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Glucocorticoids Increase Amyloid-β and Tau Pathology in a Mouse Model of Alzheimer’s Disease

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Various environmental and genetic factors influence the onset and progression of Alzheimer’s disease (AD). Dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis, which controls circulating levels of glucocorticoid hormones, occurs early in AD, resulting in increased cortisol levels. Disturbances of the HPA axis have been associated with memory impairments and may contribute to the cognitive decline that occurs in AD, although it is unknown whether such effects involve modulation of the amyloid β-peptide (Aβ) and tau. Using in vitro and in vivo experiments, we report that stress-level glucocorticoid administration increases Aβ formation by increasing steady-state levels of amyloid precursor protein (APP) and β-APP cleaving enzyme. Additionally, glucocorticoids augment tau accumulation, indicating that this hormone also accelerates the development of neurofibrillary tangles. These findings suggest that high levels of glucocorticoids, found in AD, are not merely a consequence of the disease process but rather play a central role in the development and progression of AD.

Key words: corticosterone; Aβ peptide; Alzheimer’s disease; glucocorticoids; tau; stress

Introduction

Alzheimer’s disease (AD) is a chronic neurodegenerative disorder marked by a progressive loss of memory and cognitive function. The two hallmark neuropathological features are amyloid β-peptide (Aβ) plaques and tau-laden neurofibrillary tangles. Although mutations in three different genes are known to underlie some cases of the rare, inheritable forms of the disease, the etiology of the more common sporadic cases remains unknown and likely involves complex interactions between various genetic and environmental factors, such as a stressful lifestyle or the apolipoprotein E4 allele (apoE4) (Travis, 1993). Neuroendocrine malfunctions may also be involved in the disease process, particularly because it is established that stress hormones can negatively affect neuronal survival (Stein-Behrens et al., 1994). Epidemiological evidence further supports a role for stress as a risk factor for AD because elderly individuals prone to psychological distress are more likely to develop the disorder than age-matched, non-stressed individuals (Wilson et al., 2005).

The glucocorticoid response to stressful stimuli is regulated by the hypothalamic–pituitary–adrenal (HPA) axis, which triggers the adrenal cortex to release glucocorticoids (cortisol in primates, corticosterone in mice and rats). Glucocorticoids are steroid hormones that readily cross the blood–brain barrier and bind to low-affinity glucocorticoid receptors and high-affinity mineralocorticoid receptors (Reul and de Kloet, 1985). Activity of these receptors is necessary for normal cellular metabolic activity and crucial for many CNS functions, including learning and memory (Roozendaal, 2000). There is ample evidence implicating HPA axis dysfunction in AD, reflected by markedly elevated basal levels of circulating cortisol (Davis et al., 1986; Masugi et al., 1989; Swanwick et al., 1998) and a failure to show cortisol suppression after a dexamethasone challenge (Greenwald et al., 1986; Mochan et al., 1990; Nasman et al., 1995). Of interest was the finding that, although AD patients had elevated basal cortisol levels, HPA dysfunction only seemed relevant in the early stages of the disease (Swanwick et al., 1998) because HPA dysfunction did not worsen with additional cognitive decline. In addition, more recent findings show that elevated CSF cortisol in AD patients mirrored the presence of the apoE4 allele (Peskind et al., 2001), suggesting that apoE function was influencing circulating cortisol levels. Findings from a human clinical study further suggest a detrimental role for glucocorticoids in this disorder, because AD patients treated with prednisone (a glucocorticoid used for its anti-inflammatory properties) exhibited impaired cognition compared with the placebo-treated cohort (Aisen et al., 2000). Genetic studies indicate a link between glucocorticoid function and the risk for AD, because a rare haplotype in the 5’ regulatory region of the gene encoding 11β-hydroxysteroid dehydrogenase type 1 was associated with a sixfold increased risk for sporadic AD (de Quervain et al., 2004). Furthermore, some evidence from animal studies suggests an interaction between glucocorticoids and AD pathology, including amyloid precursor protein (APP) and tau accumulation (Elliot et al., 1993; Budas et al., 1999), although the molecular mechanisms underlying these effects and the downstream consequences are unknown.
The present study sought to determine whether glucocorticoids modulate the hallmark neuropathological features of AD and, if so, the underlying mechanism. Consequently, we investigated the pathological consequences of stress-level glucocorticoid administration on Aβ formation and tau pathology using both in vitro and in vivo approaches. Here we report the novel findings that levels of the β-secretase enzyme [β-APP cleaving enzyme (BACE)] and its substrate APP are selectively increased after glucocorticoid administration, resulting in increased production of Aβ. Notably, administering glucocorticoids to the triple-transgenic (3×Tg-AD) mice, which develop both Aβ and tau pathologies in an age-dependent manner (Oddo et al., 2003), exacerbated the formation of both lesions. The present findings highlight a mechanism by which stress affects AD neuropathology and suggest that stress management or pharmacological reduction of glucocorticoids warrant additional consideration in the regimen of AD therapies.

Materials and Methods

**Immunoblotting.** Protein extracts were prepared from cells using M-per (Pierce, Rockford, IL) extraction buffer and Complete Mini Protease Inhibitor Tablets (Roche, Indianapolis, IN). Protein extracts were prepared from whole-brain samples by homogenizing in T-per (Pierce) extraction buffer and Complete Mini Protease Inhibitor Tablets (Pierce, Rockford, IL) extraction buffer and Complete Mini Protease Inhibitor Tablets (Roche), followed by high-speed centrifugation at 100,000 × g for 1 h. The supernatant was taken as the protein extract. Protein concentrations were determined by the Bradford method. Equal amounts of protein (20–50 μg depending on protein of interest) were separated by SDS-PAGE on a 10% Bis-Tris gel (Invitrogen, Carlsbad, CA), transferred to a 0.45 μm polyvinylidene difluoride membranes, blocked for 1 h in 5% (v/v) nonfat milk in Tris-buffered saline, pH 7.5, supplemented with 0.2% Tween 20, and processed as described. Antibodies and dilutions used in this study include 6E10 (1:1000; Signet, Dedham, MA) for APP in in vitro studies, CT2F0 (1:5000; Calbiochem, San Diego, CA) for C99 and C83, 22C11 (1:1000; Chemicon, Temecula, CA) and HT7 (1:3000; Innogenetics, Gent, Belgium) for APP in in vitro studies, AT8 (1:1000; Pierce), AT180 (1:1000; Pierce), anti-caspase cleaved APP (cAPP) (1:3000; Chemicon), anti-BACE (1:1000; Calbiochem), anti-glucocorticoid receptor (1:300; Affinity BioReagents, Golden, CO), and α-actin (1:10,000; Sigma, St. Louis, MO). Quantitative densitometric analyses were performed on digitized images of immunoblots with Scion Image 4.0 (Scion, Frederick, MD).

**AMP-Aβ-binding protein ELISA.** Aβ1–40 and Aβ1–42 were measured using a sensitive sandwich ELISA system. Soluble and insoluble Aβ was isolated from whole-brain homogenates using T-per extraction buffer (Pierce) and 70% formic acid (FA), respectively. Soluble fractions were loaded directly onto ELISA plates, and FA fractions were diluted 1:20 in neutralization buffer (1 μl Tris base, 0.5 μl NaH2PO4) before loading. Secreted Aβ was measured from in vitro assays by direct addition of the cell-incubated media onto the ELISA plates. MaxiSorp immunoplates (Nunc, Rochester, NY) were coated with mA2B20.1 (William Van Nstrand, Stony Bridge, NY) antibody at a concentration of 25 μg/ml in coating buffer (0.1 M NaCO3 buffer, pH 9.6) and blocked with 3% BSA. Standards of both Aβ1–40 and Aβ1–42 were made in antigen capture buffer (20 mM NaH2PO4, 2 mM EDTA, 0.4 M NaCl, 0.5 g of 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, and 1% BSA, pH 7.0), and loaded onto ELISA plates in duplicate. Samples were then loaded in duplicate and incubated overnight at 4°C. Plates were washed and then probed with either HRP-conjugated anti-Aβ1–40 (MM32-13.1.1, for Aβ1–40) or anti-Aβ1–42 (clone number MM40-21.3.4, for Aβ1–42) overnight at 4°C, 3,3’,3’-Tetramethylbenzidine was used as the chromogen, and the reaction was stopped by 30% H2SO4 and read at 450 nm on a Molecular Dynamics (Sunnyvale, NY) plate reader. Aβ readings were then normalized to protein concentrations of the samples loaded or to the protein concentration of the cell layer that the media were incubated with in the case of the in vitro assays. This takes into account any variations of cell numbers or protein concentrations that may otherwise affect Aβ readings.

**Immunostaining.** Light-level immunohistochemistry was performed using an avidin–biotin immunoperoxidase technique (ABC kit; Vector Laboratories, Burlingame, CA) and was visualized with diaminobenzidine as described previously (Oddo et al., 2003). The following antibodies were used: anti-Aβ, 6E10 (Signet), anti-tau HT7 (Innogenetics, Gent, Belgium), AT8 (Pierce), and AT180 (Innogenetics). Primary antibodies were applied at dilutions of 1:1000 for 6E10, 1:500 for AT8 and AT180, and 1:1000 for HT7. Quantification of DAB staining was performed by taking three adjacent images from each hippocampus at 20× or one image of the amygdala. These images were loaded in Scion Image 4.0 (Scion), and the DAB pixel count was measured by setting the threshold to the same value for each section. Pixel counts were averaged from the three adjacent sections for at least three animals per group, and the data were plotted.

**Confocal microscopy.** Fluorescent immunolabeling followed a standard two-way technique (primary antibody followed by fluorescent secondary antibody). Free-floating sections were rinsed in TBS, pH 7.4, and then blocked (0.25% Triton X-100, 5% normal goat serum in TBS) for 1 h. Sections were incubated in primary antibody overnight (4°C), rinsed in PBS, and incubated (1 h) in either fluorescently labeled anti-rabbit- or anti-mouse secondary antibodies (Alexa 488, 1:200; Invitrogen). Nuclear markers were added by incubating the slices in TOTO red (1:200 in PBS; Invitrogen) for 20 min. Antibodies were diluted as follows: HT7, 1:1000; and 6E10, 1:1000. Omission of primary antibody or use of preimmune IgG eliminated all labeling (data not shown). Confocal images were captured on a Radiance 2100 (Bio-Rad, Hercules, CA) confocal system. To prevent signal bleed-through, all fluorophores were excited and scanned separately using lambda strobing.

**Cell culture.** N2A cells were maintained in DMEM (Invitrogen) supplemented with 10% FBS. Cells were passaged at 1:40 when 70% confluency had been achieved and discarded after 20 passages. For experiments, equal numbers of cells were plated in six-well plates. Wells were treated 24 h later by removal of media and replacing with 2 ml of fresh media containing either dexamethasone (1 mM stock solution dissolved in H2O; Sigma) at a final concentration of 100 nm, 1 μM, or 10 μM, or corticosterone (1 mM stock solution dissolved in H2O; Sigma) at a final concentration of 100 nm, 1 μM, or 10 μM. Control wells contained fresh media only. Media were replaced every 24 h. After the treatment period, the media were taken from the wells, and secreted Aβ measurements were taken using sandwich ELISA. Protein extracts were taken as described above.

**Animal treatments.** All rodent experiments were performed in accordance with animal protocols approved by the Institutional Animal Care and Use Committee at the University of California, Irvine. The 3×Tg-AD mice were treated previously (Oddo et al., 2003). Briefly, these mice harbor a knock-in mutation of presenilin 1 (PS1M146V), the Swedish double mutation of amyloid precursor protein (APPK670N/E671K), and a frontotemporal dementia mutation in tau (tauP301L) on a 129/C57BL/6 background. Tau/PS1 mice (2×Tg-AD) harbor the same knock-in mutation of presenilin 1 (PS1M146V) and the same frontotemporal dementia mutation in tau (tauP301L) on a 129/ C57BL/6 background. Tau levels are expressed to a similar level to the 3×Tg-AD mice (Oddo et al., 2003). Four-month-old male 3×Tg-AD mice were taken and given one intraperitoneal injection of either dexamethasone (dissolved in PBS at 1 mg/ml) or PBS alone daily for 7 d. Dexamethasone was administered at 1 or 5 mg/kg bodyweight or PBS vehicle. At 24 h after the final injection, the animals were killed and the brains were removed. The brains were immediately dissected in half along the coronal line; half were frozen for biochemical analysis, and the other half were fixed in 4% paraformaldehyde. At 48 h later, brains were sliced into 40 μm sections using a vibratome. Blood was also taken and stored in EDTA-coated vacu-tubes (Fisher Scientific, Pittsburgh, PA), and centrifuged at 3000 rpm on a Beckman Instruments (Fullerton, CA) bench centrifuge for 10 min. The resultant supernatant was then taken as plasma, which was then frozen at −80°C.

**Measurement of corticosterone plasma levels.** A corticosterone competitive ELISA kit (Assay Systems, Ann Arbor, MI) was used to measure corticosterone levels as per the instructions of the manufacturer. Plasma samples were diluted 1:50 in the buffer provided.
Glucocorticoids increase 

**Results**

Glucocorticoids increase 

**Glucocorticoids increase Aβ formation in vitro**

We first determined whether glucocorticoids adversely affect APP processing in vitro using mouse neuronal N2A cells exposed to either corticosterone or the synthetic glucocorticoid dexamethasone (100 nM to 10 μM) for 24–72 h. Both corticosterone and dexamethasone increased levels of secreted Aβ40 and the longer, more amyloidogenic Aβ42 species in a concentration- and time-dependent manner as measured by sandwich ELISA (Fig. 1a,b). Dexamethasone treatment produced significantly greater increases in Aβ levels than did corticosterone treatment (~10-fold vs 3-fold higher after 72 h, respectively), presumably because of different receptor affinities (Reul and de Kloet, 1985). To determine whether the effect involved activation of glucocorticoid receptors and/or mineralocorticoid receptors, we used selective antagonists to identify the receptor underlying the glucocorticoid-mediated enhancement of Aβ levels. The glucocorticoid/progesterone receptor antagonist mifepristone (RU 38486; 1 μM) completely blocked the increase in Aβ levels produced by dexamethasone (Fig. 1c). In contrast, the mineralocorticoid receptor antagonist spironolactone (RU 28318; 100 nM) did not significantly affect the dexamethasone-induced increase in Aβ levels. Based on these studies, we conclude that the primary mode of action by which glucocorticoids modulate Aβ levels appears to be mediated by binding to stress-activated glucocorticoid receptors.

**Higher APP and BACE levels underlie the glucocorticoid-mediated enhancement of Aβ**

Aβ formation is mediated by the enzymatic cleavage of its precursor proteins APP and C99, as well as changes in the steady-state levels of the APP holoprotein. Because glucocorticoid exposure augmented Aβ levels, we next investigated whether APP processing was specifically affected. We found that glucocorticoid treatment increased APP steady-state levels by 50% as shown by Western blot (Fig. 1d,e). Glucocorticoid treatment also induced a ~40% increase in the C-terminal fragment of APP, C99, which is the immediate precursor to Aβ resulting from β-secretase cleavage of APP. These findings indicate that glucocorticoids act through low-affinity glucocorticoid receptors to increase both APP and C99 levels, which may be directly responsible for the observed elevation in Aβ levels.

Although rapid glucocorticoid effects have been reported (Dallman, 2005), the glucocorticoid–glucocorticoid receptor complex typically acts as a transcription factor, making it possible that glucocorticoid treatment increased Aβ production through a transcription-mediated pathway of its precursor, APP. The APP promoter is complex and resembles a housekeeping gene, but the presence of glucocorticoid-response elements within the APP promoter sequence has been reported (Lahiri, 2004). To determine whether these elements are responsive to glucocorticoids, we used real-time PCR to monitor APP mRNA levels after dexamethasone treatment. We found that APP mRNA levels were markedly increased, indicating that the higher steady-state levels of the holoprotein likely stemmed from either enhanced transcription and/or mRNA stabilization (Fig. 1f). Pulse-chase analysis with 35S-labeled Cys/Met revealed a similar increase in APP production during the 1 h pulse but no changes in APP degradation at the 1, 4, and 8 h chase time points (Fig. 1g). Together, these results indicate that the higher APP levels observed after glucocorticoid treatment are directly attributable to increases in APP production.

To generate Aβ, APP is cleaved at the β-secretase site by the enzyme BACE, which generates two fragments: a large ectodomain, sAPPβ, and C99. Recently, Sambamurti and colleagues identified several glucocorticoid-responsive elements within the BACE promoter (Sambamurti et al., 2004) and noted that these sites occur in a region of the promoter that positively influences transcription (Ge et al., 2004). Using real-time PCR, we found that BACE transcripts were increased significantly after dexamethasone exposure (10 μM, 72 h), accounting for the selective increase in C99 (Fig. 1f). Therefore, to our knowledge, we show for the first time, using an in vitro system, that exposure to glucocorticoids increases production of both APP and BACE, which further results in higher C99 and Aβ levels.

**Dexamethasone increases intraneuronal Aβ in the 3×Tg-AD mice**

We next investigated whether stress levels of glucocorticoids modulate Aβ and tau pathology in vivo. To address this issue, we used the 3×Tg-AD mouse model, which develops both Aβ and
Glucocorticoids elevate Aβ levels through increases in APP and BACE in vivo

Because glucocorticoid administration significantly increased Aβ levels in vivo, we attempted to elucidate the mechanism(s) under-

lying this effect. Our in vitro studies pointed to the higher Aβ levels resulting from increased production rather than diminished clearance of Aβ. Consequently, whole-brain homogenates from the 3×Tg-AD mice were analyzed by Western blot, which indicated that total APP levels were slightly increased in the 5 mg/kg dexamethasone-treated group versus the PBS-treated indicated that total APP levels were slightly increased in the 5 mg/kg dexamethasone-treated group versus the PBS-treated...
group (corresponding to the increase in the APP holoprotein). This band was not cAPP, which has a similar weight (Gervais et al., 1999; Zhao et al., 2003), because we found no differences in cAPP steady-state levels (Fig. 2i,j). This band likely represents a breakdown product of APP, and this catabolic process may be decreased by dexamethasone treatment, resulting in an additional buildup of the holoprotein, and is not attributable to glucocorticoid-mediated transcription of the Thy1.2-driven APP transgene.

We next determined whether glucocorticoid administration altered APP processing by identifying various APP fragments by Western blot. Our results revealed a marked increase in C99 levels in the dexamethasone-treated group (Fig. 2i,j). In contrast, levels of C83, the fragment resulting from α-secretase cleavage of APP, were unaffected, suggesting a selective increase in BACE activity (Fig. 2i,j). Importantly, in the dexamethasone-treated group, steady-state BACE levels were increased to a degree that can account for the selective increase in C99 levels and subsequent Aβ production (50%) (Fig. 2i,j). Thus, these findings are consistent with our in vitro data and suggest that the BACE promoter, which has been reported to contain several glucocorticoid-response elements (Sambamurti et al., 2004), is significantly activated by glucocorticoid treatment. To our knowledge, these results provide the first in vivo demonstration that glucocorticoids potently modulate Aβ levels in a transgenic mouse model of AD, and our studies identified an upregulation of BACE and C99 as the mechanism underlying this effect.

Glucocorticoids accelerate accumulation of somatodendritic tau in the 3×Tg-AD mice
The 3×Tg-AD mice develop both amyloid plaques and neurofibrillary tangles, with early tau pathology beginning within the somatodendritic region of hippocampal neurons at ~6 months of age, followed by conformational changes and hyperphosphorylation events at 9–12 months of age (Oddo et al., 2003). After dexamethasone treatment (5 mg/kg daily for 7 d), we assessed the consequences for tau pathology by immunostaining brain sections with antibody HT7, which recognizes all forms of human tau. We observed substantially increased HT7 staining in the somatodendritic compartment and along the axons in the hippocampus, cortex, and amygdala in the glucocorticoid-treated versus PBS-treated mice (compare with Fig. 3a–e; data
fluenced by the presence of Aβ. For example, reducing Aβ levels by either immunotherapy or via γ-secretase inhibitors leads to the clearance of early pathological states of tau (Oddo et al., 2004). Likewise, genetic or pharmacological manipulations of Aβ have a direct impact on tau levels (S. Oddo and F. M. LaFerla, unpublished observations). Consequently, we sought to determine whether the observed effects on tau were a direct effect of glucocorticoids or an indirect consequence of higher Aβ levels. We treated 4-month-old male Tg-AD mice increases tau accumulation. Four-month-old male 3×Tg-AD mice were treated daily for 7 d with 5 mg/kg dexamethasone (5 mg/kg, 7 d; n = 3) or PBS vehicle (PBS, 7 d; n = 3). Staining with H77 shows no immunoreactivity in 40 μm sections from vehicle-treated or 5 mg/kg dexamethasone-treated mice. H77 immunoreactivity was elevated in cell bodies of the hippocampus and throughout the neuronal processes, as also shown by confocal imaging. e, Quantification of H77 DAB immunoreactivity in hippocampal and amygdala regions from PBS-treated and 5 mg/kg dexamethasone-treated groups. f, DAB staining of no primary controls shown from hippocampus of PBS-treated and 5 mg/kg dexamethasone-treated animals, 10×. g, Western blot analyses of protein extracts from whole-brain homogenates of dexamethasone- and vehicle-treated 3×Tg-AD mice, shown as alternating lanes (P, PBS vehicle; D, 5 mg/kg dexamethasone treated) showing increases in total human tau steady-state levels (HT7) but no differences in phospho-tau (AT8 and AT180). h, Quantification of protein bands from g shown normalized to β-actin levels as a loading control. i, 2×Tg mice (PS1Δ999KI and tauP301L, lacking the human APP transgene) treated daily with dexamethasone (5 mg/kg, 7 d; n = 3) or vehicle (PBS, 7 d; n = 3). Staining with H77 shows no immunoreactivity in 40 μm sections from vehicle-treated or 5 mg/kg dexamethasone-treated mice. Hippocampus region is shown at 10× magnification. DAB H77 immunoreactivity was unchanged from vehicle.

Glucocorticoid treatment increases the insoluble Aβ load in aged 3×Tg-AD mice
AD dementia correlates best with soluble Aβ loads (McLean et al., 1999), but the most visible lesions are the Aβ plaques and tau-laden neurofibrillary tangles. As we have shown, dexamethasone treatment in prepathological 3×Tg-AD mice increases both soluble Aβ and somatodendritic accumulation of tau but...
We next investigated hyperphosphorylated tau levels in these aged, dexamethasone- and vehicle-treated animals. Western blot analysis revealed that there were no differences in tau hyperphosphorylation between dexamethasone- and PBS-treated mice (Fig. 4c,d), consistent with our findings in young, treated animals in which tau phosphorylation was unchanged with dexamethasone treatment. In contrast, HT7 tau immunostaining was increased in the cortex in cell bodies and axonal processes compared with the PBS vehicle controls, which had very little staining (Fig. 4e,f), whereas hippocampal staining was not further increased relative to PBS-treated mice (data not shown), presumably attributable to a “ceiling effect” because both PBS- and dexamethasone-treated hippocampi showed extensive staining. Interestingly, at this age when soluble Aβ levels are not increased by dexamethasone treatment, overall steady-state levels of tau, as measured by Western blot, are also not increased (Fig. 4c,d), strengthening the relationship between soluble Aβ and tau accumulation. These data once again demonstrate that short-term glucocorticoid treatment does not affect tau phosphorylation but does increase tau accumulation.

Endogenous plasma corticosterone levels are elevated in pathological 3×Tg-AD mice

In human subjects with sporadic AD, plasma cortisol levels are increased during both early and late stages (Umegaki et al., 2000). However, it remains unknown whether HPA axis dysfunction is an initiating factor in AD genesis or whether it arises as a result of decreased HPA axis regulation by limbic structures that are increasingly burdened with Aβ and tau pathology. To determine whether basal levels of circulating glucocorticoids are altered in a transgenic model of AD and whether Aβ and tau pathology precede or follow changes in circulating glucocorticoids, we investigated plasma corticosterone levels across a wide age range in 3×Tg-AD mice. Plasma was harvested from 2- to 18-month-old 3×Tg-AD mice, taken directly from their home cage. Until 9 months of age, basal plasma corticosterone levels were unchanged in 3×Tg-AD mice compared with age-matched nontransgenic (NonTg) mice, despite a steady accumulation of Aβ pathology over that period (Oddo et al., 2003; Billings et al., 2005). After 9 months of age, basal plasma corticosterone levels were significantly elevated compared with age-matched NonTg mice (Fig. 5a), indicating that AD-like pathology in this mouse model induces dysfunction of the HPA axis. It has been shown previously that hippocampal CA3 lesions increase glucocorticoid levels, because the hippocampus plays a negative feedback role in regulating the HPA axis, which in turn causes cognitive deficits (Roozenendaal et al., 2001). These higher glucocorticoid levels may then further exacerbate pathology, leading to a circle of detrimental positive feedback. We then looked at steady-state levels of glucocorticoid receptor levels in hippocampal homogenates from 4- and 15-month-old 3×Tg-AD mice and nontransgenic controls. We found no differences at either time point despite elevated corticosterone levels at 15 months (Fig. 5b,c).

Discussion

To study the link between elevated levels of stress hormones and AD genesis, we investigated the effects of glucocorticoids on APP processing in vitro, as well as on the Aβ and tau burden in vivo. Consistent with stress being a risk factor for AD, we showed that administering glucocorticoids to young 3×Tg-AD mice increased soluble and insoluble Aβ, in which in aged mice it increased the insoluble Aβ load. In both young and aged mice, glucocorticoid administration also lead to the mislocalization of tau to the somatodendritic compartment, although phospho-tau levels were not affected. We also demonstrate that elevated glucocorticoid levels increase Aβ production by augmenting steady-state levels of APP and BACE in just 7 d. Both the APP and BACE
genes contain glucocorticoid-response binding elements, making it likely that glucocorticoids directly increase transcription of the APP and BACE genes, leading to the increased Aβ production observed in vitro and in vivo. Furthermore, this effect occurs through activation of the glucocorticoid receptor, as an antagonist of this receptor type prevents glucocorticoid-mediated increases in Aβ, and the glucocorticoid receptor is widely known to mediate transcription during agonist binding, dimerization, and relocation to the nucleus (Wright et al., 1993). Increases in APP and BACE proteins lead to increased processing of APP to C99 by BACE, which is consequently cleaved by the γ-secretase to release Aβ.

We further demonstrate that glucocorticoid treatment increases tau accumulation in a manner that is dependent on the presence of the APP transgene. This finding supports our previous work showing that intraneuronal Aβ accumulation precedes the accumulation of tau (Oddo et al., 2003) and that removal of Aβ via immunotherapy also successfully clears tau provided that the tau is not yet hyperphosphorylated or the proteasome is not impaired (Oddo et al., 2004). Supporting a role for intraneuronal Aβ in AD, it has been shown that levels of soluble Aβ correlate well with cognitive decline (Lue et al., 1999; McLean et al., 1999; Naslund et al., 2000), whereas plaques correlate poorly (Braak and Braak, 1997). Furthermore, the 3×Tg-AD mouse develops intraneuronal Aβ accumulation in the cell bodies of neurons in the hippocampus before any plaque pathology develops and at an age at which both synaptic dysfunction and cognitive impairments are observed (Oddo et al., 2003; Billings et al., 2005). The sum of these findings suggests a direct relationship between intraneuronal Aβ and tau accumulation such that an increase in Aβ induces a concomitant increase in tau accumulation; likewise, a decrease in Aβ results in decreased tau accumulation. However, short-term glucocorticoid treatment does not affect the phosphorylation of tau. It is probable that the development of neurofibrillary tangles in AD results from the activation of different kinase/phosphatase pathways. Regardless, it is possible the elevated levels of tau may provide a larger pool of substrate available for hyperphosphorylation and downstream neurofibrillary tangle formation.

These findings have particular relevance to AD because it is established that early sporadic AD patients display elevated circulating cortisol (Davis et al., 1986; Masugi et al., 1989; Swanwick et al., 1998; Peskind et al., 2001). The causes underlying the higher circulating levels of cortisol are unknown and seem to be more relevant to the early stages of the disease (Swanwick et al., 1998), but clinical data suggest that a stressful lifestyle can be a risk factor for AD (Wilson et al., 2005). In addition, a strong risk factor for AD is the presence of the apoE4 allele, which has been shown to elevate CSF cortisol levels (Peskind et al., 2001) more so than the E3 or E2 allele. Taken with the present findings, these clinical data may suggest that elevated cortisol levels in early sporadic AD resulting from stress, administered exogenous glucocorticoids, or dysregulation of the HPA axis may contribute to pathology by increasing Aβ production. However, it is unlikely that increased glucocorticoids lead to AD without fail, because a number of disorders result in increased cortisol, such as Cushing’s disease, yet there is no published link between these patients and AD. Therefore, it may be that increased glucocorticoids are a risk factor for AD, along with a number of other environmental and genetic risk factors or that they may exacerbate existing AD pathologies.

The underlying cause of AD is wholly unknown, although most researchers currently agree that various environmental and genetic factors are likely to act concurrently or even synergistically to trigger sporadic AD. The Aβ cascade hypothesis states that it is the buildup of Aβ that triggers AD through its downstream effects; although multiple findings support this argument, the precise mechanism(s) underlying the initial buildup of Aβ remains unknown. According to this leading hypothesis, any regimen that increases Aβ production should therefore also increase cognitive decline. Supporting this, prednisone, a glucocorticoid used to reduce inflammation, accelerated cognitive deterioration in AD patients (Aisen et al., 2000), and, although it is not yet known why this deterioration occurred, the results from the present study suggest that prednisone may have increased the pathological burden. Given our findings here, it seems plausible that increases in circulating glucocorticoids, either through pharmacological intervention or naturally through stress or AD-related increases, could lead to accelerated cognitive decline by increasing Aβ production, in line with the amyloid cascade hypothesis. However, because we were not investigating the effects of glucocorticoid-mediated increased AD pathology on cogni-
tion in the 3×Tg-AD mouse, we cannot say for certain what the cognitive outcome of this paradigm would be.

Glucocorticoid-related genetic susceptibility for AD has been identified through the 11β-hydroxysteroid dehydrogenase type 1 gene, in which a haplotype was identified with a sixfold higher risk for developing the disease (de Quervain et al., 2004). This dehydrogenase controls biologically active levels of glucocorticoids in tissues and thus links a possible increase in circulating glucocorticoids with the development of AD. Furthermore, in transgenic mouse models of AD, environmental enrichment, a paradigm that may reduce stressful housing conditions, has been shown to reduce Aβ pathology (Lazarov et al., 2005) as well as improve cognition (Jankowsky et al., 2005). It should be noted that anti-anxiety drugs are currently in clinical trials for treatment of AD, illustrating how reducing stress may be beneficial for disease prevention and treatment.

Our finding that the 3×Tg-AD mice develop elevated corticosterone levels concurrent with advancing pathology indicates that AD neuropathology could diminish negative feedback controlling glucocorticoid levels via dysregulation of the HPA axis. This increase in circulating glucocorticoids would likely increase Aβ pathology and subsequent tau accumulation, resulting in a positive feedback loop by which pathology increases circulating glucocorticoids, which further increase pathology. Because cortisol levels are elevated in early AD, the use of therapies aimed at reducing these levels or in preventing or reducing their efficacy should be investigated because they may prevent glucocorticoid-mediated increases in Aβ. The involvement of glucocorticoids in AD is universally thought to have detrimental effects on the pathology. It has been shown previously that dexamethasone treatment of microglia in culture impedes degradation of Aβ peptides (Harris-White et al., 2001), in which in vivo application resulted in more compact Aβ plaques containing more of the peptide. It has also been shown that elevated glucocorticoid levels are associated with decreased degradation of Aβ in aged macaques, through a downregulation of insulin-degrading enzyme (Kulstad et al., 2005), an Aβ-degrading enzyme. Conversely, elevated glucocorticoid levels have been shown to increase susceptibility of cholinergic neurons to Aβ-mediated toxicity in vivo (Abraham et al., 2000) and hippocampal neuronal cultures (Goodman et al., 1996). Together with our observations, glucocorticoids mediate enhanced production of Aβ, reduce degradation, facilitate plaque formation, enhance Aβ-mediated neuronal toxicity, and increase tau accumulation. Therefore, perhaps therapies aimed at reducing glucocorticoids in the elderly and early AD patients would be beneficial, although the use of glucocorticoid-containing medications should be cautioned because they may be worsening pathology in humans. Our findings provide support for the hypothesis that elevated glucocorticoids found in AD are a consequence of the pathology and additionally, and importantly, play a significant causal role in the development of the pathology.

References


Molchan SE, Hill JL, Mellow AM, Lawlor BA, Martinez R, Sunderland T

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