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Corticosterone and Related Receptor Expression are Associated With Increased β -Amyloid Plaques in Isolated Tg2576 Mice

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Abstract

Previously, we reported that the stress associated with chronic isolation was associated with increased β -amyloid ($A\beta$) plaque deposition and memory deficits in the Tg2576 transgenic animal model of Alzheimer's disease (AD) [Dong H, Goico B, Martin M, Csernansky CA, Bertchume A, Csernansky JG (2004) Effects of isolation stress on hippocampal neurogenesis, memory, and amyloid plaque deposition in APP (Tg2576) mutant mice. *Neuroscience* 127:601–609]. In this study, we investigated the potential mechanisms of stress-accelerated $A\beta$ plaque deposition in this Tg2576 mice by examining the relationship between plasma corticosterone levels, expression of glucocorticoid receptor (GR) and corticotropin-releasing factor receptor-1 (CRFR1) in the brain, brain tissue $A\beta$ levels and $A\beta$ plaque deposition during isolation or group housing from weaning (i.e. 3 weeks of age) until 27 weeks of age. We found that isolation housing significantly increased plasma corticosterone levels as compared with group-housing in both Tg+ mice (which contain and overexpress human amyloid precursor protein (hAPP) gene) and Tg– mice (which do not contain hAPP gene as control). Also, isolated, but not group-housed animals showed increases in the expression of GR in the cortex. Furthermore, the expression of CRFR1 was increased in isolated Tg+ mice, but decreased in isolated Tg– mice in both cortex and hippocampus. Changes in the components of hypothalamic–pituitary–adrenal (HPA) axis were accompanied by increases in brain tissue $A\beta$ levels and $A\beta$ plaque deposition in the hippocampus and overlying cortex in isolated Tg+ mice. These results suggest that isolation stress increases corticosterone levels and GR and CRFR1 expression in conjunction with increases in brain tissue $A\beta$ levels and $A\beta$ plaque deposition in the Tg2576 mouse model of AD.

Keywords

stress; corticosterone; glucocorticoid receptor; corticotropin-releasing factor receptor-1; β -amyloid; Alzheimer's disease

Psychosocial stress has been suggested to be one of the environmental factors that can influence the pathogenesis of Alzheimer's disease (AD) (Wilson et al., 2003, 2006; Hasegawa 2007). Increased plasma cortisol levels have been reported in patients with dementia of the Alzheimer's type (DAT) (Davis et al., 1986; Weiner et al., 1997; Peskind et al., 2001), and in longitudinal studies of DAT patients, increased plasma cortisol levels have been correlated

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with more rapid cognitive decline (Csernansky et al., 2006). However, relatively little is known about the mechanisms by which psychosocial stressors might impact the pathogenesis of AD.

In a variety of mammals, including humans, behavioral stressors have been shown to increase the activity of the hypothalamic–pituitary–adrenal (HPA) axis (Sapolsky, 1993; McEwen, 2000; Hibbard et al., 2000). In turn, changes in various components of the HPA axis [i.e. corticotropin-releasing factor (CRF), adrenocorticotrophic hormone (ACTH), glucocorticoid and the receptor (GR)] have been linked to changes in CNS function, especially learning and memory (Cintra et al., 1994; Diamond et al., 2000; Blank et al., 2003; Orozco-Cabal et al., 2006). Some (Schrijver et al., 2004; Võikar et al., 2005), but not all (Williams et al., 2001), investigators have reported that the stress of isolation housing impairs memory in rodents. In addition, chronic stress has been shown to alter the structure and function of the hippocampus (McEwen, 1999; Kim and Diamond, 2002; Sapolsky, 2002; Bremner, 2006), as well as a variety of hippocampal-dependent behaviors (Landfield et al., 1978; Kerr et al., 1991; Dachir et al., 1993; Bodnoff et al., 1995; Alfarez et al., 2003; Kim et al., 2006). This may have particular relevance for the pathogenesis of AD, since the hippocampus is among the brain structures that are affected early in the course of this disease (Arnold et al., 1991; Price and Morris, 1999).

Recent studies of transgenic mice that overproduce amyloid precursor protein (APP) suggest that behavioral stressors may accentuate the production of β -amyloid ($A\beta$) and its incorporation into $A\beta$ plaques. For example, increases in $A\beta$ plaque deposition have been observed in the hippocampus and cortex of stressed Tg2576 and APP_{V717I}-CT100 mice (Dong et al., 2004; Jeong et al., 2006). Further, administration of dexamethasone to triple transgenic APP/PS1/MAPT mice increased brain levels of APP, $A\beta$, as well as β -amyloid precursor protein cleaving enzyme (BACE) and the β -C-terminal fragment (β -CTF) of APP (Green et al., 2006). Recently, our group showed that behavioral stressors can acutely increase basal levels of $A\beta$ via CRFR1 and increases in neuronal activity (Kang et al., 2007). Also, CRF receptors have been implicated in stress-induced hippocampal tau phosphorylation (tau-P) in rodents (Rissman and Lee, 2007). These studies suggest an interaction between stress-induced changes in elements of the HPA axis and the pathogenesis of AD.

In this study, we further investigated the effects of chronic stress on HPA axis activity as well as $A\beta$ aggregation in an animal model of AD. More specifically, we investigated associations between plasma corticosterone levels, expression of GR and CRFR1 in the cortex and hippocampus, brain tissue $A\beta$ levels and $A\beta$ plaque deposition, hippocampal volume in Tg2576 mice housed in isolation or in a group from weaning (i.e. 3 weeks of age) until 27 weeks of age (i.e. isolated for 24 weeks).

Experimental Procedures

Animals

The strain of Tg2576 mice created by Hsiao et al. (1996) was used for this study. Tg2576 mice contain a double mutation (Lys670-Asn, Met671-Leu [K670N, M671L]), driven by a hamster prion protein promoter, and over-express human APP 695. Tg2576 males (Taconic Farms Inc., Germantown, NY, USA) were bred with C57B6/SJL females (The Jackson Laboratory, Bar Harbor, ME, USA), and offspring of both sexes were distributed equally among the experimental groups. Genotyping for transgenic screening in the offspring was performed using DNA obtained from post-weaning tail biopsies (Hanley and Merlie, 1991). PCR products were visualized on a 1% agarose gel containing ethidium bromide (EB) to confirm the presence of DNA of human APP in offspring (Tg+). Transgene-negative (Tg-) littermates were used as controls.

A total of 49 Tg2576 mice of both genders were used for this study. Newborn pups were weaned at 3 weeks of age and randomly divided into four groups: isolated Tg+ ($n=14$); group-housed Tg+ ($n=12$); isolated Tg- ($n=11$); and group-housed Tg- ($n=12$). Male and female animals were equally distributed in each group. All experimental procedures involving animals were performed in accordance with guidelines established by the Animal Studies Committee at Washington University and in accordance with the National Institutes of Health and Institutional Guidelines. All efforts were made to minimize the number of animals used and their suffering.

Isolation stress

Isolated Tg+ and Tg- mice were individually housed in cages 1/3 smaller ($10 \times 14 \times 9.5 \text{ cm}^3$) than standard-sized mouse cages ($27 \times 14 \times 11 \text{ cm}^3$), placed on separate shelves, and blocked with a board to prevent visual contact, from weaning until 27 weeks of age (isolated for 24 weeks). Non-isolated animals were housed three animals per standard-sized cage of the same gender, and housed next to each other on standard racks for the same time period. Both groups of animals were maintained at $25 \pm 1^\circ \text{C}$ under a 12-h light/dark cycle (light on 6 AM), and food and water were made available *ad libitum*.

Corticosterone assay

At 27 weeks of age, at 6 AM (before lights on), blood was collected by rapid retro-orbital phlebotomy (less than 1 min). Plasma concentrations of corticosterone were measured using a radioimmunoassay kit from ICN (ICN Pharmaceuticals, Costa Mesa, CA, USA) as previously described (Jacobson et al., 1997; Boyle et al., 2005). The sensitivity of this assay is 12.5 pg and the inter-assay coefficient of variation is 7–10%.

Brain tissue A β measurement

Immediately following blood collection, half of the animals ($n=5-7$ in each group) were killed by decapitation and the hippocampus and overlying cortex were snap-frozen on dry ice, then stored at -80°C . Later, tissue levels of A β were measured using enzyme-linked immunosorbent assay (ELISA). For ELISA, the dissected brain tissues were homogenized in 20 ml Tissue Protein Extraction Reagent (T-PER), (Pierce, Rockford, IL, USA). The homogenates were centrifuged at 10,000 r.p.m. for 5 min to pellet cell/tissue debris. Supernatant was collected and total protein levels were measured using Bio-Rad Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA). Levels of A β_{1-40} and A β_{1-42} were measured using commercially available ELISA kits (Biosource International Inc., Camarillo, CA, USA) according to the manufacturer's instructions. Briefly, duplicate standards (A β_{1-40} or A β_{1-42} , from 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml, 15.65 pg/ml, 0 pg/ml) and tissue samples (120 μg), containing 1 mM AEBSF (4-(2-aminoethyl)-benzolsulfonylfluoride), were incubated overnight in antibody-coated plates at 4°C . After washing, the plates were incubated in rabbit anti-human A β_{1-40} or A β_{1-42} for 2 h at room temperature, and then with horseradish peroxidase-conjugated anti-rabbit IgG for another 2 h. Stabilized chromogen (100 μl) was added to each well for 30 min incubation in the dark. The absorbance in each well was read with Microplate Reader (SpectraMax Plus; Molecular Devices, Sunnyvale, CA, USA). The intra-assay coefficient of variation was 2.9%.

Tissue preparation and section sampling for histology

Immediately following blood collection, the remaining half of the animals ($n=5-7$ in each group) were deeply anesthetized using a 3.3 ml/kg ketamine/xylazine mixture (86:13 mg/ml) and perfused transcardially with 1% heparinized 0.01 M phosphate buffer (PBS) for 2 min and then 4% paraformaldehyde for 25–30 min. Brains were removed and post-fixed at 4°C using the same fixative with 30% sucrose for 48 h. The brains were dissected and embedded in Tissue-

Tek embedding medium (Electron Microscopy Sciences, Hatfield, PA, USA), and cut into 35 μm thick sections in the coronal plane using a cryostat (Leica CM 1850 UV, Nussloch, Germany). The cortex was nicked in the left hemisphere prior to sectioning to serve as a marker of orientation and the sections were mounted onto slides with a consistent orientation. Of the eight sets of serial sections (24–26 sections each), we used one series for $A\beta$ immunohistochemical staining, one series for Thioflavin S staining, one series for GR staining, one series for CRFR1 immunohistochemical staining and one series for Nissl (Cresyl Violet) staining. The remaining three series were stored for future studies.

GR and CRFR1 immunostaining

GR and CRFR1 immunohistochemical staining was performed using published methods (Chen et al., 2000; Klimaviciute et al., 2006). Briefly, sections were rinsed with 0.1 M PBS (pH 7.4) three times each for 5 min. The endogenous peroxidase activity was eliminated by pre-treatment with 0.3% hydrogen peroxide in methanol for 30 min followed by washing in PBS. The sections were then incubated in a blocking solution of 5% normal goat serum for 1 h and incubated overnight with mouse monoclonal IgG (MA1-510, 1:200, Affinity BioReagents, Golden, CA, USA) or goat anti-CRFR1 (1:100, sc-12381, Santa Cruz Biotech Inc., Santa Cruz, CA, USA). Subsequently, the sections were washed in PBS and incubated in a secondary antibodies that matched the primary antibodies (1:200, Vector Laboratories, Burlingame, CA, USA) diluted in blocking solution for 2 h, then in an avidin–biotin complex for 1 h at room temperature. The reaction was developed using the DAB-kit (diaminobenzidine) from Vector.

GR- or CRFR1-immunoreactive cells were counted in the cortex and the hippocampus using an optical fractionator method (West et al., 1991). The density of positive cells (per mm^3) was calculated by dividing the number of cells counted by the total volume sampled (Bonthius et al., 1992). The sampled volume was determined using the number of disectors multiplied by the volume of one disector (CAST-2; Olympus, Danmark A/S, Herstedstervej 27-29, DK 2620, Albertslund, Denmark).

$A\beta$ plaque immunostaining

For immunohistochemical staining of $A\beta$ plaques, selected sections were rinsed with 0.1 M PBS (pH 7.4), and incubated in a blocking solution of 5% normal goat serum for 1 h. Sections were then incubated overnight in the primary antibody for $A\beta$ at 4 °C (rabbit polyclonal pan antibody raised against $A\beta$ synthetic peptide). The antibody was purified by epitope-specific chromatography and was shown to recognize the sequence of $A\beta$ in the region from amino acids 15–30 (1:1000, Biosource). After PBS washing, the sections were incubated in biotinylated anti-rabbit secondary antibody for 2 h at room temperature, then in an avidin–biotin complex for 1 h at room temperature (Vector Laboratories). $A\beta$ -like immunoreactivity was visualized using a DAB kit (Vector Laboratories). To confirm that $A\beta$ -immunoreactivity represented the presence of compact (fibrillar) $A\beta$ plaques we stained selected floating sections using a 1% Thioflavine S aqueous solution for 5 min, followed by washing with 70% alcohol for 3–5 min (Guntern et al., 1992).

The number of $A\beta$ plaques was determined separately in two brain areas, the cerebral cortex and the hippocampal formation. The cortex was defined as all areas dorsal to the rhinal fissure and dorsal or lateral to corpus callosum and external capsule. The hippocampal formation was defined as the hippocampus proper plus the dentate gyrus and subiculum. $A\beta$ -immunohistochemistry was used to measure both the total $A\beta$ plaque area in each brain (i.e. total plaque burden) using the CAST-2 stereological program and total plaque number. $A\beta$ plaques were counted on every eighth section throughout the entire brain in each structure, and the total number of plaques in the selected sections was calculated for statistical analysis.

Hippocampal volume

Nissl-stained coronal sections were used to estimate the whole volume of the hippocampal formation. All sections containing the hippocampus were photographed. The final resolution of the images was one pixel²=0.00056787 mm². Using the Analyze 8.0 stereology module, the area of hippocampus on each photograph was measured in each coronal section using Cavalieri's principle. The contours of the hippocampus were identified using landmarks derived from a mouse brain atlas (Paxinos and Franklin, 2001). A grid was randomly placed over each photograph. The points on the grid were spaced eight pixels apart on the X and Y axes. Thus, the total number of grid points over the target area was multiplied by 64 to obtain an estimate of the total pixels encompassing the hippocampal formation in each brain. The total pixels were then multiplied by 0.00056787 mm² and 0.28 mm (the distance between sections) to obtain hippocampal volume.

Data analysis

All variables were compared across groups using two-way ANOVA. When genotype effects (i.e. Tg+ versus Tg-), housing condition effects (i.e. isolated versus group-housed), or genotype×housing condition interactions were found, post hoc analyses were performed using Bonferroni-Dunn tests for multiple comparisons. Pearson's correlation was used to evaluate the relationships between various measurements. For ANOVA, statistical significance was accepted for *P*-values less than 0.05. In order to estimate the precision of stereological counting in individual subjects, the coefficients of error were calculated by dividing the standard error by the mean value (Bonthius et al., 2004; Jacobs et al., 2005).

Results

Plasma corticosterone levels

There was an overall effect of genotype [$F(1,36)=11.5$, $P=0.0017$] and housing condition [$F(1,36)=6.27$, $P=0.006$], and a tendency toward a significant genotype by housing condition interaction [$F(1,36)=3.18$, $P=0.08$], on plasma corticosterone levels in Tg2576 mice. Post hoc analyses showed that Tg+ mice had significantly higher levels of corticosterone as compared with Tg- mice regardless of whether they were isolated ($P=0.012$) or group housed ($P=0.04$). Also, isolation stress was associated with significant increases in plasma corticosterone levels in both Tg+ ($P=0.0075$) and Tg- ($P=0.0056$) mice (Fig. 1).

GR-immunoreactive cell density

GR-immunoreactive nerve cell nuclei were present in the parvocellular part of the paraventricular hypothalamic nucleus, in the anterior periventricular hypothalamic nucleus, in the ventral part of the mediobasal hypothalamus, and in the CA1 and CA2 subregion of the hippocampal formation (Fig. 2A and B). Also, medium-to-high densities of GR-immunoreactive nerve cell nuclei were present across the cortex. Medium densities of GR-immunoreactive nerve cells were demonstrated in many thalamic nuclei and in the central amygdaloid nucleus. The apparent pattern of the distribution of GR was not different between Tg+ and Tg- mice.

ANOVA indicated a significant effect of housing condition [$F(1,17)=5.71$, $P=0.03$], but not genotype [$F(1,17)=1.89$, $P=0.18$], nor a genotype by housing condition interaction [$F(1,17)=2.29$, $P=0.15$], on the density of GR-immunoreactive cells in cortex among the isolated and grouped housed Tg+ and Tg- groups. Between groups, statistical testing showed a significant increase in GR-immunoreactive cell density in isolated Tg+ mice as compared with group-housed Tg+ mice ($P=0.04$, Fig. 3A). In the hippocampus, ANOVA indicated there was no effect of housing condition [$F(1,16)=3.83$, $P=0.068$] or genotype [$F(1,16)=0.60$, $P=0.45$], nor

a genotype by housing condition interaction [$F(1,16)=0.66$, $P=0.43$] on GR-positive cell density among the isolated and group housed Tg+ and Tg- groups. However, between groups statistical testing showed a significant increase in GR-immunoreactive cell density in isolated Tg+ mice as compared with group-housed Tg+ mice ($P=0.003$) (Fig. 3B).

CRFR1-immunoreactive cell density

CRFR1-immunoreactive cells were observed in the hypothalamic nuclei, sensory relay and associate thalamic nuclei, pyramidal neurons in cortex and Purkinje cells in the cerebellum, as well as the hippocampus and amygdala. CRFR1-immunoreactive cells displayed a variety of patterns of intracellular distribution of the immunoreaction product; however, most displayed cell membrane localization (Fig. 2C and D). This distribution pattern is consistent with previous reports of CRFR1 expression in rodent brain (Radulovic et al., 1998; Chen et al., 2000).

There was a significant effect of genotype [$F(1,17)=4.79$, $P=0.04$], and a significant genotype by housing condition interaction [$F(1,17)=20.75$, $P=0.003$], but not a significant effect of housing condition [$F(1,17)=0.10$, $P=0.75$], on CRFR1-immunoreactive cells in the cortex. There was a significant increase in CRFR1-immunoreactive cell density in the cortex of isolated Tg+ mice as compared with group-housed Tg+ mice ($P=0.02$) and isolated Tg- mice ($P=0.0003$) (Fig. 3C). In the hippocampus, there was a significant effect of genotype [$F(1,17)=4.79$, $P=0.05$], housing condition [$F(1,17)=8.60$, $P=0.009$], and a significant genotype by housing condition interaction [$F(1,17)=20.95$, $P=0.0003$], on CRFR1-immunoreactive cell density. Again, statistical analysis indicated a significant increase in CRFR-immunoreactive cell density in isolated Tg+ mice ($P=0.003$) as compared with group housed Tg+ mice ($P=0.0029$) and isolated Tg- mice ($P=0.001$), (Fig. 3D).

Tissue A β levels and A β plaque deposition

In Tg+ mice, isolation housing was associated with increases in tissue A β levels (Fig. 4) and in the number of A β plaques deposited in the brain (Figs. 5, 6). There was a significant effect of housing condition on both A β_{1-40} [$F(1,10)=16.15$, $P=0.001$] and A β_{1-42} [$F(1,10)=34.35$, $P<0.001$] levels in Tg+ mice (Fig. 4A and B). Since total protein was extracted for this assay and soluble A β in PBS buffer was not extracted separately, the tissue level of A β represented a combination of soluble and insoluble peptides. Human A β_{1-40} and A β_{1-42} were not detected in Tg- animals.

In isolated Tg+ mice, increases in A β plaque number and size could be seen in the cortex (Fig. 5A) and hippocampus (Fig. 5B). There was a significant effect of housing condition on the number of both A β immunoreactive plaques [$F(1,11)=14.83$; $P=0.0027$] (Fig. 6A), and Thioflavine S-stained plaques [$F(1,11)=35.68$, $P<0.0001$] (Fig. 6D), as well as overall plaque burden [$F(1,11)=8.56$, $P=0.013$] (Fig. 6E) in Tg+ mice. Further, the number of A β immunoreactive plaques disturbed mainly in the cortex. After isolation stress, the ratio of increased A β plaque was evenly distributed in the cortex (2.7 times increase, Fig. 6B) and the hippocampus (2.4 times increase, Fig. 6C). No plaques were observed in Tg- mice under any housing condition.

Hippocampal volume

There was a significant effect of housing condition [$F(1,20)=7.77$, $P=0.01$], but no effect of genotype [$F(1,20)=0.85$, $P=0.37$], nor a genotype by housing condition interaction [$F(1,20)=0.63$, $P=0.43$] on hippocampal volume (Fig. 7). Post-hoc test showed that isolation housing significantly decreased the volume of the hippocampus in both Tg+ and Tg- mice as compared with group housed Tg+ and Tg- mice (both P 's=0.03), although the magnitude of volume decrease was somewhat larger in Tg+ than Tg- mice.

Correlations between amyloid plaques and HPA-related components

The number of A β immunoreactive plaques was strongly correlated with plasma corticosterone levels ($r=0.76$, $n=11$, $P=0.02$) and GR expression in the hippocampus ($r=0.70$, $n=10$, $P=0.05$), but only weakly correlated with CRFR1 expression in the cortex ($r=0.34$, $n=11$, $P=0.39$) in Tg⁺ mice. The number of Thioflavine-stained plaques was strongly correlated with the expression of GR in the cortex ($r=0.77$, $n=11$, $P=0.02$). Also Thioflavine-stained plaques were correlated with CRFR ($r=0.61$, $n=11$, $P=0.08$) in the cortex, however, the number of Thioflavine-stained plaques was not correlated with corticosterone levels ($r=0.16$, $n=11$, $P=0.69$). Finally, hippocampal volume was inversely correlated with corticosterone levels ($r=-0.57$, $n=11$, $P=0.11$) and the number of A β immunoreactive plaques ($r=-0.71$, $n=11$, $P=0.05$) (Table 1).

Discussion

This study replicates and extends our previous findings by showing that chronic isolation stress was associated with increased tissue levels of A β_{1-40} and A β_{1-42} , as well as A β plaque burden in the cortex and the hippocampus of Tg2576 mice at 27 weeks of age. In addition, we observed that isolated Tg⁺ mice had higher corticosterone levels and increased expression of GR and CRFR1 in the cortex and hippocampus as compared with group-housed Tg⁺ mice. Furthermore, the degree of A β plaque deposition was correlated with plasma corticosterone levels, and GR and CRFR1 expression in the cortex and hippocampus. These results suggest that there is an association between changes in the activity of elements of the HPA axis and increases in brain tissue A β levels and A β plaque burden, and provide additional support for the hypothesis that stress may accelerate amyloid plaque deposition in Tg2576 mice.

Our results also suggest that Tg2576 mice may exhibit an aberrant response to chronic behavioral stress. First, Tg⁺ mice showed significantly higher levels of plasma corticosterone as compared with Tg⁻ mice after exposure to isolation housing (Fig. 1). These results were consistent with other studies using same strain (Pedersen et al., 1999; Pedersen and Flynn, 2004) and a different animal model (Touma et al., 2004) of AD. Second, isolation stress significantly increased expression of GR in the cortex and hippocampus in Tg⁺ mice as compared with group-housed Tg⁺ mice. This is unusual because in the normal brain, especially in the hippocampus, under stressful circumstances, the expression of GR or GR number is generally decreased, perhaps as an adaptation to prevent glucocorticoid-induced damage (Sapolsky et al., 1986; McEwen, 2006). Finally, the expression of CRFR1 was significantly increased in the cortex and hippocampus of Tg⁺ mice, while the expected decrease in CRFR1 expression in Tg⁻ animals was observed (Fig. 3).

The results of this study provide additional evidence to support an association between chronic stress, increased levels of brain tissue A β level and A β plaque deposition using both histological and biochemical methods. In our previous study (Dong et al., 2004), we observed abundant A β plaques throughout the hippocampus and cortex after exposure to isolation housing from weaning until 6–7 months of age. However, in the previous study, we did not measure brain tissue A β levels and correlation of A β plaque with stress related components was not evaluated. Jeong et al. (2006) found increases in A β plaque deposition and memory impairments in APP_{V717I}-CT100 mice after chronic stress. Also, the results of other studies have suggested that treatment of triple transgenic APP/PS1/MAPT mice with dexamethasone increased brain APP and A β levels as well as BACE and the β -CTF of APP (Green et al., 2006). Nevertheless, the pathways linking changes in components of the HPA axis to changes in A β pathology are still unclear. One possibility is that CRF release in response to stress may disrupt neuronal activity, which in turn triggers increases in brain A β and accelerates the deposition of A β plaques. There is now substantial evidence that A β is produced by neurons in proportion to the level of synaptic activity (Cirrito et al., 2005). Further, CRF and its receptors are widely

expressed in the brain, where it acts as an excitatory neuropeptide and modulator of neuronal activity and signaling (Chang et al., 1993; Potter et al., 1994). In a recent microdialysis study of Tg2576 mice, our group found a significant increase in $A\beta$ levels in brain interstitial fluid (ISF) after both acute restraint and chronic isolation stress. Administration of exogenous CRF, but not corticosterone, mimicked the effects of acute restraint stress, and inhibition of endogenous CRF receptors, as well as neuronal activity, blocked these effects. This work suggests that stress-induced increases in CRF function may increase neuronal activity and thereby stimulate $A\beta$ release from neurons in mice containing the APP mutation (Kang et al., 2007).

In this study, isolation stress was also associated with decreases in hippocampal volume, but this effect was similarly present in both Tg+ and Tg- mice. This result adds to a large body of literature on the effects of behavioral stressors on brain structure and function. In a variety of mammals, chronic behavioral stressors trigger increases in CRF, ACTH, and in turn, glucocorticoid levels, and changes in these components of the HPA axis have been suggested to have deleterious effects on the structure and function of various brain structures, but especially the hippocampus (Sapolsky, 1993, 1996; Fuchs and Flugge, 1998; Vyas et al., 2002; Swaab et al., 2005). In rodents, atrophy of neuronal dendrites within the hippocampus (Watanabe et al., 1992; Magarinos et al., 1997; Donohue et al., 2006) and deficits in spatial memory (Coburn-Litvak et al., 2003; Wright et al., 2006) have been reported after exposure to chronic behavioral stress or administration of the glucocorticoid, corticosterone. Moreover, the hippocampus plays a central role in inhibiting the activity of the HPA axis (Jacobson and Sapolsky, 1991; Bratt et al., 2001; Herman et al., 2005). Thus, hippocampal neuronal damage induced by stress and glucocorticoid hormones could initiate a repetitive cycle of increasing HPA dysregulation and further neuronal injury (Sapolsky et al., 1986).

Our results are relevant to ongoing efforts to understand relationships among psychosocial stressors, changes in the HPA axis, and the pathogenesis of AD in humans. Elevated plasma cortisol levels have been reported in DAT subjects (Davis et al., 1986; Weiner et al., 1997; Peskind et al., 2001), but have been generally interpreted as evidence of AD-related hippocampal degeneration on the function of the HPA axis (i.e. disinhibition). Recently, however, higher plasma levels of cortisol have been correlated with the rate of cognitive decline in AD patients (Csernansky et al., 2006), which supports a more causative role for HPA axis dysregulation in the pathogenesis of AD. Further, reductions in CRF concentrations and increases in CRFR1 density have been reported in postmortem studies of AD subjects (De Souza et al., 1986; Auchus et al., 1994; Behan et al., 1997; Davis et al., 1999).

Conclusion

In summary, the results of this study indicate that Tg2576 mice exposed to the stress of chronic isolation exhibit increases in plasma corticosterone, and increases in GR and CRFR1 expression in the cortex and hippocampus, in association with increases in brain tissue $A\beta$ level, $A\beta$ plaque deposition and hippocampal atrophy. However, the molecular mechanisms linking increases in the activity of these components of the HPA axis to the acceleration in the appearance of AD-like neuropathology in APP-transgenic mice remain unknown. Further research to clarifying these underlying mechanisms is needed, and may be helpful for designing interventions to limit the effects of psychosocial stressors on the pathogenesis of the AD disease process in humans.

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Abbreviations

ACTH	adrenocorticotrophic hormone
AD	Alzheimer's disease
APP	amyloid precursor protein
Aβ	β -amyloid
BACE	β -amyloid precursor protein cleaving enzyme
CRF	corticotropin-releasing factor
DAT	dementia of the Alzheimer's type
ELISA	enzyme-linked immunosorbent assay

GR	glucocorticoid receptor
HPA	hypothalamic–pituitary–adrenal
PBS	phosphate buffer
β-CTF	β -C-terminal fragment

Fig. 1.

Plasma corticosterone levels in Tg+ and Tg- mice. Regardless of housing condition, plasma corticosterone levels were significantly higher in Tg+ mice than Tg- mice. Plasma corticosterone levels were dramatically increased in both Tg+ and Tg- mice after 24 weeks of isolation stress.

Fig. 2. GR and CRFR1 expression in the hippocampus in Tg+ mice. Immunocytochemical staining of GR (A, B) and CRFR1 (C, D) in the hippocampus of isolated Tg+ mice (B, D) and group-housed Tg+ mice (A, C). Scale bar=40 μ m (B); applies to A. Scale bar=40 μ m (D); applies to C.

Fig. 3.

Quantitative analysis of GR and CRFR1 expression in Tg+ and Tg- mice. GR-immunoreactive cell density was significantly increased in isolated Tg+ mice as compared with group-housed Tg+ mice in the cortex and the hippocampus (A, B). CRFR1-immunoreactive cell density was significantly increased in the cortex of isolated Tg+ mice as compared with group-housed Tg+ mice and isolated Tg- mice. CRFR1-immunoreactive cell density was significantly decreased in the cortex of isolated Tg- mice as compared with group-housed Tg- mice (C). CRFR1 immunoreactive cell density was significantly increased in the hippocampus of isolated Tg+ mice as compared with group-housed Tg+ mice and isolated Tg- mice, but no significant change between group housed Tg- mice and isolated Tg- mice (D).

Fig. 4. Aβ₁₋₄₀ and Aβ₁₋₄₂ levels in brain tissue of Tg+ mice. There was a significant increase in Aβ₁₋₄₀ (A) and Aβ₁₋₄₂ (B) in isolated Tg+ mice as compared with group-housed Tg+ mice at same age.

Fig. 5. $A\beta$ plaque deposition in Tg+ mice. $A\beta$ immunocytochemical and Thioflavine S fluorescence stains were used to identify and count $A\beta$ plaques in Tg+ mice at 27 weeks of age (isolated for 24 weeks from weaning). $A\beta$ plaques were easily found in isolated Tg+ mice in the cortex (A, $A\beta$ -immunocytochemical staining) and hippocampus (B, Thioflavine S staining), but $A\beta$ plaques were rare in Tg+ mice in age-matched group-housed controls. Scale bar=50 μ m (A, B).

Fig. 6.

Quantitative analysis of A β plaque deposition in Tg+ mice. Both A β immunocytochemically positive plaques (A–C) and Thioflavine S stained plaques (D), as well as percentage of plaque loading (E) were significantly increased at 27 weeks of age after 24 weeks of isolation stress in Tg+ mice as compared with group-housed Tg+ mice. The plaques distributed mainly in the cortex. After stress, the ratio of increased plaque was evenly distributed in the cortex (B) and hippocampus (C) as compared group-housed animals.

Fig. 7. Hippocampus volume in Tg+ and Tg- mice. A significant decrease in volume of the hippocampus in both Tg+ and Tg- mice after 24 weeks of isolation stress, however, there was no significant stress by genotype interaction.

Table 1

Correlations between amyloid plaques and HPA-related components and hippocampus volume

Amyloid plaque	HPA-related components	Correlation	N of project	P-value
Amyloid ₁₋₄₀ plaque	Plasma corticosterone	0.7569	11	0.0182 *
	CRFR1 expression in cortex	0.3353	11	0.3778
	CRFR1 expression in hipp	0.1916	11	0.6214
	GR expression in cortex	0.5359	10	0.1369
	GR expression in hipp	0.7016	10	0.0524
	Hippocampal volume	-0.7143	11	0.0465 *
Thioflavine-stained plaque	Thioflavine-stained plaques	0.6070	13	0.0830
	Plasma corticosterone	0.1576	11	0.6854
	CRFR1 expression in cortex	0.6057	11	0.0838
	CRFR1 expression in hipp	0.5798	11	0.1017
	GR expression in cortex	0.7726	11	0.0147 *
	GR expression in hipp	0.4628	11	0.2482
	Hippocampal volume	-0.3952	11	0.3326

* Indicates the correlations are significant.