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Novel Protein in the Thalamus of Human Schizophrenic Brains

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Abstract

Numerous studies have found cortical and subcortical abnormalities in schizophrenic brains. Some suggested structural damage to the thalamus, while other suggested a lower metabolic rate in the thalamus or even that thalamic volume reduction may play an important role in schizophrenia. Previous studies from our lab have shown that there is an increased level of dopamine in the thalamus of schizophrenic patients when compared to controls. This study proposes that the increased concentration of dopamine could be due to synaptic disturbance and/or an abnormal distribution of growth protein in the thalamus.

To test the hypothesis, we looked at the levels of Growth-Associated Protein-43 (GAP-43). Western blots using both polyclonal and monoclonal antibodies were performed to detect the levels of GAP-43 protein in post-mortem thalamic brain tissue of schizophrenic patients and controls.

Analysis showed a decreased level of GAP-43 protein in the thalamic brain tissue of schizophrenic patients compared to control. Furthermore, we found an unknown protein with molecular weight of approximately 100kDa in the thalamic tissue of schizophrenic patients, but not in the controls. Our data may indicate impaired synaptic plasticity in the thalamus of the schizophrenic brain. We also found an unknown protein that could be a precursor/splice variant of GAP-43 or an aberrant form of GAP-43.

Keywords: Growth-Associated Protein-43; post-mortem; human brain; Western blot; GAP-43 antibodies

Introduction

The symptom complex termed schizophrenia may have existed throughout the history of mankind. Yet, the etiology of this disorder remains a conundrum. However, schizophrenia has been typically characterized as a disorder in thought. This disorder of thought seems to involve a misinterpretation of perceptions or experiences. Therefore, one might conclude that the primary pathology in schizophrenia would appear to be a disturbance in information processing. A lesion to the thalamus might explain this disturbance because a primary role of the thalamus is to relay sensory information from lower centers to the cerebral cortex. It has been described as the "gateway" to the cortex [1].

There have been a number of studies on the role of the thalamus in schizophrenia. Several suggest the size of the thalamus is smaller in individuals suffering from schizophrenia [2-5]. Others found a lower metabolic rate in the thalamus of schizophrenic patients [6,7]. The studies in our laboratory suggest an increased level of dopamine in the thalamus of schizophrenic patients when compared to controls. In some samples, the dopamine concentration was three times that of the controls, and this increase does not appear to be a failure of dopamine to be converted into norepinephrine [8]. Another possibility is that the treatment with neuroleptic medication might result in elevated dopamine levels. However, this does not appear to be the case [9]. There is some indication that thalamic dopamine might distort information processing to the cortex by reducing spontaneously evoked activity [10].

This study initially hypothesized that the increased concentration of dopamine in the thalamus of individuals who suffer from schizophrenia is due to excessive concentration of some growth protein. An increase in growth protein in the thalamus could possibly stimulate aberrant growth of dopaminergic neurons from the dopamine-enriched areas surrounding the thalamus (caudate nucleus, putamen and the substantia nigra). Thus, for the first step in our study we examined levels of growth associated protein–43 (GAP-43). Although controversy exists on this topic, some studies suggest there may be an increased concentration of GAP-43 in schizophrenic patients [11,12]. GAP-43 is a membrane phosphoprotein implicated in the initial growth and establishment of synaptic connections. It appears to support the survival and process the outgrowth of dopaminergic neurons.

Our results do not support the hypothesis that there may be an increased level of GAP-43 in the thalamus of schizophrenic patients. GAP-43 appears to be decreased when compared to controls. However, an unknown protein with molecular weight of approximately 100kDa was bound by the GAP-43 antibody and found predominantly in the thalamus of the schizophrenic patients.

Material and Methods

Design and participants

The human brain tissue utilized in this study was taken from

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the Neuroscience Brain Tissue Bank at the University of Missouri - Kansas City School of Medicine. The brain tissue was donated by individuals and family members throughout the United States. The eight brains included in this study, four controls and four diagnosed Schizophrenic, were chosen based on criteria such as age, gender, race, post mortem index, neuropathological findings, primary diagnosis, and supplemental diagnosis. The clinical diagnosis of Schizophrenia was taken from medical records and found to be consistent with the diagnostic criteria of Schizophrenia according to the Diagnostic and Statistical Manual of Mental Disorders fourth edition. Each chosen brain had a post mortem index between 6 to 24 hours. Characteristics of control brains: Ages for the control subjects were 51, 71, and two unknown. Two were male, one female, and one unknown. Two subjects were Caucasian, one African American and one unknown. One control subject was diagnosed with Alzheimer's disease. Characteristics of schizophrenic brains: Ages for the schizophrenic subjects were 67, 66, 45 and 50. Two subjects were female and two were male. Two subjects were African American and two unknown. Two also suffered from depression. All of the subjects suffering from Schizophrenia had been prescribed antipsychotic medication.

This study was approved and conducted in accordance with the Ethic Committee of the "Office of Research of University of Missouri - Kansas City".

Procedure

Each brain was sliced and analyzed separately. The following steps were repeated independently for each brain. The study was done as a single blind study and the researcher working with these brains was only given the identification number of the brain without the diagnosis until the final western blot was completed with quantitative analysis. Results with the identification numbers were then matched with the diagnosis. Brains 2–7 were studied at the University of Missouri - Kansas City. Brains 1 and 8 were studied later at the University of Kansas Medical Center. The protocol in both locations was kept constant except two different cameras were used, as noted below.

Tissue excision

The whole brain was removed from the -80°C freezer and kept in -20°C freezer overnight. The next morning it was moved to the refrigerator and kept there while preparations were made for dissection. The whole brain was taken from the refrigerator, placed on the cutting board, photographed before cutting, and was cut with a DEXTER basic (NSE) sharpened brain cutting knife. First a coronal section was made at the level of mammillary body with the base of the brain facing upwards (ventral area). The posterior portion of the brain was then cut at about 1 cm thickness from the level of mammillary body to the posterior edge of the occipital lobe of brain. This posterior portion contains thalamus of both right and left hemispheres of the brain. The anterior portion of the brain was also sectioned at approximately 1 cm thickness from the level of mammillary body to the anterior edge of the frontal lobe of the brain. The sections were then photographed and labeled individually to maintain the anatomical orientation and placed in a ziploc bag and return back to the -20°C freezer. The thalamus was identified in each slice, and as much tissue as possible was dissected out from that area. The thalamus was divided into ~200 mg samples. Each sample was placed in a 15-mL centrifuge tube, labeled according to the slice from which it came, and immediately stored in the -20°C freezer.

Sample preparation

The 15 mL centrifuge tubes with the ~200 mg samples were removed from the -20°C freezer and placed in an ice bucket. The samples of thalamus were reconstituted with a solution containing RIPA Buffer, TBS, and Halt Protease inhibitor cocktail with EDTA all from Thermo Fisher Scientific (168 Third Avenue Waltham, MA USA 02451). Each sample (~200 mg) was then homogenized twice in two 5 mL aliquots of the cold suspension buffer as above by using a Power Gen 500 Homogenizer. The blade of the homogenizer was washed twice with 1 mL of the solution to remove adherent tissue, and the runoff was collected in the tube to have a total of 12 mL of buffer. The blade was rinsed with de-ionized water and the method was repeated for each sample.

After homogenization, the samples were placed on the orbital shaker for 2 hours in ice followed by centrifugation in the IEC Centra GP8R Centrifuge at 4°C at 6000 rpm for 30 minutes. The samples were removed from the centrifuge, and the supernatant was quickly poured into a new 15 mL tube. The pellets remaining were discarded. 1 mL of the supernatant was separated from each sample and placed in -20°C freezer for protein assay. The remaining supernatant was stored in -80°C freezer. The total protein concentration was determined in each sample using a Pierce BCA protein assay Kit (Thermo Scientific). The Genesys 10s Vis spectrophotometer was set to 562 nanometers and zeroed with deionized water as a control. The standards were measured with two replicates and the samples with three replicates. The average of the measured absorbencies was found for each standard and sample. The protein concentrations of the unknown samples were determined using the Microsoft Excel Forecast equation with the known concentrations and absorbencies of the standards and the absorbency of the samples.

Quantitative western blotting

Western blot was performed to measure GAP-43. A pilot study established a loading concentration of 35 μg of protein for each lane. Protein concentration was kept constant throughout the study. Samples loaded in gel wells set up the Thermo Scientific OWL P81 apparatus. A molecular weight marker ladder (Thermo Fisher) was added to lane 1 with each run, and Sample Lane Marker Buffer (Thermo Fisher) was added to each gel run as a negative control.

Following electrophoresis, the separated proteins were transferred onto PVDF membrane using a Pierce Semi-Dry Blotter (Thermo Scientific). The membrane was prepared by briefly soaking in methanol to become activated. 10x Pierce Fast Western Transfer Buffer (Thermo Scientific) was diluted and the PVDF membrane, filter paper, and the gel were incubated in Transfer Buffer and then assembled on the anode plate in the following order, from bottom to top: two pieces of filter paper, PVDF membrane, gel, and finally two pieces of filter paper. The cathode plate was placed on top and a power source supplied 25 V for 10 minutes.

The blot was incubated overnight at 4°C with polyclonal anti-GAP-43 or monoclonal GAP-43 (clone GAP-7B10, Sigma) at 1:1000 and 1:2000 dilutions respectively. The next day, the blots were washed three times for 5 minutes each in Pierce Fast Western Wash

Buffer (Thermo Scientific). The blots were incubated overnight with secondary antibodies Rabbit (polyclonal) and Mouse (monoclonal) Optomized HRP Reagent, Pico (Thermo Scientific) respectively at 1:10 dilutions. The next day the blots were washed three times for 5 minutes each with the Wash Buffer. Finally, the blots were incubated in Chemiluminesence (SuperSignal West Pico Lumino Thermo Scientific) for 5 minutes and the blot was transfered to an Alpha Innotech MultiImage TM apparatus (at the University of Missouri - Kansas City) or the Li-Cor C-DiGit® Blot Scanner (at the University of Kansas Medical Center) for imaging.

Densitometry and Data analysis

At the University of Missouri - Kansas City (Brains 2-7): The images of the Western blots were obtained with an Alpha Innotech MultiImage TM apparatus using Fluorchem FC2 software and were saved as TIFF files. The intensity of the immunoreactive bands was determined by densitometry using ImageJ (NIH software). The images were calibrated on a grey scale, and the density of each band was measured. Mean values for GAP-43 were determined at the level of 50 kDa and 100 kDa for each sample. Statistical significance between the control and schizophrenic densitometries was calculated using IBM SPSS statistics 19 (independent sample T-Test).

At the University of Kansas Medical Center (Brains 1 and 8): The images of the Western blots were obtained using the Li-Cor C-DiGit® Blot Scanner and analyzed using the Li-Cor Image Studio Software.

Antibodies

Anti-Phospho-GAP 43 (Ser41) polyclonal antibody (Thermo Scientific) is specific for the ~50 kDa Gap-43 protein phosphorylated at Ser41. The phosphopeptide corresponds to amino acid residues surrounding the phospho-Ser 41 of GAP-43. Monoclonal anti-GAP-43 (clone GAP-7B10) (Sigma-Aldrich) recognizes an isotope present on both kinase C phosphorylated and dephosphorylated forms of GAP-43. Western blots were run using Fast Western blot kit (Super Signal West Pico, Thermo Scientific).

Results

Detection of GAP-43 protein

Western blot analysis of brain homogenate of control brains (n=4) and schizophrenic brains (n=4) showed that GAP-43 recognized a single band with molecular weight of ~50 kDa, and no protein degradation was visible (Figure 1,2). When using polyclonal antibody to GAP-43, both control and schizophrenic brains showed a higher band at the level of ~150 kDa, which may be an aggregate or oligomer.

All eight brains could not be combined into a single analysis due to the un-planned utilization of two different types of cameras. Therefore, quantitative analysis was performed separately for the six brains studied at the University of Missouri - Kansas City and for the two brains studied at the University of Kansas Medical Center. For Brains 2-7: quantitative analysis found significantly ($p < 0.001$) less GAP-43 (~50 kDa band) in the thalamus in the schizophrenic group (1992.4 ± 223) compared to the control group (4328.4 ±649) (Figure 1). Specifically, the average GAP-43 (~50 kDa band) found in the schizophrenic brains was only 46% of the amount of the average GAP-43 found in the control brains. For Brains 1 and 8: GAP-43 (~50 kDa band) in the schizophrenic brain was only 36% of the amount of GAP-43 found in the control brain.

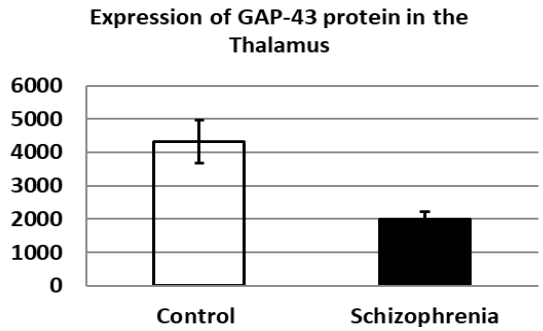

Figure 1: Quantitative analysis of the GAP-43 protein (~50 kDa) in the thalamus of Brain 2-7.

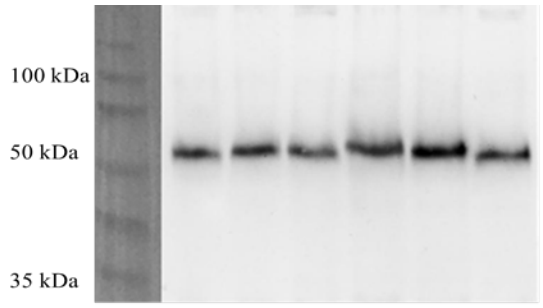

Figure 2: Western blot of GAP-43 using homogenate from the thalamus of control brain tissue shows a single band at ~50 kDa.

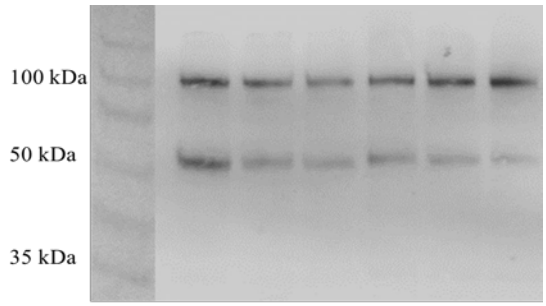

Figure 3: Western blot of GAP-43 using homogenate from the thalamus of schizophrenic brain tissue shows a single band at ~ 50 kDa and an additional band at ~ 100 kDa.

Detection of an unknown band

An unknown band was detected at a molecular weight of ~100 kDa in all four schizophrenic brain samples. However, similar molecular weight band was detected in only one control brain sample; and that was a trace amount in the brain with Alzheimer's disorder (Figure 2,3).

For Brains 2-7: quantitative analysis reveals a significantly (<0.001) high level of binding to the unknown protein (2833.3+/-410) in the thalamus of schizophrenic brain tissue when compared to very little binding in the control brain tissue (65.6+/-33) (Figure 4).

For Brains 1 and 8: a clear band was observed at 100 kDa in the schizophrenic brain while no observable band was present in the

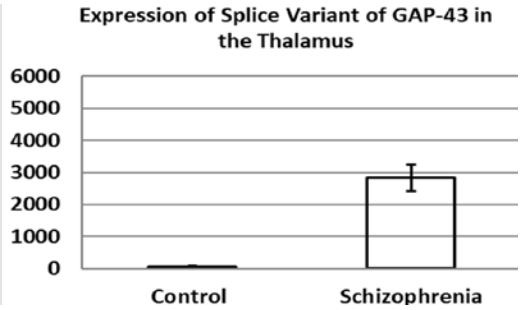

Figure 4: Quantitative analysis of the unknown protein (~100kDa) in the thalamus of Brain 2-7.

control brain.

To investigate the possibility that the higher molecular weight band could be a false positive or technical problem, monoclonal antibody was substituted for polyclonal GAP-43 antibody, and the unknown band at the molecular weight of ~100 kDa was still observed in the schizophrenic brain samples as well as the GAP-43 band at ~50 kDa. In an additional investigation, 0.1 M DTT was added to the protein/SDS mixture before boiling, and the 100 kDa band was still observed in the schizophrenic brain sample.

Discussion

The results of this study suggest there may be a novel protein in the thalamus of individuals suffering from schizophrenia. The identity of the protein remains unknown, but it appears to have a molecular weight of approximately 100kDa and can be bound by GAP-43 antibody. This protein may be a precursor/splice variant of GAP-43, dimer of GAP-43, or an aberrant form of GAP-43. A study by Oke et al [8] found a marked increase in Dopamine (DA) concentration in schizophrenic thalami when compared to that of normal/control thalami. The DA levels in a normal thalamus are usually considered to be no more than precursor levels for Norepinepthrine (NE). The DA/NE ratio in normal thalami rarely exceeded 15 to 20 percent. However, in the thalami from schizophrenic patients, the DA/NE ratio is much higher; exceeding 100 to 200 percent in some cases [8].

Perhaps the finding of an aberrant level of DA in the thalamus reflects altered thalamic activity. The DA/NE imbalance, interacting with the other neurotransmitter systems present in the thalamus, may result in variant firing patterns or neuronal assemblies that could globally alter brain functioning. As an example, Munsch et al [10] demonstrated that DA through activation of D (2)-like receptors in GABAergic interneurons increased inhibitory interactions.

The present study hypothesized that the increased concentration of DA could be due to an excessive activity of growth protein such as GAP-43. In this analysis, as noted above, levels were significantly lower in the samples from our schizophrenic brains (compared to the control brains) only to be replaced by a novel protein weighing about 100kDa and found only in the schizophrenic brains that also binds to the GAP-43 antibody.

One theory of the importance of this protein, admittedly a "hand waving theory," is that this novel protein is itself a growth factor. A growth factor may attract DA axons from the rich DA structures surrounding the thalamus, resulting in stimulation of an abnormal dopamine signal processing activity in schizophrenia patients.

Future studies will concentrate on analyzing the amino acid sequence of this protein.

Conclusion

Our data suggests a novel protein in the thalamus of individuals suffering from schizophrenia. This protein has a molecular weight of approximately 100kDa. Since this protein was bound to monoclonal GAP-43 antibodies it appears to be a precursor/ splice variant of GAP-43 or an aberrant form of GAP-43. GAP-43 is a membrane phosphoprotein imprecated in the initial growth and establishment of synaptic connections. It appears to support the survival and protect the outgrowth of dopaminergic neurons.

If this protein has similar growth attraction potential as GAP-43, it may function as a super growth factor. An increase in growth protein in the thalamus could possibly stimulate aberrant growth of dopaminergic neurons. Thus, the increase in dopamine in the thalamus. This increase may distort information processing to the cortex.

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References

1. Sherman S, Cuillery R. Thalamic functions: What is the thalamus and what is it for? Exploring the Thalamus. Academic Press. Academic Press, California. 2001: 312.

2. Andreasen NC, Arndt S, Swayze V 2nd, Cizadlo T, Flaum M, O' Leary D, et al. Thalamic abnormalities in schizophrenia visualized through magnetic resonance image averaging. Science. 1994; 266: 294-298.

3. Anderson NC. The role of the thalamus in schizophrenia. Canadian J Psychiatry. 1997; 42: 27-33.

4. Gaser C, Nenadic I, Buchsbaum BR, Hazlett EA, Buchsbaum MS. Ventricular enlargement in schizophrenia related to volume reduction of the thalamus, striatum and superior temporal cortex. Am J Psychiatry. 2004; 161: 154-156.

5. Rao NP, Kalmady S, Arasappa R, Venkatasubramania G. Clinical correlates of thalamus volumes deficits in anti-psychotics naïve schizophrenic patients: A 3-Tesla MRI study. Indian J Psychiatry. 2010; 52: 229-235.

6. Buchsbaum MS, Someya T, Teng CY, Abel L, Chin S, Najafi A, et al. PET and MRI of the thalamus in never-medicated patients with schizophrenia. Am J Psychiatry. 1996; 153: 191199.

7. Hazlett EA, Buchsbaum MS, Byne W, Wei TC, Spiegel-Cohen J, Geneve C, et al. Three-dimensional analysis with MRI and PET of the size, shape, and function of the thalamus in the schizophrenia spectrum. Am J Psychiatry. 1999; 156: 1190-1199.

8. Oke AF, Carve, LA, Adams RN. Dopamine-initiated disturbances of thalamic information processing in schizophrenia? In: Snyder, CR., & Cromwell, Rue L. (Eds.), Schizophrenia: Origins, processes, treatment, and outcome. (pp.31-47). London: Oxford University Press. 1993.

9. Oke AF, Putz C, Adams RN, Bird ED. Neuroleptic treatment is an unlikely cause of elevated dopamine in thalamus of schizophrenic subjects. Psychiatry Research. 1992; 45: 203-208.

10. Munsch T, Yanagawa Y, Obata K, Pape HC. Dopaminergic control local interneuron activity in the thalamus. Eur J Neurosc. 2005; 21: 290-294.

11. Perrone-Bizzozero NI, Sower AC, Bird ED, Benowitz,LI, Ivins KJ, Neve RL. Levels of the growth-associated protein GAP-43 are selectively increased in association cortices in schizophrenia. Proc Natl Acad Sci U S A. 1996; 93: 14182-14187.

12. Sower AC, Bird ED, Perrone-Bizzozero NI. Increased levels of GAP43 protein in schizophrenic brain tissues demonstrated by a novel immunodetection method. 1995. Mol Chem Neuropathol. 1995; 24: 1-11.