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Regional alterations in amyloid precursor protein and nerve growth factor across age in a mouse model of Down's syndrome

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Abstract

Individuals with Down's syndrome (DS) develop the pathological hallmarks of Alzheimer's (AD) disease at an early age, subsequently followed by memory decline and dementia. We have utilized an animal model for DS, mice with segmental trisomy of chromosome 16 (Ts65Dn), to study biological events linked to memory loss. Previous studies demonstrated a cognitive decline and loss of cholinergic markers after 6–8 months of age. In the current study, we found increased levels of amyloid precursor protein (APP) in the striatum by 6–8 months of age, and in the hippocampus and parietal cortex by 13–16 months of age in Ts65Dn but not in normosomic mice. Additionally, Ts65Dn mice exhibited alterations in nerve growth factor (NGF) levels in the basal forebrain and hippocampus. Ts65Dn mice demonstrated a significant decline in NGF levels in the basal forebrain with age, as well as a reduction in hippocampal NGF by 13–16 months of age. These findings demonstrate that elevated APP and decreased NGF levels in limbic areas correlate with the progressive memory decline and cholinergic degeneration seen in middle-aged trisomic mice.

Keywords: Amyloid precursor protein; Hippocampus; Ts65Dn; Down's Syndrome; Neurodegeneration; Alzheimer's disease; Nerve growth factor

1. Introduction

Individuals with Down's syndrome (DS) have a partial or complete trisomy of Chromosome 21. This chromosome contains the gene for the amyloid precursor protein (APP) in humans, and it has been shown that beta-amyloid plaques and other pathological markers for Alzheimer's disease (AD) appear early in life in some individuals with DS (Yates et al., 1980; Casanova et al., 1985; Wisniewski et al., 1985). One of the hallmarks of AD is the phenotypic loss of basal forebrain cholinergic neurons (BFCNs), which provide the major cholinergic innervation to the hippocampus and cortex (Mesulam et al., 1983), and play a key role in the processing of information involved in attentional and cognitive processes in both animals and humans (Perry et al., 1978; Bartus et al., 1982; Kasa et al., 1997; Cummings et al., 1998; Lawrence and Sahakian, 1998; Whitehouse, 1998). Interestingly, individuals with DS also undergo degeneration of BFCNs and exhibit cognitive decline with age (Yates et al., 1980; Casanova et al., 1985; Wisniewski et al., 1985; Head et al., 2001). Taken together, these observations suggest that the DS phenotype may provide important information regarding biological mechanisms for AD pathology. Therefore, the Ts65Dn mouse, an animal model for DS, may provide a unique model for studying mechanisms related to AD.

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Ts65Dn mice are trisomic for a segment of murine chromosome 16 homologous to the 'critical region' of human chromosome 21, including the APP gene (Davisson et al., 1990; Galdzicki et al., 2001). They are born with intact BFCNs (Holtzman et al., 1996) and spatial memory deficits that may be indicative of the delayed brain development reported in young children with DS (Hyde and Crnic, 2001b; Crnic and Pennington, 2000). However, when Ts65Dn mice reach 6–12 months of age, they undergo a progressive degeneration of cholinergic phenotype in the BFCNs, coupled with additional deterioration of spatial working and reference memory (Demas et al., 1996, 1998; Holtzman et al., 1996; Granholm et al., 2000; Cooper et al., 2001; Hyde et al., 2001a; Hyde and Crnic, 2001b; Bimonte et al., 2002; Hunter et al., 2003). These animals also display neurochemical imbalances (Dierssen et al., 1996, 1997), along with changes in synaptic structure (Kurt et al., 2000) and neuronal plasticity (Siarey et al., 1997, 1999).

BFCNs are dependent on neurotrophins, most notably nerve growth factor (NGF), for development and maintenance of function (Mufson et al., 1995, 1999; Charles et al., 1996; Bäckman et al., 1996; Rossner et al., 1997; Jaffar et al., 2000). A decline in the function of neurotrophic systems has been shown in both DS and AD, and it has been hypothesized that these changes may play a role in the diminished function of BFCNs and subsequent memory impairments in AD (Mufson et al., 1993, 1995, 2000; Sendera et al., 2000). After NGF is synthesized in the hippocampus and cortical regions, it is bound to high affinity TrkA receptors and transported retrogradely to the BFCN cell bodies (Seiler and Schwab, 1984; Hefti and Will, 1987; Ehlers et al., 1995). Alterations in the NGF transport system have been reported in aged rats, (Cooper et al., 1994) and in Ts65Dn mice (Cooper et al., 2001). A deficit in the retrograde transport of NGF from its cortical and hippocampal production sites to its BFCN target neurons may explain the decline in BFCN phenotype reported in Ts65Dn mice (Holtzman et al., 1996; Granholm et al., 2000; Cooper et al., 2001).

One of the factors that may affect the neurotrophic system is APP and its amyloid cleavage products. APP, a large transmembrane pre-protein present in most neurons of the central nervous system, is involved in cell-to-cell signaling and plays an integral role in normal brain processing (Perez et al., 1997; Mattson et al., 1999; Selkoe, 1999; Neve et al., 2000). APP cleavage by α, β or γ-secretases results in at least two different peptide families: the soluble or secreted APP (sAPP), or the aggregating amyloid fragments (Selkoe, 1999). One prominent theory in AD research proposes that it is the processing of APP into aggregating amyloid fibrils that initiates pathological changes in the brain resulting in neuronal death and memory loss (Selkoe, 1999; Price, 1999; Neve et al., 2000). Although they do not develop actual amyloid plaques, Ts65Dn mice show increased levels of full-length APP mRNA and protein in the cortex (Reeves et al., 1995). This upregulation may lead to alterations in APP processing and/or functioning, possibly playing a role in the regional degeneration of BFCNs and cognitive decline. Previous reports have also suggested that there is a close relationship between the cholinergic, neurotrophic, and APP systems, all of which are altered in AD and DS (Isacson et al., 2002). However, it is not known whether the genetic over-expression of APP (1.5 gene dose) leads to a temporally or regionally stable over-expression of the APP protein.

Due to the interactions between NGF and APP, and the prominent role they appear to play in DS and AD, evaluation of these proteins may provide valuable information about the Ts65Dn phenotype. Previous investigation of APP protein levels in Ts65Dn mice examined only one brain region at one time point (Reeves et al., 1995), and there have been conflicting reports of NGF levels in the limbic system (Cooper et al., 2001; Bimonte et al., 2002). Furthermore, there have been no previous studies evaluating NGF or APP protein levels in animals that were younger than 6 or older than 12 months of age. In order to confirm and extend prior investigations, we examined the temporal and regional levels of both proteins in multiple age groups of Ts65Dn mice.

2. Materials and methods

2.1. Animals

Ts65Dn mice contain an extra chromosome (Chr) attached to the centromeric region of mouse Chr 17, consisting of a segment of mouse Chr16 homologous to human Chr 21. The trisomy is maintained by mating female carriers of the partial trisomy (males are sterile) to C57Bl/6 jeicher X C3H/HeSnJ F1 males on a segregating background (see Davisson et al., 1990). For the present studies, female mice were bred in the laboratory of Dr Linda Crnic from stock obtained from The Jackson Laboratories, and were genotyped by fluorescence in situ hybridization (FISH) using a probe for the telomeric end of mouse Chr 16 (Korenberg et al., 1994). Animals with retinal degeneration (rd) due to homozygosity for the mutation carried by C3H mice (detected by amplifying the rd mutation, Bowes et al., 1993) were discarded. Mice were maintained on a 12/12 light dark cycle (light onset 07:00 h), had ad lib access to food and water, and were group housed until sacrificed. All procedures were approved by the local Animal Care Committee, and were performed according to the NIH standards for animal care and use.

In order to determine the regional and temporal expression of full length APP protein, 57 animals were

used (24 Ts65Dn animals and 33 normosomic littermates). NGF levels in the hippocampus and basal forebrain were obtained from 77 animals (35 Ts65Dn and 42 normosomics) and 94 animals (45 Ts65Dn and 49 normosomics), respectively. Sham treated mice from a previous study (Granholm et al., 2002; 11 Ts65Dn and 12 normosomic) were included in the data analysis for this experiment since their values did not differ from those in naïve animals.

Ts65Dn mice are difficult to breed, so the collection of tissue from mice in the different age groups was performed over a time period of more than 12 months from multiple pools of animals. Animals from both groups (Ts65Dn and normosomic) were included in each batch of animals. For data analysis, the animals were grouped in ages of 2–3-month increments: 1–4, 6–8, 9–12, and 13–16 months old. Data for all measures were not available for all animals; the numbers used for each analysis are provided in Section 3.

2.2. Western blots for APP protein detection

The brains used for APP Western blots were removed for dissection following an overdose of chloral hydrate (dosage according to NIH guidelines for euthanasia). Tissue samples consisting of the striatum, hippocampus, and parietal cortex were collected according to landmarks previously established in our laboratory. Immediately after dissection, tissue samples were frozen in dry ice and maintained in a −70 °C freezer.

The antibody 22C11 (Boehringer Mannheim, Indianapolis, IN) raised against the N-terminal epitope of APP was used to determine the APP level in protein extracts obtained from brain tissue (Lin et al., 1999). This antibody recognizes full-length APP, as well as several variants that arise from splicing of amyloid precursor-like protein. The tissue was homogenized using a hand-held homogenizer in cell lysis buffer (50 mM Tris pH 8.0, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 10 μg/ml Aprotinin, 25 μg/ml Leupeptin, 10 μg/ml Pepstatin, 1 mM PMSF; all protease inhibitors purchased from Sigma, St. Louis, MO) and then sonicated until all viscosity was lost. Homogenates were centrifuged at $14\,000 \times g$ for 30 min at 4 °C. The supernatant was collected and aliquots were stored at −70 °C. Samples containing equal amounts of total protein were boiled with SDS sample buffer and electrophoresed on 10% SDS-polyacrylamide gels. Proteins were electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes (BIO-RAD, Hercules, CA). Membranes were blocked with 2.5% non-fat dried milk in 0.05 M Tris-buffered saline (pH 7.4) containing 0.1% Tween 20 (TBS-T) and then incubated with 22C11 antibody (1:500) in 1% non-fat dried milk overnight at 4 °C. After incubation with the secondary horseradish peroxidase (HRP)-linked anti-mouse IgG antibody (dilution 1:6000, Jackson Lab, Bar Harbor, ME) in 0.25% non-fat dried milk, the membranes were visualized by enhanced chemiluminescence (Amersham, Arlington Heights, IL) using Kodak X-Omat films. The hippocampus from a young rat was used as the same internal standard for all blots, and values were thus expressed as percent of the standard value, and will be referred to as 'relative APP levels' in the text.

2.3. Densitometric analysis

Quantification of APP immunoreactive bands was performed using densitometry. Films of Western blots were scanned (Scanner UMAX ASTRA 1200S) using Adobe Photoshop (version 5.5, Adobe Systems) and the optical density (OD) of the APP bands was measured using NIH Image (Version 1.61). The relative APP values were calculated by subtracting the background OD-value from the measured OD of the APP bands. Each immunoreactivity was within linearity range, and the results were confirmed by duplicate measurements of the same sample.

2.4. Enzyme linked immunosorbent assay (ELISA) for NGF

The brains used for ELISA were removed for dissection following an overdose of chloral hydrate (dosage according to NIH guidelines for euthanasia). Tissue samples consisting of the basal forebrain (containing the medial septal nucleus and the ventral diagonal band (MS/VDB)), and the CA1/CA2 region of the hippocampus were harvested according to landmarks previously established in our laboratory (see Albeck et al., 1999). For the basal forebrain piece, the rostral border consisted of the medial orbital cortex (at the level of the midline fusion of the corpus callosum), the caudal border consisted of the midline fusion of the anterior commissure, and the lateral borders consisted of the shell of the nucleus accumbens. Hippocampal tissue pieces contained all layers of the CA1/CA2 excluding the alveus and the dentate gyrus. All tissue pieces were placed in pre-weighed Eppendorf tubes, weighed, and kept in −70 °C until assayed. The tissue was analyzed for NGF levels using an ELISA kit (Promega, Madison, WI) according to our previously described protocol (Albeck et al., 1999). The NGF ELISA kit has been tested for specificity (see Weskamp and Otten, 1987) and has less than 3% cross-reactivity with other neurotrophins. Briefly, 96-welled flat-bottom NUNC-Immuno maxisorp plates were incubated with carbonate-coating buffer containing polyclonal anti-NGF antibody (Promega) overnight at 4 °C. Non-specific binding was blocked by incubating the plate with 1 × 'block and sample' buffer for 1 h at room temperature. A standard

curve was generated from serial dilutions of known concentrations of NGF ranging from 0 to 500 pg/ml. Dissected brain tissue was homogenized in lysis buffer supplemented with protease inhibitors according to manufacturer's recommendations. Samples were placed in coated wells and the plate was incubated for 6 h at room temperature. A secondary anti-NGF antibody raised in rat was incubated overnight at 4 °C, followed by an anti-rat IgG antibody conjugated to HRP for 2.5 h at room temperature. A TMB/peroxidase solution was used as the chromogen to visualize the reaction product in the plate wells. The reaction was terminated with 1N HCL. Optical density was measured at 450 nm in an ELISA plate reader (Molecular Devices SpectraMax 340 PC). (Softmax Pro version 3.1.2; see Albeck et al., 1999; Bimonte et al., 2002a,b), for further details regarding the ELISA method).

2.5. Statistical analysis

All statistical analyses of Western blot and ELISA data were analyzed using 2 way (Age and Genotype) ANOVAs with Tukey–Kramer posthoc analyses (Statview), and differences between groups were considered statistically significant when $P < 0.05$. For statistical analysis between only two groups (Ts65Dn and normosomics), Student's T-test was employed.

3. Results

3.1. Regional and temporal distribution of APP protein in the brain

3.1.1. Striatum

Western blot analysis using the 22C11 antibody was performed on 57 mice from 1 to 16 months of age (1–4 month group: 8 Ts65Dn and 9 normosomics, 6–8 month group: 6 Ts65Dn and 15 normosomics, 9–12 month group: 6 Ts65Dn and 5 normosomics, 13–16 month group: 4 Ts65Dn and 4 normosomics). Fig. 1A depicts the distribution of APP protein levels in the striatum across age groups in Ts65Dn and normosomic mice. ANOVA demonstrated a main effect of Age [$F(3,49) = 6.50$; $P < 0.001$] and Genotype [$F(1,49) = 17.24$, $P < 0.0001$] (see Fig. 1A). The significant Age × Genotype interaction [$F(3,49) = 3.33$; $P < 0.05$] was due to APP levels fluctuating across age in Ts65Dn, but not normosomic mice. In fact, post-hoc analysis revealed that Ts65Dn mice had significantly higher levels of APP at 6–8 months of age [$F(1,19) = 13.87$; $P < 0.01$], and marginally higher levels at both 9–12 [$F(1,9) = 4.10$; $P < 0.10$] and 13–16 [$F(1,6) = 3.83$; $P < 0.10$] months of age. Collectively, these data suggest that there was an increase in APP in Ts65Dn, but not

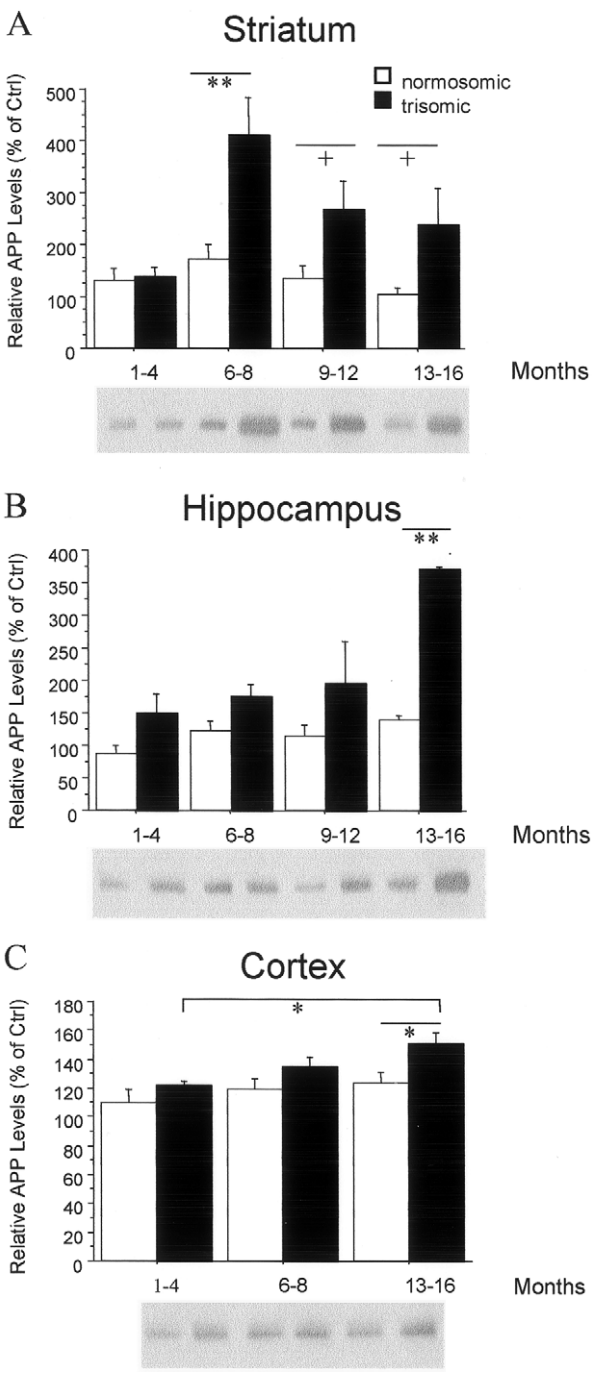

Fig. 1. Regional and temporal distribution of full-length APP in Trisomic mice. Densitometric analysis with corresponding immunoblots; (A) Striatum, (B) hippocampal CA1, (C) parietal cortex. APP levels in the striatum were significantly elevated in the trisomic animals compared to normosomics at 6–8 months of age, and were marginally significant at 9–12 and 13–16 months of age (A). In the hippocampus, trisomic mice had significantly higher APP levels at 13–16 months of age, while the controls remained constant (B). The only age group where differences were found between the trisomics and normosomics in the parietal cortex was at 13–16 months of age, where trisomics had higher APP levels than age-matched controls (C). Asterisk indicates $P < 0.05$, double asterisk; $P < 0.01$, cross; $P < 0.10$.

normosomic mice, that emerged around 6–8 months of age (see Fig. 1A).

3.1.2. Hippocampus

Twenty-two Ts65Dn mice and 25 normosomic mice were assessed for APP in the hippocampus (1–4 month group: 9 Ts65Dn and 8 normosomics, 6–8 month group: 6 Ts65Dn and 11 normosomics, 9–12 month group: 4 Ts65Dn and 4 normosomics, 13–16 month group: 3 Ts65Dn and 2 normosomics). Results showed a main effect of Age [$F(3,39) = 5.79$, $P < 0.01$] and Genotype [$F(1,39) = 24.46$; $P < 0.0001$] (see Fig. 1B). As revealed by the marginal Age × Genotype interaction [$F(3,39) = 2.64$; $P = 0.06$], the Age effect was primarily due to higher APP levels at 13–16 months in the Ts65Dn group only. Additional analyses indicated that trisomics had significantly higher relative levels of APP in the hippocampus at 13–16 months of age [$F(1,3) = 1104.84$; $P < 0.0001$]. Thus, hippocampal APP was elevated in Ts65Dn mice by 13–16 months of age, while normosomic levels did not change with age.

3.1.3. Cortex

The data available from 41 mice (1–4 month group: 4 Ts65Dn and 4 normosomics, 6–8 month group: 6 Ts65Dn and 7 normosomics, 13–16 month group: 10 Ts65Dn and 10 normosomics) demonstrated a main effect of Age [$F(2,35) = 3.43$; $P < 0.05$] and Genotype [$F(1,35) = 7.21$; $P < 0.05$] for APP levels in the parietal cortex (Fig. 1C). However, Ts65Dn mice only showed a significant elevation of APP levels in the oldest (13–16 month) group [$F(1,18) = 7.06$; $P < 0.05$]. There was no significant Age × Genotype interaction, but 13–16 month-old Ts65Dn mice showed significantly higher APP levels than those in the 1–4 month-old group [$F(1,12) = 6.99$; $P < 0.05$]. Thus, APP levels increased by 13–16 months of age in this brain region as well (Fig. 1C), albeit to a lesser degree than the elevation seen in striatum and hippocampus (compare with Fig. 1A and B, respectively).

3.2. Regional and temporal brain distribution of NGF protein

3.2.1. Hippocampus

Thirty-five Ts65Dn mice and 42 normosomic mice (1–4 month group: 4 Ts65Dn and 10 normosomics, 6–8 month group: 8 Ts65Dn and 9 normosomics, 9–12 month group: 8 Ts65Dn and 7 normosomics, 13–16 month group: 15 Ts65Dn and 16 normosomics) were assessed for NGF protein levels. Results showed an effect of Age [$F(3,69) = 4.09$, $P < 0.01$] but no effect of Genotype or Age × Genotype interaction [Ps > 0.23]. Indeed, NGF levels increased with age from (1–4) to (9–12) months for both groups (Fig. 2A). Interestingly, after 12 months normosomic mice continued to show

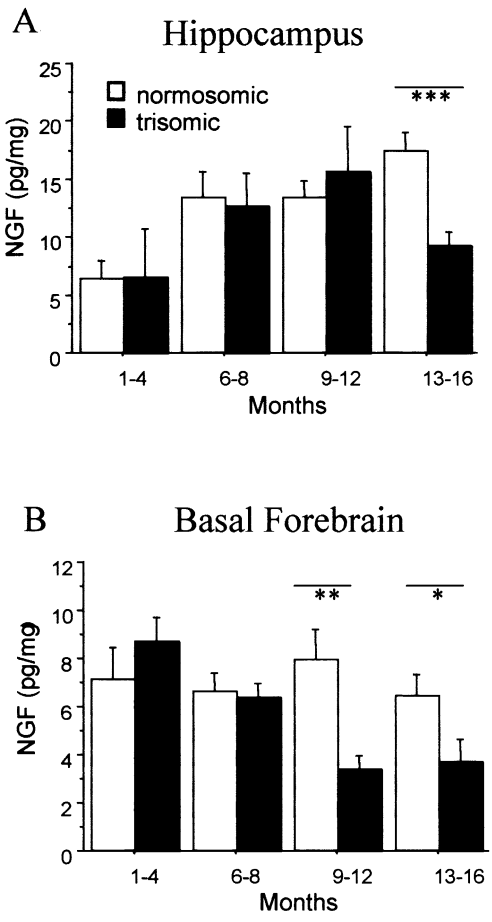

Fig. 2. Temporal pattern of NGF levels in the hippocampus and basal forebrain of trisomic and normosomic mice (A) hippocampus, (B) basal forebrain. In the hippocampus, there was an age-related elevation of NGF for both trisomic and normosomic mice from 1–4 to 9–12 months of age. However, trisomics had significantly lower NGF levels at 13–16 months (A). In the basal forebrain, the significant Age × Genotype interaction reflects that NGF levels steadily declined with age in trisomics while remaining at the same levels in normosomic mice (B). Asterisk indiactes $P < 0.05$, double asterisk; $P < 0.01$, triple asterisk; $P < 0.001$.

elevated NGF levels, while Ts65Dn mice exhibited a marginally significant decline between the 9–12 and 13–16 month-old period [$F(1,22) = 3.94$: $P < 0.10$]. There was a significant genotype difference in the 13–16-month-old group [$F(1,29) = 14.16$; $P < 0.001$], indicating that by 13–16 months of age Ts65Dn mice displayed lower levels of NGF in the hippocampus than age-matched normosomics.

3.2.2. Basal forebrain

Forty-five Ts65Dn mice and 49 normosomic mice (1–4 month group: 6 Ts65Dn and 10 normosomics, 6–8 month group: 15 Ts65Dn and 16 normosomics, 9–12 month group: 8 Ts65Dn and 6 normosomics, 13–16 month group: 16 Ts65Dn and 17 normosomics) were

used for the assessment of NGF protein levels in the basal forebrain. An ANOVA demonstrated a main effect of Age [$F(3,86) = 2.90$: $P < 0.05$] and Genotype [$F(1,86) = 4.13$: $P < 0.05$] (see Fig. 2B). As revealed by the significant Age × Genotype interaction [$F(3,86) = 2.94$: $P < 0.05$], NGF levels declined across age for the trisomic group, while levels in the normosomics remained constant. Further, there were significant Genotype differences in both the 9–12 [$F(1,12) = 13.11$: $P < 0.01$] and the 13–16 month-old groups [$F(1,31) = 4.36$: $P < 0.05$], while there were no detectable differences between groups in NGF levels from 1–4 or 6–8 months of age.

4. Discussion

The data presented in the current report demonstrate age-related alterations in APP and NGF protein levels in Ts65Dn mice. There was an age-related overexpression of full-length APP in all brain regions of Ts65Dn mice examined, and progressive alterations of NGF levels leading to an overall reduction of the protein compared to normosomics by 13–16 months of age. While basal forebrain NGF levels in Ts65Dn mice declined steadily with age, levels of NGF in the hippocampus increased concurrently with normosomics until the animals were 9–12 months old, after which there was a significant decline. The peak APP protein expression in the hippocampus corresponded to the age-related decline of NGF levels in this region, while striatal APP elevations appeared to peak much earlier-around 6–8 months of age.

It was of interest to note differences in the regional and temporal pattern of APP overexpression in Ts65Dn mice. Reeves et al. (1995) have previously shown a greater than two-fold increase in APP mRNA and protein levels in the cerebral cortex of Ts65Dn mice. However, longitudinal studies of APP holoprotein and mRNA expression in trisomics were not performed, nor were other brain regions examined. Interestingly, while there is an increased APP gene dosage in Ts65Dn mice, they do not demonstrate stable increases in APP protein levels in the cortex or hippocampus until 13–16 months of age, suggesting a mechanism regulating transcription and/or translation of the protein. It has been previously reported that a regionally defined lack of concordance between growth factor mRNA expression and protein levels exists, suggesting specific translational control at least for those proteins (Das et al., 2001). Moreover, it is conceivable that similar mechanisms may be in place for APP in both healthy and/or pathologically dysfunctional brains. Thus, it is clearly important to correlate gene dosage and mRNA levels with protein expression when studying disease processes occurring in DS and AD. Increased APP levels may occur in response to cellular insults in limbic regions (Wallace et al., 1997), or other factors such as altered growth factor synthesis and decreased neuronal activity (Milward et al., 1992; Isacson et al., 2002). The spatiotemporal elevation of hippocampal and cortical APP may thus play a direct or indirect role in the age-related synaptic alterations and cognitive impairments reported in Ts65Dn mice; especially if they are coupled with increased Aβ(1–42) production.

In terms of temporal distribution of APP levels in Ts65Dn mice, the earliest and greatest increase occurred in the striatum. The functional consequences of this elevation of APP remain to be investigated. Previous studies of 2 (Escorihuela et al., 1995) and 4–6-month-old (Klein et al., 1996) Ts65Dn mice did not reveal motor abnormalities. However, when older Ts65Dn mice (6–8 months old), were evaluated, Costa et al. (1999) found specific motor deficits, suggesting that such alterations appear during young middle age. This age correlates with the sharp increase in APP levels seen in striatum at 6–8 months of age in the present study. Further investigation into striatal physiology and function in older Ts65Dn mice is crucial to understanding the role of APP striatal levels and motor function, since this brain region plays a role in motor control.

The decreased basal forebrain NGF levels seen in middle-aged Ts65Dn mice are in accordance with human literature reporting a decline of this growth factor in the basal forebrain of AD-affected brains (Scott et al., 1995; Mufson et al., 1995, 2000). Previously, Cooper et al. (2001) reported a trend towards reduction of basal forebrain NGF in 12-month-old Ts65Dn mice, which the current data substantiates. Studies have shown increased NGF levels in the hippocampus of AD-affected brains (Scott et al., 1995), suggesting an attempt at the level of the NGF production sites to up-regulate trophic expression in response to BFCN dysfunction. Cooper et al. (2001) reported that hippocampal NGF increased in both Ts65Dn and normosomic mice between 6 and 12 months of age, and that 6, but not 12-month-old Ts65Dn mice had higher NGF levels than normosomics in this region. Similarly, in the present study NGF was elevated in the hippocampus of Ts65Dn and normosomic mice simultaneously from 1–4 to 9–12 months of age. However, no genotype differences were found until 13–16 months, when there was a sharp decline in the Ts65Dn group. Interestingly, Ts65Dn mice did not have higher hippocampal NGF levels than normosomics at any age, including the 6–8-month-old group. These results are consistent with some (Bimonte et al., 2002), but conflicting with other (Cooper et al., 2001) previous reports. This discrepancy is not likely to be due to gender differences alone (the present study used females, Cooper et al. (2001) used males), since Bimonte et al. (2002) found results similar to ours using 6-month-old males. Possible explanations may be differ-

ences in dissection procedures (in the current study and Bimonte et al. (2002) only the CA1/CA2 region, excluding the dentate gyrus, was utilized as hippocampal tissue while Cooper et al. (2001) did not specify exact landmarks), or the grouping of ages (the current study pooled 6–8-month-old animals together while Cooper et al. (2001) used only 6-month-old animals).

The significant reduction of NGF in the hippocampus and basal forebrain of Ts65Dn mice occurred at the same age as the most dramatic alterations in BFCN phenotype (>12 months) (Holtzman et al., 1996; Granholm et al., 2000; Cooper et al., 2001). However, detectable differences in BFCN phenotype and cognitive ability between Ts65Dn and normosomic mice appear at a much earlier age, approximately 6–8 months old (Holtzman et al., 1996; Granholm et al., 2000; Cooper et al., 2001; Bimonte et al., 2002; Hyde and Crnic, 2001b; Hunter et al., 2003). This suggests a functional deficit in the cholinergic/trophic factor system months before changes in protein levels and phenotypic expression become apparent.

A considerable body of evidence suggests that retrograde transport of NGF from the cortical areas to the basal forebrain is necessary for its trophic actions (for review see Mufson et al., 1999). Previous reports have demonstrated a disruption of NGF retrograde transport in Ts65Dn mice (Cooper et al., 2001). The present study provides indirect support for this hypothesis, showing that NGF levels in the basal forebrain of Ts65Dn mice declined between 1–4 and 9–12 months of age as levels in the hippocampus increased. This may be in response to impaired transport of NGF from the hippocampus to the basal forebrain. Beyond 12 months of age Ts65Dn mice had significantly less NGF in both regions suggesting a possible overtaxing of the neurotrophic system in general, or a disruption of the neurons synthesizing the protein in the target region. In fact, we have recently observed considerable morphological damage to the hippocampal dendritic structure of Ts65Dn mice at that age (Granholm et al., 2002). Interestingly, the decline of hippocampal NGF at 13–16 months did not lead to further loss of NGF in the basal forebrain. It has been demonstrated that destruction of hippocampal neurons leads to NGF release from basal forebrain glial cells (Bakhit et al., 1991). Thus, the maintenance of basal forebrain NGF levels in Ts65Dn mice from 9–12 to 13–16 months of age may be due to glial-derived NGF release in response to failed transport of the growth factor to this region.

Recently, studies reported that sAPP can act synergistically with NGF in culture to potentiate its neurotrophic and neuroprotective activities (Milward et al., 1992; Wallace et al., 1997; Akar and Wallace, 1998; Luo et al., 2001). In the present study we have shown imbalances in APP and NGF protein levels in the limbic system of Ts65Dn mice, which reportedly undergo progressive neuronal degeneration. These imbalances may alter the interactions between sAPP and NGF, resulting in an improperly functioning neurotrophic system. Furthermore, transgenic mice expressing a neutralizing dose of anti-NGF antibody have been reported to display beta-amyloid immunoreactive deposits and cholinergic deficits, suggesting a strong relationship between cholinergic, neurotrophic, and APP systems (Capsoni et al., 2000). Based on the findings described above, it cannot be ruled out that imbalances of APP levels or processing in Ts65Dn mice may somehow alter the neurotrophic system during neurodegeneration.

In conclusion, we have reported regional and age-related alterations in APP and NGF protein levels in Ts65Dn mice. These alterations, as well as interactions between the two proteins may, at least in part, play a role in the age-dependent decline of BFCN phenotype and consequent cognitive impairments in Ts65Dn mice. However, future studies are necessary to determine the interaction between APP fragments, NGF transport, and cholinergic neuronal function in the Ts65Dn mouse.

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References

Akar, C.A., Wallace, W.C., 1998. Amyloid precursor protein modulates the interaction of nerve growth factor with p75 receptor and potentiates its activation of trkA phosphorylation. Mol. Brain Res. 56, 125–132.

Albeck, D.S., Bäckman, C., Veng, L., Friden, P., Rose, G.M., Granholm, A.-Ch., 1999. Acute application of NGF increases the firing rate of aged rat basal forebrain neurons. Eur. J. Neurosci. 11, 2291–2304.

Bäckman, C., Rose, G.M., Hoffer, B.J., Henry, M.A., Bartus, R.T., Friden, P., Granholm, A.-Ch., 1996. Systemic administration of a nerve growth factor conjugate reverses age-related cognitive dysfunction and prevents cholinergic neuron atrophy. J. Neurosci. 16, 5437–5442.

Bakhit, C., Armanini, M., Bennett, G.L., Wong, W.L., Hansen, S.E., Taylor, R., 1991. Increase in glia-derived nerve growth factor following destruction of hippocampal neurons. Brain Res. 560 (1–2), 76–83.

Bartus, R.T., Dean, R.L., Beer, B., Lippa, A.S., 1982. The cholinergic hypothesis of geriatric memory dysfunction. Science 217, 408–414.

Bimonte, H.A., Hunter, C.L., Nelson, M.E., Granholm, Ach., 2002. Frontal cortex BDNF levels correlate with working memory in an animal model of Down Syndrome. Behav. Brain Res. (in press).

Bowes, R., Li, W.N., Frankel, M., Danciger, J.M., Coffin, M.L., Applebury, Farber, D.B., 1993. Localication of a retroviral element within the rd gene coding for the β subunit of cGMP phosphodiesterase. Proc. Natl. Acad. Sci. 90, 2955–2959.

Capsoni, S., Ugolini, G., Comparini, A., Ruberti, F., Berardi, N., Cattaneo, A., 2000. Alzheimer-like neurodegeneration in aged antinerve growth factor transgenic mice. Proc. Natl. Acad. Sci. 97 (12), 6826–6831.

Casanova, M.F., Walker, L.C., Whitehouse, P.J., Price, D.L., 1985. Abnormalities of the nucleus basalis in Down's syndrome. Ann. Neurol. 18, 310–313.

Charles, V., Mufson, E.J., Friden, P.M., Barus, R.T., Kordower, J.H., 1996. Atrophy of cholinergic basal forebrain neurons following excitotoxic cortical lesions is reversed by intravenous administration of an NGF conjugate. Brain Res. 728, 193–203.

Cooper, J.D., Lindholm, D., Sofroniew, M.V., 1994. Reduced transport of nerve growth factor by cholinergic neurons and down-regulated TrkA expression in the medial septum of aged rats. Neuroscience 62 (3), 625–629.

Cooper, J.D., Salehi, A., Delcroix, J.-D., Howe, C.L., Belichenko, P.V., Chua-Couzens, J., Kilbridge, J.F., Carlson, E.J., Epstein, C.J., Mobley, W.C., 2001. Failed retrograde transport of NGF in a mouse model of Down's Syndrome: Reversal of cholinergic neurodegenerative phenotypes following NGF infusion. Proc. Natl. Acad. Sci. USA 98, 10439–10444.

Costa, A.C.S., Walsh, K., Davisson, M.T., 1999. Motor dysfunction in a mouse model for Down syndrome. Phys. Behav. 68, 211–220.

Crnic, L.S., Pennington, B.F., 2000. Contributions of mouse models to understanding Down's syndrome. Prog. Infancy Res. 1, 69–111.

Cummings, J.L., Vinters, H.V., Cole, G.M., Khachaturian, Z.S., 1998. Alzheimer's disease: etiologies, pathophysiology, cognitive reserve, and treatment opportunities. Neurology 51 (Suppl.), S2–S17.

Das, K.P., Chao, S.L., White, L.D., Haines, W.T., Harry, G.J., Tilson, H.A., Barone, S., Jr, 2001. Differential patterns of nerve growth factor, brain-derived neurotrophic factor and neurotrophin-3 mRNA and protein levels in developing regions of the rat brain. Neuroscience 103 (3), 739–761.

Davisson, M.T., Schmidt, C., Akeson, E.C., 1990. Segmental trisomy of murine chromosome 16: a new model system for studying Down syndrome. Prog. Clin. Biol. Res. 360, 263–280.

Demas, G.E., Nelson, R.J., Krueger, B.K., Yarowsky, P.J., 1996. Spatial memory deficits in segmental Trisomic mice. Behav. Brain Res. 82, 85–92.

Demas, G.E., Nelson, R.J., Krueger, B.K., Yarowsky, P.J., 1998. Impaired spatial working and reference memory in segmental trisomy (Trisomic) mice. Behav. Brain Res. 90, 199–201.

Dierssen, M., Vallina, I.F., Baamonde, C., Lumbreras, M.A., Martinez-Cue, C., Calatayud, S.G., Florez, J., 1996. Impaired cyclic AMP production in the hippocampus of a Down Syndrome murine model. Dev. Brain Res. 95, 122–124.

Dierssen, M., Vallina, I.F., Baamonde, C., Garcia-calatayud, S., Lumbreras, M.A., Florez, J., 1997. Alterations of central noradrenergic transmission in Ts65Dn mouse, a model for Down syndrome. Brain Res. 749, 238–244.

Ehlers, M.D., Kaplan, D.R., Price, D.L., Koliatsos, V.E., 1995. NGF-stimulated transport of trkA in the mammalian nervous system. J. Cell Biol. 130, 149–156.

Escorihuela, R.M., Fernandez-Teruel, A., Vallina, I.F., Baamonde, C., Lumbreras, M.A., Dierssen, M., Tobena, A., Florez, J., 1995. A behavioral assesment of Ts65Dn mice: a putative Down syndrome model. Neurosci. Lett. 199, 143–146.

Galdzicki, Z., Siarey, R., Pearce, R., Stoll, J., Rapoport, S., 2001. On the cause of mental retardation on Down syndrome: extrapolation from full and segmental trisomy 16 mouse models. Brain Res. Rev. 35, 115–145.

Granholm, A.-Ch., Sanders, L.A., Crnic, L.S., 2000. Loss of cholinergic phenotype in basal forebrain coincides with cognitive decline in a mouse model of Down's syndrome. Exp. Neurol. 161, 647–663.

Granholm, Ach., Sanders, L., Seo, H., Lin, L., Ford, K.A., Isacson, O., 2002. Estrogen alters amyloid precursor protein as well as dendritic and cholinergic markers in a mouse model of Down's syndrome. Hippocampus (in press).

Head, E., Azizeh, B.Y., Lott, I.T., Tenner, A.J., Cotman, C.W., Cribbs, D.H., 2001. Complement association with neurons and b-amyloid deposition in the brains of aged individuals with Down's syndrome. Neurobiol. Dis. 8, 252–265.

Hefti, F., Will, B., 1987. Nerve growth factor is a neurotrophic factor for forebrain cholinergic neurons: implications for Alzheimer's disease? J. Neural. Transm. 24 (Suppl.), 309–315.

Holtzman, D.M., Santucci, D., Kilbridges, J., Chua-Couzens, J., Fontana, D.J., Daniels, S.E., Johnson, R.M., Chen, K., Sun, Y., Carlson, E., Alleva, E., Epstein, C.J., Mobley, W.C., 1996. Developmental abnormalities and age-related neurodegeneration in a mouse model of Down syndrome. Proc. Natl. Acad. Sci. USA 93, 13333–13338.

Hunter, C.L., Bimonte, H.A., Granholm, Ach., 2003. Behavioral comparison of 4 and 6 month-old Ts65Dn mice: Age-related impairments in working and reference memory. Behav. Brain Res. 138, 121–131.

Hyde, L.A., Frisone, D.F., Crnic, L.S., 2001a. Trisomic mice, a model for Down Syndrome, have deficits in context discrimination learning suggesting impaired hippocampal function. Behav. Brain Res. 118, 53–60.

Hyde, L.A., Crnic, L.S., 2001b. Age-related deficits in context discrimination learning in Ts65Dn mice that model Down's syndrome and Alzheimer's disease. Behav. Neurosci. 115 (6), 1239–1246.

Isacson, O., Seo, H., Lin, L., Albeck, D.A., Granholm, A.-Ch., 2002. Alzheimer's disease and Downs syndrome: roles of APP, trophic factors and ACh. TINS 25 (2), 79–84.

Jaffar, S., Counts, S.E., Ma, S.Y., Dadko, E., Gordon, M.N., Morgan, D., Mufson, E.J., 2000. Neuropathology of mice carrying mutant APP(swe) and/or PS1(M146L) transgenes: Alterations in the p75(NTR) cholinergic basal forebrain septohippocampal pathway. Exp. Neurol. 170 (2), 227–243.

Kasa, P., Rakonczay, Z., Gulya, K., 1997. The cholinergic system in Alzheimer's disease. Prog. Neurobiol. 52, 511–535.

Klein, S.L., Kriegsfield, L.J., Hairston, J.E., Rau, V., Nelson, R.J., Yarowsky, P.J., 1996. Characterization of sensorimotor performance, reproductive and aggressive behaviors in Segmental Trisomic 16 (Trisomic) mice. Physiol. Behav. 60, 1159–1164.

Korenberg, J.R., Chen, X.N., Schipper, R., Sun, Z., Gonsky, R., Gerwehr, S., Carpenter, N., Daumer, C., Dignan, P., Disteche, C., Graham, J.M., Jr, Hugdins, L., McGillivray, B., Miyazaki, K., Ogasawara, N., Park, J.P., Pagon, R., Pueschel, S., Sack, G., Say, B., Schuffenhauer, S., Soukup, S., Yamanaka, T., 1994. Down syndrome phenotypes: theconsequences of chromosomal imbalance. Proc. Natl. Acad. Sci. USA 91, 4997–5001.

Kurt, M.A., Davies, D.C., Kidd, M., Dierssen, M., Florez, J., 2000. Synaptic deficit in the temporal cortex of partial trisomy 16 [Ts65Dn] mice. Brain Res. 858, 191–197.

Lawrence, A.D., Sahakian, B.J., 1998. The cognitive psychopharmacology of Alzheimer's disease: focus on cholinergic systems. Neurochem. Res. 23, 787–794.

Lin, L., Georgievska, B., Mattsson, A., Isacson, O., 1999. Cognitive changes and modified processing of amyloid precursor protein in the cortical hippocampal system after cholinergic synapse loss and muscarinic receptor activation. Proc. Natl. Acad. Sci. USA 96, 12108–12113.

Luo, J.J., Wallace, M.S., Hawver, D.B., Kusiak, J.W., Wallace, W.C., 2001. Characterization of the neurotrophic interaction between nerve growth factor and secreted alpha-amyloid precursor protein. J. Neurosci. 63, 410–420.

Mattson, M.P., Guo, Z.H., Geiger, J.D., 1999. Secreted form of amyloid precursor protein enhances basal glucose and glutamate transport and protects against oxidative impairment of glucose and

glutamate transport in synaptosomes by a cyclic GMP-mediated mechanism. J. Neurochem. 73, 532–537.
Mesulam, M.M., Mufson, E.J., Levey, A.I., Wainer, B.H., 1983. Cholinergic innervation of cortex by the basal forebrain: cytochemistry and cortical connections of the septal area, diagonal band nuclei, nucleus basalis (substantia innominata) and hypothalamus in the rhesus monkey. J. Comp. Neurol. 214, 170–197.
Milward, E.A., Papadopoulos, R., Fuller, S.J., Moir, R.D., Small, D., Beyreuther, K., Masters, C.L., 1992. The amyloid protein precursor of Alzheimer's disease is a mediator of the effects of nerve growth factor on neurite outgrowth. Neuron 9, 129–137.
Mufson, E.J., Cochran, E., Benzing, W., Kordower, J.H., 1993. Galaninergic innervation of the cholinergic vertical limb of the diagonal band (Ch2) and bed nucleus of the stria terminalis in aging, Alzheimer's disease, and Down's syndrome. Dementia 4 (5), 237–250.
Mufson, E.J., Conner, J.M., Kordower, J.H., 1995. Nerve growth factor in Alzheimer's disease: defective retrograde transport to nucleus basalis. Neuroreport 6 (7), 1063–1066.
Mufson, E.J., Kroin, J.S., Sendera, T.J., Sobreviela, T., 1999. Distribution and retrograde transport of trophic factors in the central nervous system: functional implications for the treatment of neurodegenerative diseases. Prog. Neurobiol. 57 (4), 451–484.
Mufson, E.J., Ma, S.Y., Cochran, E.J., Bennett, D.A., Beckett, L.A., Jaffar, S., Saragovi, H.U., Kordower, J.H., 2000. Loss of nucleus basalis neurons containing trkA immunoreactivity in individuals with mild cognitive impairment and early Alzheimer's disease. J. Comp. Neurol. 427, 9–30.
Neve, R., McPhie, D.L., Chen, Y., 2000. Alzheimer's disease: a dysfunction of the amyloid precursor protein. Brain Res. 886, 54–66.
Perez, R.G., Zheng, H., Van der Ploeg, L.H.T., Koo, E.H., 1997. The β-amyloid precursor protein of Alzheimer's disease enhances neuron viability and modulates neuronal polarity. J. Neurosci. 17, 9407–9414.
Perry, E., et al., 1978. Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. Br. Med. J. 2, 1457–1459.
Price, D.L., 1999. New order from neurological disorders. Nature 399 (Suppl.), A3–A5.
Reeves, R.H., Irving, N.G., Moran, T.H., Wohn, Q., Kitt, C., Sisodia, S.S., Schmidt, C., Bronson, R.T., Davisson, M.T., 1995. A mouse model for Down syndrome exhibits learning and behavioral deficits. Nat. Genet. 11, 177–184.
Rossner, S., Wortwein, G., Gu, Z., Yu, J., Schliebs, R., Bigl, V., Perez-Polo, J.R., 1997. Cholinergic control of nerve growth factor in adult rats: evidence from cortical cholinergic deafferentation and chronic drug treatment. J. Neurochem. 69, 947–953.
Scott, S.A., Mufson, E.J., Weingartner, J.A., Skau, K.A., Crutcher, K.A., 1995. Nerve growth factor in Alzheimer's disease: increased levels throughout the brain coupled with declines in nucleus basalis. J. Neurosci. 15, 6213–6221.
Selkoe, D.J., 1999. Translating cell biology into therapeutic advances in Alzheimer's disease. Nature 399 (Suppl.), A23–A31.
Sendera, T.J., Ma, S.Y., Jaffar, S., Kozlowski, P.B., Kordower, J.H., Mawal, Y., Saragovi, H.U., Mufson, E.J., 2000. Reduction in trkA immunoreactive neurons is not associated with an overexpression of galaninergic fibers within the nucleus basalis in Down's syndrome. J. Neurochem. 74, 1185–1196.
Seiler, M., Schwab, M.E., 1984. Specific retrograde transport of nerve growth factor receptor from neocortex to nucleus basalis of the rat. Brain Res. 300, 33–39.
Siarey, R.J., Stoll, J., Rapoport, S.I., Galdzicki, Z., 1997. Altered long-term potentiation in the young and old Trisomic mouse, a model for Downs Syndrome. Neuropharmacology 36, 1549–1554.
Siarey, R.J., Carlson, E.J., Epstein, C.J., Balbo, A., Rapoport, S.I., Galdzicki, Z., 1999. Increased synaptic depression in the Ts65Dn mouse, a model for mental retardation in Down syndrome. Neropharmacology 38, 1917–1920.
Wallace, W.C., Akar, C.A., Lyons, W.E., 1997. Amyloid precursor protein potentiates the neurotrophic activity of NGF. Brain Res. Molec. Brain Res. 52, 201–212.
Weskamp, G., Otten, U., 1987. An Enzyme-linked immunoassay for nerve growth factor (NGF): a tool for studying regulatory mechanisms involved in NGF production in the brain and in peripheral tissues. J. Neurochem. 48, 1779–1986.
Whitehouse, P.J., 1998. The cholinergic deficit in Alzheimer's disease. J. Clin. Psychiatry 59 (Suppl. 13), 19–22.
Wisniewski, K.E., Wisniewski, H.M., Wen, G.Y., 1985. Occurrence of neuropathological changes and dementia of Alzheimer's disease and Down's syndrome. Ann. Neurol. 17, 278–282.
Yates, C.M., Simpson, J., Maloney, A.F., Gordon, A., Reid, A.H., 1980. Alzheimer-like cholinergic deficiency in Down Syndrome [Letter]. Lancet 2, 979.