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Altered expression of KIF17, a kinesin motor protein associated with NR2B trafficking, may mediate learning deficits in a Down syndrome mouse model

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Abstract

Objective—Down syndrome (DS) a major cause of mental retardation affects 1/800 newborns. Mouse models for Down syndrome have been studied and found to have developmental and learning deficits, including the Ts65Dn (DS) mouse model. NMDA receptor NR2B subunit enhances synaptic plasticity. Upregulation of KIF17, a motor protein which transports NR2B to the synaptic region parallels upregulation of synaptic NR2B. Down regulation of KIF17 reflects upregulation of less plastic NR2A subunit. We evaluated NR2B, NR2A and KIF17 in Ts65Dn and control mice.

Study Design—Ts65Dn (4) and control (4), adult brains were collected, NR2A, NR2B and KIF17 measured by western blot and quantified using NIH Image software. Comparisons were made using ANOVA, $P < 0.05$ was considered significant.

Results—There was a significant decrease in KIF17 ($P = 0.04$) level in Ts65Dn mice as compared to the control animals but there were no significant differences in the levels of NR2A ($P = 0.79$) and NR2B ($P = 0.96$).

Conclusion—The significant decrease of KIF17 in Ts65Dn animals may in part mediate cognitive defects in DS.

Keywords

Down syndrome; KIF17; NR2B; synaptic plasticity; Ts65Dn

Background and Hypothesis

Down syndrome (DS, trisomy 21) is a major cause of mental retardation affecting 1/800 newborns (1) due to the partial or entire triplication of chromosome 21 (2). The clinical sequelae in DS include mild to severe mental retardation as well as a spectrum of cognitive and physical complications as abnormal central nervous system development and function, neonatal delay in achieving developmental milestones, hypotonia, as well as the early onset of Alzheimer disease (3,4,5 and 6).

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Condensation: Using an established mouse model for Down syndrome we demonstrate differential levels of the motor protein KIF17 responsible for NR2B trafficking.

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The Ts65Dn mouse, is a model for DS and has a triplicated segment of chromosome 16 which shares >50% homology with human chromosome 21.(7) The Ts65Dn mice exhibit many of the learning, developmental and behavioral deficits found in DS (8)(9). Previously, we have reported on the known delay in developmental milestones and learning deficits using this model (11). *These abnormalities are thought to be mediated through abnormal neuronal development* resulting in cognitive impairment(12). Alterations in NMDA receptors that are critical for learning/memory through long term potentiation (LTP) may contribute to the cognitive impairment

Previously, we found a decrease in the gene expression (message) of NMDA (NR2A and B) receptors in brain of DS animals (12). The objective of this study was to evaluate protein levels of NR2A and NR2B as well as KIF17, a motor protein which transports NR2B subunits into the synaptic region.

Materials and Methods

Ts65Dn females and wild-type (B6C3Hf1) male mice (The Jackson Laboratory, Bar Harbor, ME) were bred to obtain the Ts65Dn and control (wild type) mice for this study. Animals were kept in a temperature controlled environment with 12-hour light/12-hour dark regimen and ad lib access to standard laboratory chow and water at all times. The mice received humane animal care in compliance with the National Institutes of Health (NIH) guidelines for care and use of experimental animals. The protocol was approved by the National Institute of Child Health and Human Development (NICHD) Animal Care and Use Committee. Pups were delivered naturally and were reared by their mothers until weaning on postnatal day 20. To ensure anonymity, weaned pups were gender segregated, ear tagged, housed at four littermates per cage and genotype was established using rt-PCR for amyloid precursor protein(10) after completion of all studies. Male animals aged 49-66.5 weeks old that had completed the Morris water maze to evaluate learning and memory testing were used (11).

After completion of the water maze, four Ts65Dn and four control mice from four litters were decapitated while deeply anesthetized with CO₂, the brain immediately dissected from the skull, quickly frozen on liquid N₂ and kept at -80°C until homogenization. To prepare for western blotting, frozen tissue was resuspended in lysis buffer [consisting of 200 mM NaCl, 2.5mM EDTA, 0.1% sodium dodecyl sulfate (SDS) and 50 mM Tris-Cl (pH 9.0) and protease inhibitor cocktail (Sigma-Aldrich, St Louis, MO, USA P8340) homogenized with three ten second burst using a Polytron while the temperature was maintained at 4°C (12). The homogenate was centrifuged at 12 000 g for 30 min at 4°C and an aliquot of the homogenate supernatant extract removed for determination of protein concentration. The Pierce BCA (Pierce Biotechnology, Rockford, IL, USA) protein assay was used to measure the protein concentration and the homogenate extract diluted with NuPage™ lithium dodecyl sulfate (Invitrogen, Carlsbad, CA, USA) sample running buffer at 1 : 4 (v/v) and reducing agent at 1 : 10 (v/v) and heated to 70°C for 10 min. 20 µg protein aliquots were loaded onto pre-cast NuPage™, 4–12% Tris-Bis gel, and electroblotted onto Immobilon-P blotting membrane. After blocking the membranes for 30 minutes in 3% non-fat dry milk diluted in Tris-buffered saline (TBS pH=7.4) with 0.1% Tween 20 (TTBS), followed by an overnight incubation at 4°C with the specific primary antibodies diluted in TTBS containing 3% milk. These include anti-NR2A (1 µg /ml; Upstate Biotechnology, Lake Placid, NY, USA), anti-NR2B (1 µg /ml; Upstate Biotechnology), anti-KIF17 (1 : 200; Sigma-Aldrich, St Louis, MO, USA), anti-Actin (1 : 100; Cell Signaling). Membranes then were incubated in either goat anti-rabbit IgG (1 : 5000) or donkey anti-goat IgG (1 : 2000) horseradish peroxidase-conjugated secondary antibody for at least 1 hr in TTBS/milk, washed three times for 5 min each in TTBS. Signal was detected using SuperSignal Chemiluminescent kit (Pierce Biotechnology, Rockford, IL, USA) and quantified. Actin was used as the internal control. An anti-actin antibody (Cell

Signaling 1:100) was used to probe each immunoblot simultaneous to the probe for NR2A, NR2B or the KIF17 antibody probes. The relative densities of bands were assessed using NIH Image software. The intensity of anti Actin the bands were quantitated and their value used to establish a ratio of the NR2A/actin, NR2B/actin and KIF17/actin. Statistical analysis was performed using one-way ANOVA and a $P \leq 0.05$ was considered statistically significant.

Results

We found no difference in the protein levels of NR2A $p=0.79$ or NR2B $p=0.96$ between Ts65Dn and control mice (Figure 1). There was a difference in the levels of KIF17, with a significant decline in the brains of the Ts65Dn animals as compared to the controls ($P=0.02$) (Figure 1). As previously reported, these brains were from animals that were tested in the Morris Water maze, with the Ts65Dn animals demonstrating learning deficits as compared to the control animals (10).

Comment

Levels of KIF17, a motor protein that transports NR2B subunits into the synaptic region, were significantly lower in Ts65Dn than control animals, whereas the levels of NR2A and NR2B were unchanged. Since the NR2B subunit is the most plastic subunit of the NMDA receptors, it is associated with enhanced learning as compared to the less plastic NR2A subunit. Having a deficit in KIF17 in the Ts65Dn animals may thus explain the learning impairment as it precludes the transport and use of the more plastic NR2B subunit, and has been reported to be indicative of the upregulation of less plastic synaptic NR2A subunit (16). These results suggest that the memory and learning deficits seen in the adult Ts65Dn mice may be in part mediated through a deficit in level of the motor protein KIF17.

KIF17, a motor protein and a member of the kinesin superfamily proteins has been shown to transport and regulate NR2B in living hippocampal neurons (14,15 and 16). Moving specifically along dendrites, KIF17 vesicles are associated with and deliver NR2B subunits into dendrites (16). Transport of NR2B via KIF17 therefore, may in part play a important regulatory role in the presence of NMDAR in synaptic regions during cascades of learning and memory. We have previously reported that Ts65Dn mice show a relative decrease of the NR2B/NR2A ratio gene expression as compared to control (12) which correlates with the learning deficit, while in this study we did not find a difference in protein levels for both NR2A and NR2B between Ts65Dn and controls. These apparently conflicting results could be explained by increased translation efficiency or by decreased protein turnover. A deficit in KIF17 may be indicative of a deficient transport of NR2B to the cell surface, resulting in the possible deficit of NMDAR synaptic localization essential in synaptic plasticity from sustained induction of LTP. Ts65Dn mice show developmental delays and exhibit increased cognitive decline (10) with age in comparison to their wild-type littermates. The underlying mechanisms regulating neurological dysfunction in DS have not been clearly elucidated, however, it has been reported that neurological dysfunction in DS are underscored by hippocampal dysfunction (10,18 and 19) which affects short and long term memory. Long-term potentiation (LTP), the cellular equivalent of long lasting memory in paradigms of cognition and learning, remains the focus of ongoing studies of learning and memory impairment in the Ts65Dn murine model. Hippocampal synaptic field potential studies have demonstrated a reduction in LTP in Ts65Dn hippocampal slices as compared to controls (20). However, recent studies have demonstrated in Ts65Dn hippocampal slices that the LTP mechanism is unchanged, and the apparent decrease in LTP is concomitant with and possibly a direct result of increased synaptic inhibition (LTD). To test this hypothesis researchers restored the LTP induction rate by pharmacologically blocking enhanced synaptic inhibition transmission (LTD) (21). Given that LTP mechanism is intact in the Ts65Dn murine model, a recent study found that impaired LTP resulting from

hippocampal dysfunction in Ts65Dn in part is a direct consequence of the altered connectivity in the hippocampal network. Despite an overabundance of active synaptic connections between pyramidal neurons in the Ts65Dn CA3 region as compared to WT this study demonstrated a general decrease in the function of the Ts65Dn excitatory and inhibitory synaptic inputs. (22).

Other regulators of NR2B include the calcium-calmodulin-dependent protein kinase II (CAMKII) dependent phosphorylation (23). Recently, Guiland (24) demonstrated that the CAMKII dependent phosphorylation of Ser 1029 on KIF17 is responsible for the ultimate release of NR2B, the KIF17 cargo. Taken together these findings lend to the idea that NR2B and KIF17 are co-regulated by similar mechanisms. Supporting this hypothesis is a recent finding by (25) that demonstrates that Ts65Dn mice are more sensitive to the psychotomimetic effects of NMDA receptor antagonist MK-801, and that some behavioral deficits in these animals can be at least partially reversed with the use of memantine, which is a lower affinity NMDA receptor antagonist. In this study, deficits in calcineurin signaling were identified as a potential mechanism. Another possibility, given our findings, is that KIF17 may have a role.

This study sought to provide evidence of the KIF17 signaling mechanism that is concurrent to and co-regulated with NMDAR activation, specifically the transport of NR2B into the synaptic region. The transport of NR2B by KIF17 into the synaptic region is an event that may directly affect induction of LTP which is prior to synaptic events that are directly impacted by alterations in the Ts65Dn synaptic anatomy and circuitry. Therefore this may offer insight into mechanisms controlling signaling pathways that can impair the induction of LTP and sustained synaptic plasticity that may in part be responsible for learning and memory deficits in the Ts65Dn, a mouse model for DS.

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Fig. 1.

NR2A, NR2B and KIF17 levels of protein brain lysates from the Ts65Dn and WT (wild type) brain. Brain proteins from Ts65Dn and WT mice were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) followed by Western blot analysis. Whole brain representative blots are shown for KIF17, NR2A and NR2B in the upper panel; cumulative data from four Ts65Dn and four WT brains are shown in the graph. KIF17 is decreased in the Ts65Dn as compared to the WT ($P=0.04$). Level of NR2A and NR2B were unaltered in the two groups ($P=0.79$ and $P=0.96$, respectively). Bars represent target protein/actin $\pm$ SD.