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Altered DARPP-32 expression in the superior temporal gyrus in schizophrenia

Yasuto Kunii *, Hirooki Yabe, Akira Wada, Qiaohui Yang, Keisuke Nishiura, Shin-ichi Niwa

Department of Neuropsychiatry, Fukushima Medical University School of Medicine, 1 Hikarigaoka, Fukushima City, Fukushima 960-1295, Japan

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ABSTRACT

Many neuroimaging studies have revealed structural abnormalities in the superior temporal gyrus (STG) in schizophrenia (Kasai et al., 2003a, 2003b; Sun et al., 2009). Neurophysiological studies of mismatch negativities (MMN) generated in the STG have suggested impaired function of *N*-methyl-D-aspartate (NMDA) receptors (Javitt et al., 1996). Although many postmortem studies have been conducted on the pathogenesis of schizophrenia, relatively few reports have studied molecular alterations in the STG (Bowden et al., 2008; Deng and Huang, 2006; Kang et al., 2009; Katsel et al., 2005; Le Corre et al., 2000; Nudmamud and Reynolds, 2001; Sokolov et al., 2000). The STG shows pronounced changes in gene expression when compared to other regions implicated in schizophrenia (Katsel et al., 2005). Dopamine and a cAMP-regulated phosphoprotein of molecular weight 32 kDa (DARPP-32) is thought to be closely associated with pathophysiological changes in the dopamine and glutamate systems in schizophrenia because, when activated by phosphorylation, DARPP-32 acts as a critical regulator of D1 dopamine receptor and NMDA receptor activity (Greengard et al., 1999). The molecular pathways involving DARPP-32 appear important in the pathogenesis of schizophrenia. Here, we show dramatic alterations in DARPP-32 expression in the STG of postmortem brains from patients with schizophrenia. To clarify the detailed histological and cellular expression of DARPP-32 in the STG in schizophrenia, we immunohistochemically examined postmortem brains by using specific antibodies. We compared the density of immunoreactive cells of the STG (BA22) from 11 schizophrenia patients with those from 11 age- and sex-matched controls, and found significantly lower densities of DARPP-32-immunoreactive (IR) cells and threonine (Thr) 34-phosphorylated DARPP-32-IR cells in the STG in the schizophrenia group. Thus, the DARPP-32-related pathogenesis in schizophrenia may be more severe in the STG than previously found in the prefrontal cortex.

1. Introduction

The molecular pathways containing DARPP-32 (Greengard et al., 1999) is thought to be important in the pathogenesis of schizophrenia. A frequent haplotype PPP1R1B, which encodes DARPP-32, was associated with enhanced performance on several cognitive tests that depend on frontostriatal function and the risk for schizophrenia in one family based association analysis (Meyer-Lindenberg et al., 2007).We previously used immunohistochemistry to examine detailed histological and cellular DARPP-32 expression in several regions of postmortem brains, including the dorsolateral prefrontal cortex (DLPFC), hippocampus, caudate, putamen, and nucleus accumbens (Kunii et al., 2011). We found decreased density of DARPP-32-IR neurons and a slightly increased density of Thr34-phosphorylated DARPP-32-IR neurons in the DLPFC in schizophrenia. Western blot studies reporting decreased DARPP-32 expression in the DLPFC in postmortem brains of patients with schizophrenia are consistent with these findings (Albert et al., 2002; Ishikawa et al., 2007). In areas of postmortem brains other than DLPFC, in situ hybridization revealed no changes in the anterior cingulate gyrus and thalamus (Clinton et al., 2005; Baracskay et al., 2006). Functional and structural studies have also documented abnormalities associated with schizophrenia in the STG (Kasai et al., 2003a, 2003b; Sun et al., 2009), an area that we and other researchers have not previously examined. The STG is the main generator of MMN, which occurs by a process of comparison between sound deviation and neural traces of preceding sounds stored in the auditory memory, located in the STG (Näätänen et al., 2007). The generation of MMN is assumed to involve neurotransmission via NMDA receptors, since NMDA antagonists reduce the magnitude of MMN (Javitt et al., 1996). Indeed, there is some evidence for dysfunction of glutamate systems in the STG. For example, postmortem in situ hybridization investigations of the STG in schizophrenia have reported increased mRNA expression of the NMDA receptor NR1 subunit splice variant (Le Corre et al., 2000), and ligand-binding studies have found increased NMDA receptor density (Nudmamud and Reynolds, 2001). To clarify the detailed histological and cellular expression of DARPP-32 in the STG patients with schizophrenia, we immunohistochemically examined

Abbreviations: DAB, diaminobenzidine; DARPP-32, dopamine and cAMP-regulated phosphoprotein 32KD; DLPFC, dorsolateral prefrontal cortex; DOI, duration of illness; IR, immunoreactive; MMN, mismatch negativities; NMDA, N-methyl-D-aspartate; PBS, phosphate buffered saline; PMI, postmortem interval; STG, superior temporal gyrus; Thr, threonine.

* Corresponding author. Tel.: +81 24 547 1331; fax: +81 24 548 6735.
E-mail address: kunii@fmu.ac.jp (Y. Kunii).

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postmortem brains by using specific antibodies. We found dramatic alterations in DARPP-32 expression associated with schizophrenia in the STG.

2. Methods

2.1. Brain tissue

Postmortem schizophrenia brain tissue was obtained from the Fukushima Brain Bank, and normal postmortem brain tissue was obtained from the Section of Pathology, Fukushima Medical University Hospital. The use of these tissues was approved by the Ethics Committee of Fukushima Medical University and complied with the Declaration of Helsinki. The collection consisted of tissue from 11 patients with schizophrenia and 11 controls (Table 1). No significant differences were seen in the mean age, postmortem interval (PMI), or tissue preservation time between the 2 groups. All patients with schizophrenia fulfilled the diagnostic criteria established by the American Psychiatric Association (Diagnostic and Statistical Manual of Mental Disorders: DSM-IV) and did not have a history of other neurological disorders or substance abuse. None of the normal cases had any history of mental disorders, neurological disorders, or substance abuse. Through neuropathological examination, there were no indications of neurological disorders in any of the brain tissues used in the present study, although mild senile changes were found in some brains. Tissue blocks of the BA22 were taken at the level of the superior temporal gyrus between above superior temporal sulcus and below lateral sulcus and fixed in 10% formalin, embedded in paraffin, and sliced into 5-μm sections.

2.2. Immunohistochemistry

Immunohistochemistry was performed in accordance with our previous protocol (Kunii et al., 2011). Sections were subjected to immunohistochemical analysis using rabbit polyclonal antibodies against human DARPP-32 (Santa Cruz Biotechnology, California, USA) by the LSAB (labeled streptavidin biotin) method. The sections were deparaffinized in xylene and then rehydrated in a series of 100%, 90%, 80%, and 70% ethanol. Endogenous peroxidase activity was blocked by adding methanol containing .3% hydrogen peroxide at room temperature for 20 min. Sections were then washed using phosphate buffered saline (PBS). For antigen activation, sections were microwaved for 15 min in 10 mM citrate buffer and washed using PBS. To reduce nonspecific binding, sections were soaked in 5% skim milk in PBS for 30 min at room temperature. Sections were incubated overnight at 4 °C with the primary antibody diluted 50-fold using PBS. As a negative control, normal rabbit serum was used instead of the primary antibody. Sections were incubated with secondary antibody (biotinylated anti-rabbit IgG, Nichirei Corporation, Tokyo, Japan) for 20 min at room temperature. Sections were treated with peroxidase-labeled streptavidin for 15 min at room temperature. Sections were then treated with diaminobenzidine (DAB) (Wako Pure Chemical Industries) for 5 min at room temperature in order to detect immunoreactivity, and were washed using PBS to terminate the reactions. Hematoxylin was used for nuclear counter staining (Fig. 1A and B). The results using rabbit polyclonal antibodies against human DARPP-32 were shown to illustrate the similar results using another goat polyclonal antibody (Santa Cruz Biotechnology, California, USA) against human DARPP-32 by the same methods. In addition, the sections were subjected to immunohistochemical analysis using goat polyclonal antibodies against human Thr34 phosphorylated DARPP-32 (Santa Cruz Biotechnology, California, USA) using similar standard methods (Fig. 1C and D).

2.3. Image acquisition and statistical analysis

DARPP-32-IR cells were examined under a microscope equipped with a digital camera (OLYMPUS BX51; Olympus, Tokyo, Japan) at ×100 magnification, and parenchymal cell numbers were counted using WinROOF version 5.5 software (Mitani Corporation, Tokyo,

Table 1
Demographic information of schizophrenic patients and matched controls. PMI = postmortem interval, DOI = Duration of illness (years). Estimated total lifetime drug consumption = DOI × dose of drugs (during 3 months before death). Neuroleptic doses are represented as chlorpromazine-equivalents (mg). Anticholinergic doses are represented as promethazine-equivalents (mg).

Subject	Sex	Age	DOI (yr)	PMI (h)	Preservation (y)	Cause of death	Estimated total dosage of neuroleptics and anticholinergics prescribed (mg)	
Control								
1	M	72		16.0	4	Lung cancer		
2	M	72		9.0	4	Lung cancer		
3	F	80		8.0	1	Septic shock		
4	M	71		5.0	3	Malignant mesothelioma		
5	M	71		6.0	5	Pneumonia		
6	M	50		24.0	4	Acute heart failure		
7	F	40		16.0	2	Acute heart failure		
8	M	62		15.0	5	Tongue cancer		
9	M	44		12.0	2	Acute heart failure		
10	M	53		7.0	2	Lung cancer		
11	M	46		25.0	1	Sudden death		
Total	9 M/2 F	60 ± 13.9		13 ± 6.9	3 ± 1.5			
Schizophrenia								
1	M	70	39	29.0	6	Pancreatic cancer	21,450	5343
2	M	75	47	18.0	5	Pneumonia	10,575	10,575
3	F	87	49	5.0	5	Suffocation	0	0
4	M	70	39	20.0	4	Pneumonia	20,787	5850
5	M	66	36	8.0	3	Pneumonia	0	0
6	M	73	52	19.0	2	Pneumonia	6240	0
7	F	39	23	21.0	2	Gastric sarcoma	13,915	1438
8	M	71	48	14.0	1	Pneumonia	14,544	7200
9	M	48	33	9.0	2	Pneumonia	68,640	2475
10	M	39	25	33.0	1	Suicide	3875	2500
11	M	58	30	6.0	1	Gastric cancer	42,810	10,500
Total	9 M/2 F	63 ± 15	38 ± 10	16.5 ± 9.2	2.9 ± 1.8			