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Postmortem brain calcineurin protein levels in schizophrenia patients are not different from controls

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Abstract

Calcineurin (CaN), also designated as protein phosphatase 2B, is a major Ca^{2+}/calmodulin-binding protein in the brain and the only serine/threonine phosphatase under the control of Ca^{2+}/calmodulin. CaN activity has been implicated in downstream regulation of dopaminergic signal transduction and in NMDA receptor-dependent synaptic plasticity. Thus, it serves as a point of convergence for the abnormalities of these two neurotransmitter systems in schizophrenia. The aim of the present study was to determine if levels of CaN were altered in two schizophrenia- and CaN-related brain regions — the dorsolateral prefrontal cortex and hippocampus from subjects with schizophrenia compared to that in tissue from age and sex matched controls. CaN protein levels were measured by Western-blot analysis in samples from 15 schizophrenia patients vs. 15 control subjects. No significant differences in CaN protein levels were found either in the prefrontal cortex or in the hippocampus of schizophrenia patients compared to matched control subjects. Our result of lack of difference does not support the concept that brain CaN levels are a pathophysiological factor in this disorder. Further studies with antibodies against specific CaN catalytic subunit isoforms (presently unavailable) are required to resolve this issue.

Keywords: Postmortem brain; Calcineurin; Protein levels; Schizophrenia

1. Introduction

Available data suggest that changes in both dopaminergic and glutamatergic function are perturbed in schizophrenia (Seeman, 1987; Carlsson et al., 2001; Coyle and Tsai, 2004). Calcineurin (CaN) activity has been suggested to regulate both dopaminergic (Greengard, 2001) and glutamatergic neurotransmission (Zeng et al., 2001) and could therefore affect functioning of both dopamine and glutamate.

CaN, also designated as protein phosphatase 2B, is a major Ca^{2+}/calmodulin-binding protein in the brain and the only serine/threonine phosphatase under the

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control of Ca^{2+}/calmodulin (Rusnak et al., 1999). CaN is a heterodimer, consisting of a 61 kDa catalytic subunit (CaN A) and a 19 kDa regulatory subunit (CaN B) (Klee et al., 1979; Aramburu et al., 2000). There are three mammalian CaN catalytic isoforms (α, β and γ) and two regulatory isoforms (CaNB1 and CaNB2). CaN has a number of substrates including nitric oxide synthase, Elk-1, dopamine and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32), tau and the heat-shock protein 25 (for review see Rusnak and Mertz, 2000; Winder and Sweatt, 2001). CaN is regulated by intracellular calcium, calmodulin, calcineurin inhibitor (CaNI) (Lai et al., 2000), FK-506 binding protein (FKBP12) (Steiner et al., 1992) and protein kinase A anchoring protein 79 (AKAP79) (Coghlan et al., 1995). CaN has been implicated in neuronal functions such as regulation of long-term potentiation and long-term depression (Malenka, 1994), regulation of neuronal cytoskeleton and recycling of synaptic vesicles (Liu et al., 1994).

Recent results in CaN knockout mice have shown behavioral changes such as increased locomotor activity, decreased social interaction, impaired prepulse inhibition and impaired working memory (Miyakawa et al., 2003; Zeng et al., 2001). These alterations in behavior resemble behavioral characteristics present in schizophrenia. In addition, an association study of CaN-related genes and schizophrenia suggests that the CaN gene may increase susceptibility to schizophrenia (Gerber et al., 2003). These findings prompted us to determine if levels of CaN were altered in the dorsolateral prefrontal cortex (DLPFC) and hippocampus from subjects with schizophrenia compared to that in tissue from age and sex matched controls.

2. Materials and methods

2.1. Patients

Permission to carry out this study was obtained from the Ethics Committee of the Victorian Institute of Forensic Medicine and the North Western Mental Health Program Behavioral and Psychiatric Research and Ethics Committee. Postmortem brain samples from 15 patients meeting DSM-IV criteria for chronic schizophrenia and 15 matched normal controls were obtained from the Rebecca L. Cooper Research Laboratories at the Mental Health Research Institute of Victoria, Australia. Samples studied were of frontal cortex [Brodmann's Area (BA) 9] and hippocampus from the left hemisphere of the same subjects. The final diagnosis according to DSM-IV criteria was established by consensus between two senior psychiatrists and a psychologist following an extensive case history review using the Diagnostic Instrument for Brain Studies (Hill et al., 1996; Roberts et al., 1998). Control subjects had no contact with any psychiatric service prior to death, had not received anti-psychotic medication, had not died by suicide or had any neurological disorder. The groups were matched by age [controls: 46.6 years ± 15.2 (SD); schizophrenia patients: 46.6 ± 15.3, $p = 1.0$], sex (4F/11M both groups), postmortem interval (PMI) [controls: 42.0 h ± 16.4 (SD); schizophrenia patients: 43.6 ± 13.3, $p = 0.44$] and pH of brain tissue [controls: 6.3 ± 0.22 (SD); schizophrenia patients: 6.3 ± 0.16, $p = 0.74$]. All experimental parameters were measured (by N. K.) in a balanced way but blind to diagnosis and demographic data.

2.2. Immunoblotting procedures

Crude homogenates, prepared as described previously (Kozlovsky et al., 2000), containing 6 μg total protein (within the linear range of detection) of either frontal cortex or hippocampus were resolved by SDS-PAGE (10% polyacrylamide gels were used). Blots were blocked in 5% non-fat dried milk (w/v) in Tris–buffered saline with 0.1% (v/v) Tween 20 (TBS-T) for 60 min at room temperature. The proteins on each gel were transferred electrophoretically to PVDF membrane (0.2 μm pore size; Bio-Rad) and incubated with anti- all isoforms (α,β,γ) of calcineurin catalytic subunit antibody (BD transduction Laboratories: dilution 1: 250) in TBS-T overnight at 4 °C on a shaker. After washing the blots (3 × 10 min) in TBS-T, the resulting immuno-complex was detected by incubation with 1 : 2000 dilution of horse-radish peroxidase-conjugated goat anti-rabbit immunoglobulin antibody (Upstate Biotechnology). The bands were detected using an enhanced chemiluminescence Kit (Amersham) and an X-ray film (Kodak X-Omat LS). Densitometry analysis was performed using AIDA II (Dinco and Renium). The results presented are of a 61 KDa band established as the catalytic subunit of CaN