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REVIEW ARTICLE

Endogenous glucocorticoids: role in the etiopathogenesis of Alzheimer's disease

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Abstract

Endogenous glucocorticoids (eGCs) are steroid hormones with a wide spectrum of physiological effects. However, enhanced basal eGCs levels have been observed in patients affected by Alzheimer's disease (AD) and they have been correlated with dysregulation of the hypothalamic-pituitary-adrenocortical axis, hippocampal degeneration and reduced cognitive/memory performance. Therefore, it has been proposed that elevated concentration of eGCs might have a role in AD pathogenesis. AD is the most common form of dementia, characterized by the pathological accumulation of two proteins: the Amyloid Beta (Aβ) and the microtubule-associated protein tau in the neurons of the hippocampus and prefrontal cortex. In particular, the hippocampus, the cerebral area involved in learning and memory, is the brain region more vulnerable to chronic eGCs exposure. Although clinical studies have failed to establish a direct causative link between eGCs e and AD pathogenesis, evidences from pre-clinical studies have shown that increased eGCs levels accelerate the formation of Aβ in AD animal models by promoting the amyloidogenic pathway, and in parallel by reducing Aβ clearance, through transcriptional mechanisms involving the Glucocorticoid receptors. Instead, the effects of stress on tau phosphorylation seem to be mainly mediated bv the corticotropin-releasing factor receptor (CRFR1) and independent from stress-induced eGCs elevation.

INTRODUCTION

Endogenous glucocorticoids (eGCs) are a class of steroid hormones produced by the adrenal gland and released into the systemic circulation. Basal levels of GCs are known to play a key role in the maintenance of an organism's homeostasis by regulating energy metabolism, by coordinating immune responses and by orchestrating the adaptive responses to stress.

In physiological conditions, eGCs secretion occurs in an ultradian pulsatile manner and it is tightly regulated by the hypothalamic-pituitary-adrenocortical (HPA) axis. However, eGCs secretion can increase in response to different stimuli, such as low plasma levels of corticosteroids, psychological or physical stress. When eGCs blood concentrations rise a certain threshold, their secretion is generally suppressed by a negative feedback loop, mainly regulated by the hippocampus.

According to the glucocorticoid cascade hypothesis the exposure to elevated concentrations of eGCs affects the ability of the hypothalamus to inhibit eGCs release, leading to the

over-activation of the HPA axis. The over-activation of the HPA axis results in a chronic eGCs release, which leads to hippocampal atrophy, in a vicious cycle (Sapolsky *et al.* 1986). Elevated eGCs levels by binding the glucocorticoid receptors (GRs), exert both genomic and non-genomic deleterious effects on the hippocampus. Instead, according to the hippocampal vulnerability hypothesis, prolonged eGCs exposure makes the hippocampus more susceptible to subsequent neurotoxic effects, accelerating the age-related cognitive decline (Conrad 2008).

Evidences from clinical studies have reported increased basal eGCs levels in Alzheimer's Disease (AD) patients that have also been correlated with dysregulation of the HPA axis, hippocampal degeneration and reduced cognitive and memory performance (Martignoni *et al.* 1992; Sapolsky *et al.* 1986). Therefore, it has been proposed that elevated concentrations of circulating eGCs may play a role in AD pathogenesis. AD is a the most common form of dementia characterized by memory loss and cognitive impairment, which reflect the loss of cholinergic neurons in the hippocampus and entorhinal cortex (Nestor *et al.* 2004), likely due to the extracellular accumulation of amyloid Beta (Aβ) and intracellular aggregation of the microtubule-associated protein tau. However, it has not been established whether eGCs alterations precede AD onset or whether they are a consequence of the disease. In this review, we have tried to clarify the molecular mechanism by which eGCs might play a role in AD pathogenesis.

ALZHEIMER'S DISEASE PATHOGENESIS

Alzheimer's disease (AD) is the most prevalent cause of dementia in the elderly. It is characterized by cognitive dysfunctions likely due to the loss of cholinergic neurons, especially in the hippocampus, the brain area involved in cognition, learning and memory (Dhikav & Anand 2011).

The main neuropathological hallmarks of AD are the senile plaques caused by the aggregation of Amyloid Beta (Aβ) in the extracellular matrix and the neurofibrillary tangles which are due to the hyperphosphorylation of the microtubule-associated protein tau and its consequent intracellular precipitation. It is thought that these two insoluble protein aggregates cause chronic neuronal damage leading to the progressive loss of cholinergic neurons.

Aβ is a 38–43 kDa peptide derived from the sequential cleavage of the Amyloid Precursor Protein (APP) by the β-secretase (BACE-1), which generates the C-terminal fragment 99 (C99) (Vassar *et al.* 1999), while the subsequent proteolysis of the C99 by the gamma (γ)-secretase complex results in the generation of amyloid peptides (Cupers *et al.* 2001). The γ-secretase complex is composed of at least four components: Presenilin 1 and Presenilin 2 (PS1/PS2), Nicastrin (NCSTN), Anterior Pharynx Defective 1 (APH-1), and Presenilin Enhancer 2 (PSENEN) (Zhang *et al.* 2014). In physiological conditions, basal levels of Aβ are thought to be involved in neurogenesis and synaptic plasticity (Parihar & Brewer 2010). However, excessive Aβ production can cause several deleterious effects which lead to neuronal death (Rajasekhar *et al.* 2015). Aβ levels depend on the balance between its production and degradation. In particular, microglia and astrocytes play a pivotal role in Aβ clearance through the production of specific proteases, such as neprilysin, the insulin degrading enzyme (IDE), and the endothelin-converting enzymes, which hydrolyze Aβ at specific residues, or enzymes which degrade Aβ, such as matrix metalloproteinases (Ries & Sastre 2016). An impaired function of the enzymes involved in Aβ clearance has been frequently observed in AD. Although, the mechanisms responsible of Aβ and tau pathology are not still understood, it is well-accepted that AD results from the interaction between environmental factors and genetic predisposition. Mutations in genes coding for APP, PS1 and PS2 could be responsible of Aβ over-production and these mutations have been associated with the familiar form of AD (Tang & Gershon 2003). Moreover, individuals carrying the polymorphic APOE ε4a allele have an increased risk to develop AD, whereas the ε2 allele decreases the risk (Liu *et al.* 2013).

In physiological conditions, tau is a microtubule-stabilizing protein that maintains neuronal cell structure and regulates axonal transport (Rodriguez-Martin *et al.* 2013). Instead, during AD tau is aberrantly phosphorylated by multiple kinases, including the glycogen synthase kinase-3 Beta (GSK3β) (Sperber *et al.* 1995), the cyclin-dependent protein kinase-5 (CDK5) (Kimura *et al.* 2014), the dual specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A) (Ryoo *et al.* 2007), the calmodulin-dependent protein kinase II (CAMKII) (Yoshimura *et al.* 2003) and the mitogen-activated protein kinases (MAPKs) (Leugers *et al.* 2013). Tau phosphorylation affects its ability to interact with microtubules leading to cytoskeletal destabilization (Mietelska-Porowska *et al.* 2014). Moreover, hyperphosphorylated tau oligomerizes and accumulates in the intracellular compartment, causing neuronal degeneration (Johnson & Stoothoff 2004). Of interest, most of the kinases involved in tau phosphorylation are also involved in the regulation of the glucocorticoid receptor activity and their expression can be induced by acute stress.

According to the "cascade amyloid hypothesis", Aβ production precedes tangle formation, but according to "tau hypothesis", tau tangles appear before the Aβ plaque formation (Lansdall 2014). Although to date the temporal relationship between Aβ and tau in AD pathogenesis is still controversial, it is well-accepted that both Aβ and tau accumulation in the frontal cortex and hippocampus are neurotoxic and their interaction may have a synergistic effect on AD development and progression (Ittner & Gotz 2011) (Nisbet *et al.* 2015).

THE ENDOGENOUS GLUCOCORTICOIDS CASCADE

Endogenous glucocorticoids (eGCs) are a class of steroid hormones derived from cholesterol, called corticosteroids, produced by the adrenal gland and released into the systemic circulation. eGCs are distinct in cortisol in mammals, also known as hydrocortisone, and corticosterone in rodents. Normal levels of eGCs are known to play a key role in the maintenance of an organism's homeostasis through the regulation of energy metabolism, by coordinating immune responses and by orchestrating the adaptive responses to stress. eGCs are secreted in an ultradian pulsatile manner, but also in response to different stimuli, such as low plasma levels of corticosteroids, psychological or physical stress.

eGCs secretion and release is regulated by a complex neuroendocrine system, the hypothalamic-pituitary-adrenocortical (HPA) axis, which is formed by the hypothalamus, the anterior pituitary, and the adrenal cortex (Smith & Vale 2006). Under physiological conditions, the neuroendocrine neurons of the paraventricular nucleus (PVN) of the hypothalamus release the corticotropin-releasing factor (CRF). In turn, CRF hormone travels to the anterior pituitary where it induces the pituitary gland to produce the adrenocorticotropic hormone (ACTH). ACTH via type 2 melanocortin receptors, triggers the systemic release of eGCs from the zona fasciculata of the adrenal gland (Herman *et al.* 2003) (Figure 1). However, when eGCs blood concentrations rise to a certain threshold, eGCs secretion is suppressed by a negative feedback loop. Specifically, eGCs themselves inhibit CRH secretion from the hypothalamus, which turns off ACTH secretion, stopping the eGCs secretion from the adrenal gland.

The control of HPA axis activation is mediated by multiple brain areas, in particular the hippocampus and the medial prefrontal cortex mediate the termination of eGCs secretion, mitigating in this way their effects on the brain (Mizoguchi *et al.* 2003). The combination of positive and negative control on CRH secretion results in a cyclic secretion of eGCs. Typically, eGCs are released according to the circadian rhythm, displaying greater circulating levels during the awake phase (~ 800 nM) and low levels in the night (~ 200 nM) in humans, while the opposite occurs in rodents with higher levels of eGCs in the night and lower in the morning. In response to stress, eGCs levels can increase up to 10 fold respect to the basal values.

eGCs are secreted by the adrenal grand directly into the bloodstream, but only the 10% of circulating eGCs are free and biologically active with a plasma half-life of 60 to 90 minutes, instead the main part of eGCs (approximately 95% of cortisol/corticosterone in the circulation) are transported by plasma proteins, known as corticosteroid-binding globulins (CBG)/transcortins or by albumins (Gardill *et al.* 2012). In addition to the normal homeostatic regulation of eGCs secretion, various types of stress can stimulate the CRF release, increasing the circulating levels of eGCs and mobilizing the energy reserves. Indeed, eGCs are also called "stress hormones" because they prepare the body for a "fight-or-flight" response for combating physical or emotional acute stress. eGCs supply an immediate energy source to respond to the increased metabolic demand of the body and elevate blood pressure. The transiently raising blood glucose is important to promote maximal brain function.

eGCs action depends not only on their plasma concentrations, but also on their intracellular availability. This mechanism of eGCs intracellular level regulation is operated by the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD), which is expressed particularly in the cortex and hippocampus. This enzyme catalyzes the enzymatic conversion of cortisol/corticosterone in their active forms, arising from the inert 11-keto derivatives (cortisone in humans, 11-dehydrocorticosterone in rodents) (Seckl 1997). The 11β-HSD1 can protect neurons against glucocorticoid excess. On the contrary, its increased expression can amplify eGCs cascade, potentiating the eGCs-mediated detrimental effects.

Circulating free-GCs are liposoluble molecules that can cross easily the cellular membranes. The eGC effects on the brain are mediated by two main gluco-

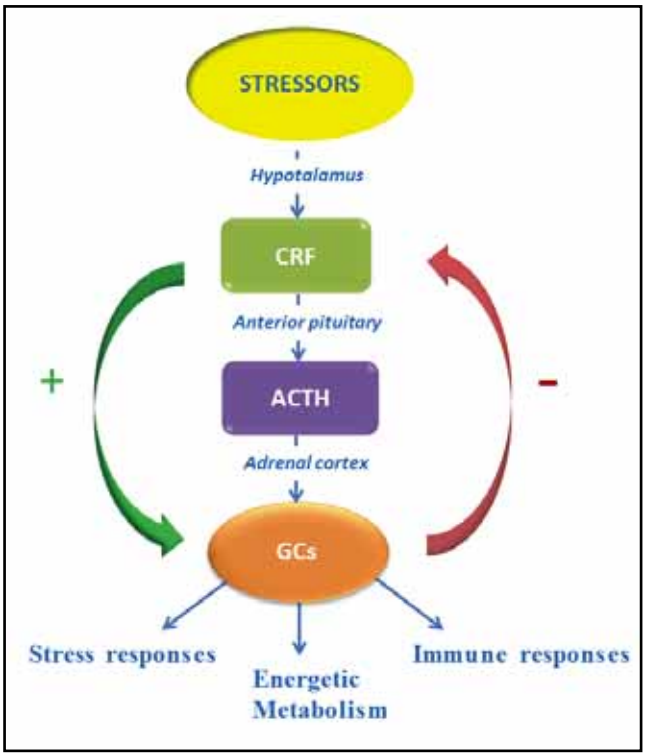

Fig. 1. The HPA regulation of eGCs secretion. eGCs secretion is regulated by the HPA axis. Under physiological conditions, the neuroendocrine neurons of the PVN of the hypothalamus release CRF. In turn, CRF induces the pituitary gland to produce ACTH that triggers the adrenal gland to release eGCs in the bloodstream. eGCs inhibit both CRF and ACTH release by the pituitary and hypothalamus through a negative feedback loop.

corticoid receptors: the Mineralcorticoid receptors (MRs) or type I and the Glucocorticoid receptors (GRs) or type II, that show a different distribution in the body. eGCs possess a 10-fold greater binding affinity for the MRs than for the GRs. Therefore, basal/low levels of eGCs bind MRs with high affinity, while they bind GRs when their concentration reached the circadian peak (late at night in humans and early morning in rodents) or under conditions of severe stress (Reul & de Kloet 1985). However, the most known isoforms are the GR-α and GR-β. GR-α activity is regulated by phosphorylation operated by different kinases, such as MAPKs, CDKs, and GSK-3β. In the absence of the ligand, GR is phosphorylated and it becomes hyper-phosphorylated upon ligand binding (Bodwell *et al.* 1998). Phosphorylation of GRs has been reported to occur on several serine residues located in the N-terminal region of the protein (S113, S134, S141, S143, S203, S211, S226, and S404). Interestingly, most of these kinases involved in GR regulation are also involved in tau stability. In particular, CDK5 and GSK3β kinases are thought to be involved in eGCs-induced tau (Joshi *et al.* 2012). In turn, acute stress has shown to activate these kinases, that are also known as "stress kinases"(Feng *et al.* 2005; Planel *et al.* 2004). GR-α is localized in the cytoplasm, and after ligand binding it translocates into the nucleus, whereas GR-β isoform resides ever in the nucleus. eGCs by binding the glucocorticoid receptors (GRs) modulate the expression of approximately 10% of the genome (Buckingham 2006). GRs can bind directly chromatin in specific DNA consensus regions within DNA gene promoter elements, called Glucocorticoid response elements (GREs), or they can regulate target gene transcription indirectly by forming protein-protein interactions with specific transcriptional factors. The latter process is called "tethering" and can result in transactivation or trans-repression (Ratman *et al.* 2013). In addition to genomic regulation by eGCs, non-genomic effects of eGCs have been found. These include eGCs interactions with cellular membranes, that can be mediated by cytosolic GRs, or by specific interactions with membrane-bound glucocorticoid receptors (Groeneweg *et al.* 2011; Stahn *et al.* 2007).

The systemic actions of eGCs include the regulation of energy metabolism, playing an important role in carbohydrate, fat and protein metabolism (Tataranni *et al.* 1996). In the liver, when the glucose levels are low, eGCs maintain normal blood glucose concentrations by promoting the gluconeogenesis, a process that converts amino acids into glucose. Specifically, eGCs promote gluconeogenesis through GR activation, which increases the expression of the enzymes involved in this process, such as the phosphoenolpyruvate carboxykinase (Friedman *et al.* 1993) and the glucose-6-phosphatase (Vander Kooi *et al.* 2005). Instead, in skeletal muscle and white adipose tissues, eGCs decrease glucose uptake and utilization by antagonizing the insulin action on glycogen synthesis. eGCs prevent glucose storage *via* the inhibition of insulin release, in favor of glucose immediate use. Therefore, eGCs increase the glucose circulating levels by inhibiting both glucose uptake and utilization, and in parallel by promoting glucose synthesis. Moreover, eGCs increase the amount of circulating fatty acids by promoting lipolysis, through the enhancement of lipase expression, by triggering the differentiation of pre-adipocytes into adipocyte, and by inducing *de novo* lipid production in hepatocytes, through the induction of fatty acid synthase (Peckett *et al.* 2011).

Although the rate of corticosteroid secretion is usually maintained within limits because it is tightly regulated by the HPA axis, the negative feedback mechanism does not work during massive stress responses. The inadequate or excessive secretion of eGCs can cause metabolism problems. Indeed, elevated eGCs secretion for long term can result in insulin-resistance and hyperglycemia(Geer *et al.* 2014). Moreover, tissue injury or pathogen invasion can represent the kinds of physic stresses that can stimulate the secretion of eGCs. Indeed, eGCs are known to exert also immunomodulatory and anti-inflammatory actions in order to restore the body's homeostasis, through a number of distinct mechanisms (Bellavance & Rivest 2014). These include the interference with leukocyte trafficking, chemotaxis, phagocytosis and inflammatory activation and thereby reducing the synthesis and release of many secreted mediators of inflammation (Hardy *et al.* 2012).

EFFECTS OF ELEVATED EGCS LEVELS ON AD PATHOGENESIS

Higher morning plasma cortisol and CRF levels have been found in the cerebro-spinal fluid of patients suffering from AD compared to controls aged-matched (Martignoni *et al.* 1990). Elevated levels of cortisol were also observed in the plasma of 33 patients affected by mild AD compared to healthy controls, and they have been correlated with the degree of cognitive impairment (Csernansky *et al.* 2006). Of interest, basal cortisol plasma levels were found to increase with the age of AD patients and correlated with the progressive cognitive impairment and lower hippocampal volume in a clinical study carried out on 172 AD patients followed up for 2-years (Huang *et al.* 2009). Likewise, enhanced cortisol circadian secretion was found in the saliva of a cohort of 18 patients affected by AD and correlated with cerebral atrophy (Giubilei *et al.* 2001). Hippocampus atrophy is an early event in AD that compromise the transfer of signals through the hippocampus and thereby reducing the capacity to learn and memorize information (Magarinos & McEwen 1995; Sapolsky 1996; Watanabe *et al.* 1992).

According to the glucocorticoid cascade hypothesis, a prolonged exposure to elevated concentrations of eGCs impairs the ability of the hippocampus, the brain area involved in learning and memory, to exert a negative feedback on the HPA axis. Indeed, the hip-

pocampus is the brain area most susceptible to eGCs damages (McEwen 1997), probably because it shows a high density of GRs. eGCs can exert different effects on cognition and memory processes in a dose- and receptor-dependent manner. Basal eGCs levels show a higher affinity for MRs in the hippocampus and promote memory consolidation. On the contrary, higher eGCs levels have an increased affinity for GRs. GRs binding by eGCs in the hippocampus has been associated with memory impairment in rats (Fietta *et al.* 2009; Roozendaal *et al.* 2009).

Prolonged eGCs exposure can lead to hippocampal volume loss by inducing biochemical, morphological and electrophysiological alterations (Conrad 2008). A daily injection of 10 mg of corticosterone (CORT) for 21 days in healthy rats led to alterations of the number and length of apical dendritic branch points in the neurons of the CA3 hippocampal region (Woolley *et al.* 1990). Moreover, a pre-treatment with high doses of CORT (100 nM) enhanced cell excitability in the CA1 hippocampal region of rats and it has been associated with an increased expression of the glutamate receptors (NMDA and AMPA). Interestingly, the administration of a GR intracellular agonist reversed these effects, indicating its direct involvement (Karst & Joels 2005). In addition, chronic CORT treatment (40 mg/kg/day) in rats for 21 days led to impaired Long-Term Potentiation (LTP) (Pavlides *et al.* 1993), a mechanism involved in learning and the formation of memories (Joels *et al.* 2004; Pavlides & McEwen 1999).

Another mechanism by which chronic eGCs exposure can contribute to hippocampal volume loss is the inhibition of neurogenesis, an endogenous process of neural plasticity that occurs in the dentate gyrus and through which new neurons are generated and incorporated into the adult connections within the CA3 (Reagan & McEwen 1997). In this regard, it has been found that elevated eGCs levels stimulated apoptosis of the newly generated neurons in the hippocampus. Indeed, GRs activation was shown to promote neuronal apoptosis in the granule cells of the rat hippocampus by increasing the expression of the pro-apoptotic molecule Bax and by reducing the anti-apoptotic ones, such as Bcl-2 (Almeida *et al.* 2000). Moreover, it has also been reported that eGCs can affect neuronal survival and neuronal plasticity by downregulating the expression of neutrophins, such as the brain-derived neurotrophic factor (BDNF) in rat hippocampus (Chao & McEwen 1994).

Coherently, it was found an inverse correlation between serum BDNF levels and total urinary cortisol secretion at 24 hours in 18 AD patients compared with 22 healthy elderly controls. The decreased ratio between cortisol and BDNF was correlated with the extent of cognitive impairment observed in these AD patients (Curto *et al.* 2016).

AD is an age-related disease and according to the hippocampal vulnerability hypothesis, chronic exposure to elevated eGCs levels can accelerate the cognitive decline associated with aging. A prospective study performed on 197 elderly people (between 65 and 90 years) has found that increased diurnal cortisol secretion in elderly people was correlated with cognitive deficits in visual and verbal memories (Beluche *et al.* 2010). Similarly, increased basal cortisol levels were associated with reduced cognitive performance in a correlation study performed on 19 healthy elderly subjects over a 4 year period (Lupien *et al.* 1994). Ennis *et al.* 2017 (Ennis *et al.* 2017) proposed the increased the urinary free-cortisol levels in conjunction with urinary free-creatinine as pre-clinical markers of AD, following a prospective longitudinal study performed over ten years on healthy elderly subjects. Similarly, a screening study performed on 754 healthy subjects and 207 AD patients has found that cortisol belongs to a panel of blood biomarkers that could be useful for the diagnosis of AD (Doecke *et al.* 2012), suggesting that monitoring eGCs levels may predict the risk to develop AD.

Conversely, Schrijvers *et al.* (Schrijvers *et al.* 2011) performed a large prospective study by monitoring plasma cortisol levels in 3,341 individuals not demented. After seven years of follow-up, they found that 210 of the enrolled subjects developed AD, however, they failed to find any correlation between serum cortisol levels and AD. Likewise, Popp *et al.* (Popp *et al.* 2009) observed elevated cortisol in the CSF of 66 AD patients compared to 34 control subjects, but no substantial differences in plasma levels were found between 33 subjects affected by mild cognitive impairment (MCI) and controls. Since MCI is a cognitive deterioration which can precede AD onset (Boyle *et al.* 2006), it has been suggested that elevated eGCs are not an early event in AD. However, since clinical studies have obtained controversial results, it has not been yet established whether eGCs alterations precede AD or they are a consequence of the degenerative process associated with the disease.

The discovery of a 11β-HSD1 haplotype associated with a sixfold higher risk to develop AD in humans (de Quervain *et al.* 2004), has raised the potential involvement of these enzymes in driving the adverse effects of eGCs in neurons. Indeed, intracellular eGCs tissue-specific concentration depend on the 11β-HSD1 activity. The hippocampal expression of 11β-HSD1 was found to increase with aging in wild-type mice and to be correlated with the spatial memory deficits (Wyrwoll *et al.* 2011), suggesting that 11β-HSD1 over-expression may contribute to AD pathogenesis. Soy *et al.* (Sooy *et al.* 2010) demonstrated that 11β-HSD1 null-mice are not affected by spatial memory impairments associated with aging, while Yau (Yau *et al.* 2015) showed that pharmacological inhibition of the 11β-HSD1 reduced hippocampal CORT levels and prevented spatial memory deficits in aged wild type mice. In another study, Soy and coworkers, subjected the old (aged 14 months) transgenic AD mouse model "Tg2576" to the treatment

with the 11β-HSD1 inhibitor (UE2316) for four weeks. 11β-HSD1 inhibition was found to decrease Aβ plaque deposition in the cerebral cortex and to improve cognitive functions in AD mice (Sooy *et al.* 2015). However, a double blind phase II clinical trial (ClinicalTrials.gov NCT01137526) performed on 267 subjects with mild-to-moderate AD treated with HSD-11 inhibitor (ABT-384, 10 or 50 mg/daily, for 12 weeks) did not produce symptomatic improvements in these patients (Marek *et al.* 2014).

High cortisol levels in the cerebro-spinal fluid in AD patients have also been associated with the presence of the APOE ε4 allele (Bruno *et al.* 2012), a well-known genetic susceptibility factor for the development of AD. Peskind *et al.* (Peskind *et al.* 2001) by measuring cerebro-spinal fluid cortisol levels in 64 AD patients and 34 older control subjects, found that the high cortisol concentration in the cerebro-spinal fluid correlated with increased frequency of the APOE ε4 allele in AD subjects.

It has been also proposed that elevated eGC levels could promote Aβ and tau pathologies (Elliott *et al.* 1993) (Budas *et al.* 1999). A prospective cohort study performed on 416 normal elderly in 6-years has shown that increased plasma cortisol levels correlated with increased Aβ production and cognitive decline in these subjects (Pietrzak *et al.* 2015). It was also found a positive correlation between increased cortisol and $A\beta_{1-42}$ in the cerebro-spinal fluid, by comparing 26 patients with mild AD with 20 healthy age-matched controls. Although, it was found an inverse association between serum cortisol level in AD subjects, $A\beta_{1-42}$ and tau in the CSF (total tau, tau phosphorylated at threonine 181 and 231), indicating a protective role of mild increased serum cortisol levels in patients with moderate AD (Laske *et al.* 2009). In order to better understand the contribute of eGCs on Aβ and tau pathologies and the underlying molecular mechanisms, researchers have administered high doses of eGCs or alternatively they have induced various kinds of stresses in animals in order to mimic the endogenous GCs elevation observed in AD In particular, Green *et al.* (Green *et al.* 2006) evaluated the contribute of elevated eGCs levels in Aβ generation both *in vitro and in vivo*. *In vitro* Neuro2A cells treated with high doses of corticosterone (CORT, 10μM/72 h) released an increased level of $A\beta_{40}$, AB_{42} and C99 fragment. However, the administration of the GR antagonist mifepristone reversed these effects, suggesting a direct GR involvement in promoting Aβ production. In order to understand by which mechanism GR influenced Aβ formation, they evaluated the expression levels of APP and BACE-1 expression in these cells, finding that they were increased after CORT administration. Moreover, by monitoring corticosterone plasma levels from 2- to 18-month in the triple transgenic AD mouse model 3xTg-AD carrying the transgenes PS1(M146V), APP(Swe) and tau (P301L) 13-month-old, which generally develops Aβ deposition at 9–12 months of age and later tau tangles, they find that eGCs levels were increased from the 9 months of age, thus after Aβ and tau appearance in this AD mouse model. These findings indicated that GCs alterations were a secondary event respect to Aβ and tau aggregation. Sambamurti *et al.* (Sambamurti *et al.* 2004) proposed that the existence of a GRE within the APP promoter and BACE sequences, suggesting that their transcription was mediated by GR transcriptional activation by eGCs. On the other hand, Baglietto-Vargas *et al.* 2013 demonstrated that administration of the GR receptor antagonist (mifepristone) in 10 month-old 3xTg-AD mice, reversed the deposition of Aβ plaque in the hippocampus. Interestingly, it was found that mifepristone precluded Aβ formation by generating a new APP fragment of 17 kDa that interferes with the cleavage mediated by α- or β- secretase. Moreover, mifepristone treatment reduced also tau phosphorylation at Thr181 and Ser396/404, an effect that was correlated with the inactivation of CDK5/p25 in treated-mice (Baglietto-Vargas *et al.* 2013). Coherently with these findings, daily mifepristone treatment for 6-months lowered cortisol levels and was found to ameliorate the cognitive functions in AD patients (Belanoff *et al.* 2002).

Interestingly, Wang *et al.* (Wang *et al.* 2011) demonstrated that *in vitro* GRs activation by CORT treatment (1 μm for 72 h) not only enhanced the production of Aβ in primary astrocytes derived from wild-type middle-aged mice, by increasing APP and BACE-1 expression, but also suppressed the ability of these cells to remove Aβ deposits through the downregulation of the proteases involved in Aβ clearance, such as IDE enzyme and the matrix metalloproteinase-9. In support of this hypothesis, another study *in vivo* found a reduced IDE expression in the frontal cortex and in the dentate gyrus, as well as an increase in $A\beta_{42}$ levels in the frontal cortex, of aged macaques exposed to chronic cortisol treatment (orally administered 5.78 mg/kg twice-daily for 12 months). However, these restricted positivity in only the dentate gyrus region of the hippocampus could be due to the technical limitation to detect little changes in areas with lower IDE expression (Kulstad *et al.* 2005). The preclinical studies regarding the role of eGCs in AD pathogenesis are summarized in Table 1.

Stress and eGCs are considered two inseparable factors, because stress increases the release of eGCs, which in turn mediate the effects of stress. In order to obtain elevated eGCs levels in animals, researchers have induced various kinds of experimental stress. However, the type of stress applied, as well as, the timing and duration of stress exposure are critical for determining eGCs impact. One of the most validated paradigm of stress is known as "Unpredictable chronic stress" (UCS) which consists in animal exposure to an unpredictable recurrent physical, psychological or social stress (food or water deprivation, immobilization, overcrowding or isolation stress, or placed on a vibrating platform) that is applied in a random manner for a period of minimum

Tab.1 Preclinical studies about the role of eGCs in AD pathogenesis.

Models	Experimental conditions	Receptor involved	Observed effects	Putative mechanisms	Ref.
hippocampal rat neurons	Pre-treatment with CORT	GR	↑cell excitability	↑AMPA and NMDA	(Karst & Joels 2005)
Wt rats	Daily injection of CORT for 21 days	not specified	↓number and length of hippocampal dendrites	not specified	(Woolley *et al.* 1990)
Wt rats	CORT treatment	not specified	↓LTP	not specified	(Pavlides *et al.* 1993)
Wt rats	none	GR	↑neuronal death	↑Bax ↓BCL2 ↓BDNF	(Almeida *et al.* 2000) (Chao & McEwen 1994)
Wt mice	11β-HSD1 inhibition	not specified	↓CORT	not specified	(Yau *et al.* 2015)
Tg2576 mice aged 14 months	11β-HSD1 inhibition	not specified	↓Aβ plaque	not specified	(Sooy *et al.* 2015)
Neuro2A cells	CORT treatment	GR	↑$Aβ_{40}$, $Aβ_{42}$ and C99 fragment	↑APP; BACE-1	(Green *et al.* 2006)
3xTg-AD mice 10-months old	GR antagonist treatment	GR	↓Aβ plaque ↓ tau phosphorylation	APP fragment of 17 kDa ↓CDK5/p25	(Baglietto-Vargas *et al.* 2013)
Primary astrocytes	CORT treatment	not specified	↑Aβ production	↑APP; BACE-1 ↓IDE	(Wang *et al.* 2011)
Aged macaques	CORT treatment	not specified	↑$Aβ_{42}$ levels	↓IDE	(Kulstad *et al.* 2005)

four weeks (Frisbee *et al.* 2015; Monteiro *et al.* 2015). Although, the unpredictable chronic stress paradigm is considered the best model to mimic everyday stress in humans (Cuadrado-Tejedor *et al.* 2012), animals could be also subjected to a chronic variable single type of stress (CVS) or to chronic restraint single type of stress (CRS).

The exposure of young (6 months) or aged (18 months) wild-type mice to a chronic variable single type of stress daily for 2 weeks, led to increased CORT plasma levels in stressed animals. Moreover, stress-induced eGCs elevation enhanced BACE-1 and GSK3β expression in the hippocampus of both young and aged mice, lowering also BDNF in aged mice. Whereas in the prefrontal cortex, BACE-1 and GSK3β levels were found to be higher only in aged mice (Cordner & Tamashiro 2016). These findings may indicate that stress-induced eGCs can accelerate the age-related degenerative processes.

Conversely, wild-type middle-aged rats of 14 months were subjected to 1 month of chronic, random and unpredictable stress prior to be infused with Aβ. Interestingly, these stressed animals showed higher serum cortisol levels and increased tau phosphorylation in specific amino acidic residues in both hippocampus and prefrontal cortex (pSer202, pThr231, pSer396/404, and pSer409) compared to unstressed group and to the another experimental group which has received Aβ alone. The aberrant tau phosphorylation was correlated with the increased expression of the kinases pERK1/2, pGSK3β, p35, pJNK, and CaMKII (Sotiropoulos *et al.* 2011). Similarly, Catania and coworkers (Catania *et al.* 2009) showed that exposure of wild-type rats aged 14 months to chronic and unpredictable stress for 4 weeks, led to significantly increased CORT plasma levels compared to unstressed animals. Moreover, five days after stress exposure, these rats were infused with Aβ for a period of 2 weeks. Both Aβ-infused and stressed rats showed increased production of the C99 fragment of APP both in the CA1 and CA3 regions of the hippocampus compared to unstressed rats. Moreover, it was observed that stress-induced eGCs elevation increased BACE-1 and NCST expression both in the hippocampus and prefrontal cortex of stressed rats.

In order to evaluate the impact of stress/eGCs on Aβ deposition, Cuadrado-Tejedor and coauthors, used as AD animal model the transgenic mouse "Tg2576" carrying the Swedish mutation hAβPP(Swe), which generally displayed Aβ deposition around at 10–12-months. Six-weeks of exposure of Tg2576 mice aged 4-months to chronic, unpredictable mild stress led to increased rates of $Aβ_{40}$, $Aβ_{42}$ and C99 fragment in the cerebral cortex and enhanced plaque deposition (Cuadrado-Tejedor *et al.* 2012). Moreover, it was observed an increase of 2,5 folds in tau phosphorylation at Ser202 in the hippocampus of stressed mice, likely ascribed to the increased activity of GSK3β in this brain area. These results indicated that stress contributed to accelerate the onset of Aβ deposition in a transgenic mouse which normally develops Aβ plaques and tau tangles at later stages (Kitazawa *et al.* 2012). However, CORT measurement two-months after suspension of stress protocol revealed that CORT levels were not increased in stressed animals. Thus, it was suggested that eGCs might return to normal levels or decrease over time,

although their effects may persist for a longer period. Similarly, Huang *et al.* (Huang *et al.* 2010) showed that stress enhanced Aβ toxicity. Specifically, wild-type mice (6–8 weeks old) were prior subjected to 2-days of electric foot shocks followed by 3-weekly of situational reminders, and then infused with $A\beta_{1-40}$ oligomers in the CA1 region. Stressed animal showed increased plasma CORT levels, enhanced GRs and CRF expression compared to unstressed, as well as increased Aβ accumulation in the whole hippocampus.

Conversely, other studies have underlined the potential role of CRF in mediating the neurotoxic effects of stress. CRF modulates the HPA activity via two G-protein-coupled receptors, CRFR1 and CRFR2, which show a different distribution in the brain (Van Pett *et al.* 2000). A reduced CRF expression has been reported in the early stage of AD progression (Bayatti & Behl 2005).

Carrol *et al.* (Carroll *et al.* 2011) evaluated the effect of stress and CRF on two different AD transgenic mouse models: the Tg256 and the PS19 mice. As previously said, the Tg256 mouse is characterized by a specific mutation in the gene coding for APP and it is characterized by Aβ deposition around at 10–12-months of age. Instead, the PS19 is a mouse model of tauopathy carrying the P301S mutation (PS19) which develops tau fibrils at 6 months of age (Yoshiyama *et al.* 2007). Both mouse models were adrenalectomized subjected to one month of restraint/isolation stress. Chronic stress was found to exacerbate amyloid plaque deposition in the Tg256 mice, as well as tau phosphorylation in the PS19 mice. Conversely, the administration of a CRFR1 antagonist, prior stress exposure, was able to antagonize the effect of stress on tau phosphorylation. These results corroborated the hypothesis that stress can influence both Aβ and tau pathology and suggested that stress-induced tau phosphorylation involve a CRFR1-dependent mechanism which is independent from eGCs levels. The Tg256 mice aged 27 weeks were divided in two main experimental groups: one group was genetically manipulated in order to overexpress human APP (Tg+), while another group was not manipulated, and used as control (Tg^-). Both Tg^+ and Tg^- were further exposed to chronic isolation stress (mice were individually housed from weaning until 27 weeks of age), while grouped mice represented the unstressed control. Both Tg^+ and Tg^- exposed mice to isolation stress showed decreased hippocampal volume. Notably, Tg^+ stressed mice displayed also increased $A\beta_{1-40}$ and $A\beta_{1-42}$ levels, both in the cortex and the hippocampus, as well as increased amyloid plaque deposition and plasma CORT concentration, enhanced GRs expression in the hippocampus, as well as increased CRFR1 levels in hippocampus and prefrontal cortex (Dong *et al.* 2008).

Interestingly, it has been found that early stressful experiences can increase the susceptibility to develop AD (Daskalakis *et al.* 2015). In this regard, Zhang *et al.* (2012) and created a model of neonatal isolation in mice before weaning followed by social isolation after weaning. It was found an increased tau phosphorylation in cultured primary hippocampal neurons derived

Tab.2 Preclinical studies about the role of stress-induced GCs in AD pathogenesis.

Models	Stress	Treatment	Receptor involved	Observed effects	Putative mechanisms	Ref.
wt mice 6 months old	CVS for 2 weeks	none	not specified	↑CORT	↑BACE-1 ↑GSK3β	(Cordner & Tamashiro 2016)
Wt mice 18 months old	CVS for 2 weeks	none	not specified	↑CORT	↑BACE-1 ↑GSK3β ↓BDNF	(Cordner & Tamashiro 2016)
Wt rats 14 months old	UCS for 1 month	none	not specified	↑CORT ↑p-tau	↑pERK1/2, pGSK3β, p35, pJNK, and CaMKII	(Sotiropoulos *et al.* 2011)
Wt rats 14 months old	UCS for 4 weeks	Aβ infusion	not specified	↑C99 fragment of APP	↑BACE-1 and NCST	(Catania *et al.* 2009)
Tg2576 mice aged 4-months	UCS for 6 weeks	none	not specified	↑$A\beta_{40}$, $A\beta_{42}$ and C99 ↑plaque deposition ↑p-tau	↑GSK3β	(Kitazawa *et al.* 2012)
Tg256 and PS19 adrenalectomized mice	CRS for 1 month	CRF antagonist	CRFR1	↓amyloid plaque ↓p-tau	not specified	(Carroll *et al.* 2011)
Tg256 mice aged 27 weeks	CRS for 24 weeks	none	not specified	↑CORT ↑$A\beta_{1-40}$ and $A\beta_{1-42}$ ↑CRFR1	not specified	(Dong *et al.* 2008)
Wt mice	CRS	CRFR1 antagonist	CRFR1	↓p-tau	GSK3β	(Zhang *et al.* 2012)
Murine primary hippocampal neurons	none	CRF treatment for 2hr	CRFR1	↑p-tau	GSK3β	(Le *et al.* 2016)

from the hippocampus of chronically stressed mice. Tau phosphorylation was inhibited by the treatment of hippocampal neurons with lithium, a well-known GSK3β inhibitor. Moreover, they observed that tau phosphorylation was decreased *in vivo* through the administration of CRFR1 antagonists (CP154,526) but not by the GR antagonist (RU486) treatment, indicating that tau phosphorylation in this chronic model of stress was mediated by CRFR1. Recently, Le *et al.* (Le *et al.* 2016) proposed a mechanism to explain how CRF mediates the deleterious effect of stress on the brain. Specifically, murine primary hippocampal neurons were treated with 10 μM CRF for 2hr to reproduce chronic stress. Chronic CRF treatment induced p-tau in a GSK3β dependent-manner. Moreover, they found that tau accumulation in hippocampal neurons led to the decreased axonal transport of BDNF that in turn correlated with neuronal degeneration (Saura & Valero 2011).

The preclinical studies regarding the role of stress-induced eGCs in AD pathogenesis are summarized in Table 2.

CONCLUSIONS

Elevated eGCs basal levels have been correlated with AD progression, however clinical studies have failed to find a causative link between eGCs levels and AD onset. Larger follow-up studies with longer duration are required to clarify the role of eGCs in AD pathogenesis. However, evidences from preclinical models have shown that increased eGCs contribute to Aβ formation and tau phosphorylation by triggering different molecular mechanisms. Specifically, elevated eGCs influence the amyloidogenic pathway by accelerating or exacerbating the formation of senile plaques, and in parallel by reducing Aβ clearance in wild-type animals, as well as in both transgenic and non transgenic AD animal models, through mechanisms mediated by the transcriptional activation of GR. However, is not clear whether these eGCs/stress alterations precede or not AD onset, due the heterogeneity of AD experimental models used. Although stress and eGCs have been considered for a long time as two inseparable factors, new evidence seems to indicate that chronic stress, promotes tau pathology by increasing the expression of kinases involved in tau phosphorylation, through a mechanism which is mainly mediated by the CRFR1. However, doses and duration of eGCs exposure, as well as the kind of stressor and timing, may give differential results. Therefore, new standardized conditions are required to better understand the role of eGCs in AD pathogenesis.

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