms through which antidepressants display their differential modes of action [174].

Several other non-serotonergic classes of antidepressants including norepinephrine selective reuptake inhibitors, monoamine oxidase inhibitors, phosphodiesterase inhibitors, lithium and electroconvulsive shock therapy augment cell proliferation and neurogenesis as well [175-179] and often restore HPA function in both humans [21, 180-183] and animal models [7, 184-188]. In fact, this correlation between HPA axis function and AD effects is reinforced by the observation that distorted HPA axis diurnal rhythms prevented antidepressants to stimulate cell proliferation and hippocampal neurogenesis in rats [189].

Remarkably, the delayed therapeutic actions of all major classes of marketed ADs (which take two to four weeks to develop [190]) coincides with the timescale of hippocampal neurogenesis and neuroplasticity [191, 192]. It is notable that the induction of cell proliferation and neurogenesis is contingent upon chronic but not subchronic (acute) SSRI treatment [37, 169, 179, 193-195]. Moreover, the unique physiological properties of adult-born dentate granule neurons, in terms of their location within the hippocampal neuronal circuitry and their functional plasticity, suggests adult neurogenesis as a potential common pathway underlying the functional effects of antidepressants [162, 196].

Mature adult-born neurons may also contribute to the behavioral effects of SSRIs. This is in line with the observations that enhancing neurogenesis is necessary to exert antidepressant-like effects in animal models [163, 194, 197-199]. A recent study has shown that from a group of rats exposed to chronic stress, only a subset responded behaviourally to SSRI treatment [200]. Interestingly, neurogenesis was restored to normal levels only in the behaviourally identified responders. However, in another study, a dissociation was found between the effects of stress on adult neurogenesis and learned helplessness [201].

The consistent observation that different classes of ADs with distinct mechanisms of action block the behavioral effects of stress and restore normal levels of adult hippocampal neurogenesis supports the possibility that increasing neurogenesis is a common pathway through which ADs exert their behavioral and therapeutic effects [162, 171, 202, 203]. However, arguing against a crucial role for neurogenesis in anti-depressant action are studies by Meshi *et al.* [204] and Holick *et al.* [205] who found that AD-like behavioral effects can also be achieved in the absence of neurogenesis.

Interestingly, the positive effects of both fluoxetine and the nitric oxide inhibitor L-NAME on hippocampal neurogenesis require rhythmic changes in corticosterone levels, strongly suggesting that concurrent manipulation of the glu-

cocorticoid system may improve sensitivity to AD treatment [206, 207].

SECTION IV: NEW ANTI-DEPRESSANTS ARE NEEDED

Introduction

Although current antidepressants offer many patients marked improvement of the disease state, there is still a high unmet need in the treatment of depression. Foremost amongst these is the need to improve treatment of patients who fail to respond to any of the currently marketed antidepressants. The NIMH uses figures of 20-30% for depressive patients who do not respond to current treatments, with a further 30-40% (i.e. a total of 50-70%) of patients not recovering satisfactorily [31]. Another area for improvement is the poor side effect profile of many of the 'serotoninergic' antidepressants. One of the major side effects of some current antidepressants is sexual dysfunction, but common side effects also include nausea, headache, tremor, vomiting and weight gain.

Similar to most ADs, the beneficial effects of antiglucocorticoids may be mediated by interference with neurogenesis. Importantly, pharmacological blockade of the GR can fully normalize the reduction in cell proliferation and/or survival produced by elevated corticosterone [208-210]. Similar effects on increased cell proliferation and adult neurogenesis were found in animal models using other methods of inhibiting HPA axis activity, such as blockade of CRF-1 or V1b receptors [153, 211]. But contrary to other compounds interfering with the effects HPA axis activation on neurogenesis, the normalizing effects of mifepristone are rapid and selectively potent in a high-stress environment [209]. The short time frame for the cellular effects of mifepristone on neurogenesis after chronic stress parallels its fast acting effects in clinical studies where 4 days of treatment was reported to relieve symptoms of psychotic depression [62, 65, 66]. Thus, the hypothesis emerging from these studies is that blockade of glucocorticoid receptor in neuronal progenitor cells might relieve depressive symptoms.

Targeting the Glucocorticoid Receptor in Neuronal Progenitor Cells: The Role of the Doublecortin-like Kinase (DCLK) Gene

Most antidepressants will enhance neurogenesis and it has been shown for some of them that this enhancing effect is a prerequisite for their antidepressant effects. As mentioned before, the anti-glucocorticoid mifepristone quickly normalizes chronic stress-induced or chronic corticosterone-induced reduction of adult neurogenesis [208, 209]. Thus blockade of the GR in neuronal progenitor cells might be of clinical importance for the treatment of depression. If this is the case, neuronal progenitor cell-specific genes, which are involved in GR action may form alternative targets for pharmaceutical experimentation aiming to block GR activity in a cell-specific manner. Interestingly, we recently have characterized one such gene-product, doublecortin-like (DCL), that controls activated GR transport to the nucleus and which is specifically expressed in neuronal progenitor cells [212]. Here, we will briefly review our understanding of DCL and its interaction with the GR.

The mouse DCL mRNA encodes a protein of 362 amino acids that shares 73% sequence identity over the entire length with doublecortin (DCX), a microtubule-associated protein (MAP, [213, 214]) that is involved in neuronal migration. As DCX, DCL is a MAP specifically expressed during corticogenesis. However, we have demonstrated that unlike DCX, DCL is expressed at embryonic day (ED) 8 onwards throughout the early neuroepithelium. It is localized in mitotic cells, radial glia cells (RGC), particularly in their radial cellular processes [212]. DCL knockdown using RNA-interference *in vitro* and *in vivo* induces spindle collapse in dividing neuroblasts, whereas overexpression results in elongated and asymmetrical mitotic spindles. These indicate a role for DCL in the stability of mitotic spindle [212, 215]. DCL knockdown by in utero electroporation and RNA-interference, significantly reduces cell number in the inner proliferative zone and dramatically disrupts most radial processes, indicating a role in the stability and formation of the radial glia scaffold that guides the migration of newly born cells [212]. Interestingly, radial glia cells themselves have been shown to be the major source of neurons during brain development [216-220]. Together with its specific spatio-temporal expression, these data strongly indicate that DCL is a highly specific marker for radial glia and neuronal progenitor cells. Besides having overlapping roles in neuronal migration [221], DCX and DCL may control different aspects of neurogenesis.

GR Interacts with DCL in Neuronal Progenitor Cells: A Potential Therapeutic Intervention Point

Activated GR is translocated to the nucleus of neuronal cells guided by the microtubule cytoskeleton. In the cytosol, GR is part of a protein complex that consists of a number of chaperone proteins, such as various heath-shock proteins (hsp), immunophilins and the motor-protein dynein [222-226].

DCX has been implicated in intracellular transport, interacts with dynein [227] and structural analysis of DCX suggests a role for its C-terminus in microtubule-guided transport [228]. DCL is highly homologous to DCX and may therefore be involved in microtubule-guided retrograde transport of signaling proteins in neuronal progenitor cells. Recently, we have shown that this is indeed the case [229]. Co-immunoprecipitation and FRET studies showed that DCL directly interacts with the GR. GR and DCL are co-expressed in neuronal progenitor cells and colocalize to microtubules. Moreover, loss and gain of function studies in neuroblastoma cell lines and in hippocampal organotypic slice cultures showed diminished GR translocation and impaired GR-dependent transcription. As predicted by structural analyses, the C-terminus of DCL is involved in microtubule-based GR transport [229].

Because DCL is specific for neuronal progenitor cells, blockade of GR-DCL interactions might be an interesting alternative for current anti-depressant drug developments aiming to block GR actions in neuronal progenitor cells (Fig. **6**). However, a deeper analysis of GR-DCL interactions is required to pinpoint specific molecular interaction sites. Although it is clear that the C-terminus of DCL is involved, the matching GR domain is presently obscure. Also, the type of

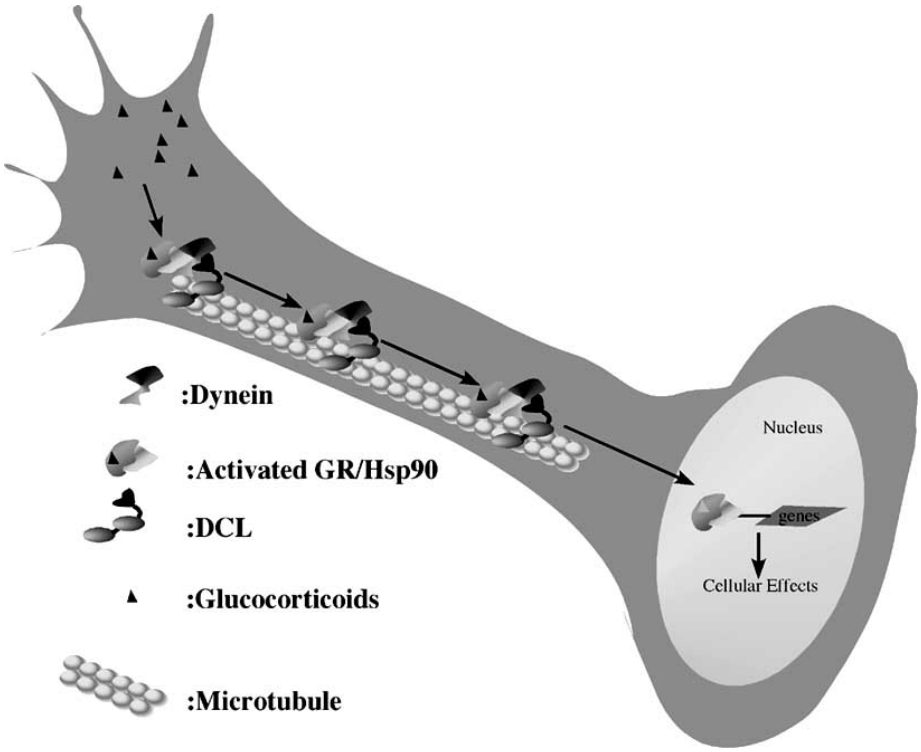

Fig. (6). Hypothetical model to explain DCL action on GR translocation in neuronal cells. The GR is a ligand-activated transcription factor. DCL is involved in cytoskeleton-mediated GR transport by directly interacting with the GR, or alternatively, with other yet unidentified component(s) of the GR transportosome. Once in the nucleus, the GR induces its well-characterized genomic effects. The C-terminus of DCL, involved in direct interactions with the GR might or the corresponding interaction domain in the GR, could be pharmaceutical intervention points. See text for further details.

intervention strategy needs to be defined. Beside small molecules that block GR-DCL interaction specifically (presently unknown), one may consider alternatives like peptidomimetics, that mimic e.g. the C-terminus of DCL or nucleic acid based drugs like small interfering RNAs that could knockdown DCL in neuronal progenitors.

CONCLUSIONS

Stress and GCs have both beneficial and damaging effects on the hippocampus. Long periods of chronic stress characterized by high levels of circulating adrenal GCs lead to structural changes in the hippocampus that resemble those observed in depressed patients. Therefore, modulation of the glucocorticoid system in the hippocampus could prevent or revert these structural changes, providing a therapeutic intervention point for severe mood disorders such as depression. Moreover, in rodents, chronic stress and subsequently elevated glucocorticoid levels reduce neurogenesis in the hippocampus. Concurrently, hippocampal neurogenesis seems to be linked with the etiology and treatment of depression, as the vast majority of the existing antidepressants increase neurogenesis in the dentate gyrus.

Short-term treatment with anti-glucocorticoids such as mifepristone, has been demonstrated to exhibit antidepressant effects in clinical trials. This has suggested that interfering with glucocorticoid receptor signaling has therapeutic potential for the treatment of depression. However, these drugs appear to have severe side-effects and novel therapeutic strategies seem necessary to antagonize glucocorticoid receptor functions for the treatment of severe mood disorders. To this end, detailed knowledge on the role of neurogenesis in mood disorders and the consequent role of GR therein, is a prerequisite. We propose to focus on cell-type specific factors, like DCL, that are involved in glucocorticoid signaling in neuronal progenitor cells.

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ABBREVIATIONS

ACTH	=	Adrenocorticotropic hormone
AD	=	Antidepressant
ADX	=	Adrenalectomy
CHO	=	Chinease hamster ovary
CNS	=	Central nervous system
CRF	=	Corticotropin-releasing factor
DCL	=	Doblecortin like

DCX = Doublecortin

DCLK = Doublecortin-like kinase

ER = Oestrogen receptor

GC = Glucocorticoids

GR = Glucocorticoid receptor

HPA = Hypothalamo-pituitary-adrenal

LBD = Ligand binding domain

MDD = Major depressive disorder

MR = Mineralocorticoid receptor

NOD = Normalized optical density

PMD = Psychotic major depression

PR = Progesterone receptor

SEM = Standard error of the mean

SSRI = Serotonin selective reuptake inhibitor

THC = R,R enantiomer of 5,11-cis-diethyl-5,6,11, 12-tetrahydrochrysene-2,8-diol.

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