CALCINEURIN ACTIVITY IN CHILDREN WITH MENTAL HANDICAP

L. Hema Bindu, P. Usha Rani* and P. P. Reddy

Departments of Environmental Toxicology and *Clinical Psychology and Special Education, Institute of Genetics and Hospital for Genetic Diseases, Ameerpet, Hyderabad, Andhra Pradesh, India-500 016.

Abstract

Calcineurin, a Ca^{2+} - Calmodulin dependent protein phosphatase, is important for Ca^{2+} mediated signal transduction. The main objective of this study was to examine the potential role of calcineurin in idiopathic mental handicap. Calcineurin levels were estimated in 20 children in the age group of 5-16 years with idiopathic mental handicap attending the Special Education Centre for the Mentally Handicapped in Hyderabad. The results of the present study showed decreased activity of serum calcineurin in children with idiopathic mental handicap compared to those of normal subjects in the same age group. The observations thus suggest impaired calcineurin activity in children with mental handicap. Calcineurin that is involved in biosynthesis and release of neurotransmitters at the synaptic terminal brain is affected thereby causing brain damage and leading to mental handicap. Impaired calcineurin activity was already indicated in many human diseases such as Down's syndrome, Alzheimers, Brain ischemia, cardiac hypertrophy etc. It is therefore necessary to check the calcineurin levels in children with mental handicap to understand the role of calcineurin in the causation of Mental handicap.

KEYWORDS

Calcineurin, Calmodulin, Mental Handicap, Neurotransmitters, CaN Inhibitors.

INTRODUCTION

Mental Handicap (MH) is a condition of developmental disability where the mental functions are not developed with the age of the affected individuals when compared with the normal subjects. It may be defined as a condition of arrested or incomplete development of intellectual capacity arising from inherited causes or induced by disease or injury. According to World Health Organization, 3% of the world's population is affected with mental handicap. Mentally challenged condition is due to interplay of several biochemical, genetic, socio-cultural and psychological factors. The etiology of mental retardation can be distributed under three major heads namely prenatal, perinatal and postnatal conditions. Prenatal condition includes genetic (chromosomal or single gene disorders), environmental (infections, drugs, toxemia, maternal diseases, fetal growth retardation, etc) and multifactorial (recognized

syndromes and multiple congenital anomalies with mental retardation). Perinatal causes of MH are anoxia, low birth weight etc and postnatal causes include infections, socio-cultural disintegration, etc. Majority of the cases of mental retardation are idiopathic.

Calcineurin (CaN) or protein phosphatase 2B is a Ca^{2+} and a major calmodulin binding protein that is thought to play an important role in the neuronal response to changes in the intracellular Ca^{2+} concentration. It is originally identified in extracts of mammalian brain. It is a Calcium-Calmodulin dependent protein phosphatase, which plays an active role in calcium signalling pathways and is involved in many different pathways in multiple organ systems. It is distributed in brain, testis, liver, kidney, muscles, pancreas, thyroid, adrenal, lungs, spleen, prostate and blood cells such as erythrocytes, lymphocytes and platelets. Among the neuronal tissue, CaN content is highest in caudate and putamen, neurons, photoreceptors, bipolar and ganglion cells (1,2). This serine/threonine phosphatase protein can regulate several intracellular metabolic events such as neuronal development, glycogenolysis in skeletal muscle, lymphocyte activation, maturation of hematopoietic cells, sperm motility and exocytosis. Calcineurin is also known to regulate calcium

Address for Correspondence :

Prof. P. P. Reddy

Institute of Genetics and Hospital for
Genetic Diseases, Ameerpet, Hyderabad-500 016
E-mail : reddydenagalu@rediffmail.com

sequestration, cytokinesis, sporulation and mating (3). Calcineurin has also been implicated in certain diseases such as neuronal neoplasms, Down's syndrome, Alzheimers disease (AD), brain ischemia and cardiac hypertrophy (4,5,6). Induction of long term depression of the hippocampal neurons, modulation of the activities of the sodium channels, L-type calcium channels, NMDA receptors and heat stable inhibitors of protein phosphatase I, calcium mediated apoptosis, redistribution of integrins and regulation of intracellular calcium channels are few other cellular events influenced by calcineurin. CaN plays a major role in hippocampus based long term memory and certain pharmacologic and nonpharmacologic inhibitors reduce the activity of calcineurin mediated signalling pathways impairing the memory (7). CaN also has been implicated in the transduction of signals that control the hypertrophy of cardiac muscle and slow fiber gene expression in skeletal muscle (8,9,10). The immunosuppressive drugs such as Cyclosporin A and FK506 inhibit CaN which is necessary for nuclear import of NF-ATc (Nuclear Factor of Activated T-cells) (11). These CaN inhibitors are believed to play a major role in the reduction of serum calcineurin levels, thus leading to potential disease conditions.

Approximately 24 million children all over India are affected with mental handicap and as mentioned earlier in many cases, the cause is not known. Studies carried out in India to understand the role of calcineurin protein in the causation of idiopathic mental handicap are meager and therefore an attempt is made.

MATERIALS AND METHODS

20 children with idiopathic mental handicap in the age group of 5-16 years from both the sexes attending Special Education Center for Mentally Handicapped in Hyderabad formed the study group. Children in the same age group and socioeconomic status attending the normal schools formed the control group in the present study.

Psychometric analysis was carried out in these children in order to assess the Intelligence Quotient (IQ)/ Developmental Quotient (DQ) using verbal and non verbal tests, Seguin Form Board test and Raven's Colored Progressive Matrices. The degree of mental handicap was calculated in each child. Children were categorised into different groups depending on the presence or absence of associated problems with mental handicap.

Serum calcineurin levels were estimated in these children and compared with the levels of control subjects and its role in

mental handicap was analyzed. Calcium-calmodulin dependent phosphatase activity of CaN present in the samples was assayed according to the procedure of Wang et al (1997) in the presence and absence of trifluoroperazine (TFP). The assay was conducted in a reaction mixture (100ul volume) containing 250mM tris (pH 7.2), 250mM morpholino ethane sulphonic acid (MES pH 7.0), 10mM $MnCl_2$, 8.8mg/ml paranitrophenol phosphate (p-NPP) and 13% dipotassium hydrogen phosphate. The serum was added to the assay mixture. Reactions were initiated by addition of p-nitrophenyl phosphatase followed by incubation for 10min at 30°C. The reaction was terminated by the addition of 10ul of 13% dipotassium hydrogen phosphate. Samples were then transferred to microtitre plates and absorbance was measured at 405nm in the ELISA reader. The difference between the amounts of p-nitro phenol released in the presence and in the absence of TFP indicated the activity of the calcineurin in both the groups.

RESULTS AND DISCUSSION

In the present study the role of calcineurin was assessed in children with congenital idiopathic mental retardation. The psychometric analysis of the study group indicated that 25% of the children had borderline degree of mental retardation followed by 5% of them with mild, 35% of them with moderate, 20% of them with severe and 15% of them with profound degree of mental retardation out of 20 children analyzed (Table1). All the subjects included in the study were distributed as per their presenting symptoms. Among them 50% of the mentally challenged children showed no associated problems, while 20% of them had cerebral palsy, 15% of them had congenital anomalies, 10% of them had convulsions and the remaining 5% had autism associated with mental retardation (Table 2). The calcineurin levels in children with mental handicap and in MH cases with associated problems such as convulsions, cerebral palsy, congenital anomalies or autism were estimated and since there was no significant difference

Table-1: Degree of mental retardation in children with mental handicap

Degree of Retardation	IQ levels	No. of children (n=20)	Percentage
Dull to normal	84-90	—	—
Border line	68-83	5	25
Mild	52-71	1	5
Moderate	36-51	7	35
Severe	20-35	4	20
Profound	20	3	15

Table-2: Associated problems in children with Mental Handicap (n=20)

Type	No. of children	Percentage
Mental Retardation	10	50
MR with cerebral palsy	4	20
MR with congenital-anomalies	3	15
MR with convulsions	2	10
MR with autism	1	5

in the calcineurin levels between groups, the data for all cases was pooled and the mean values were presented in table-3.

Table-3: Estimation of Calcineurin levels in children with Mental Handicap

Group	Calcineurin activity u mol/dl
Control subjects	96.8 +/- 40.03
Children with MH	59.6 +/- 15.02 *

*P>0.01

The serum calcineurin levels showed a decrease in children with mental handicap (59.6 +/- 15.02m mol/dl) when compared with the levels observed in control subjects (96.8 +/- 40.03u mol/dl). Statistical analysis of the results by 't'-test indicated that the decrease in CaN levels in children with mental handicap was highly significant (P>0.01). In the current study, significant decrease of calcineurin activity in the probands indicates a definite role of this protein in mental retardation. It can be concluded that the biosynthesis and release of neurotransmitters was affected in the children with mental retardation and this might either be due to genetic inhibition of calcineurin activity or due to over expression of calcineurin inhibitors in the body. The impaired calcineurin activity would affect calcium signaling pathways to the brain affecting various cellular events.

Calcineurin is highly localized in the central nervous system and therefore is considered to play an important role in neuron specific functions. CaN (PP2B), is the best enzyme to unveil the phosphatase function since it was shown to be the direct target for immunosuppressants such as Cyclosporin A and FK506, which are considered as powerful tools for understanding the function of CaN in diseases as well as in various tissues and organs (11).

Chronic overexpression of the calcineurin inhibitor gene, DSCR1 is associated with Alzheimers disease (AD) and Downs syndrome. The DSCR1 gene product is an inhibitor of CaN and its signaling pathways. In Down's syndrome patients,

DSCR1 is highly expressed in neurons within the cerebral cortex, hippocampus, substantia niagra, thalamus and medulla oblongata and interacts physically and functionally with CaN A, the catalytic subunit of the Ca^{2+} /CaM dependent protein phosphatase PP2B thus leading to the above condition (4,5,6). A disturbance of calcium homeostasis is believed to play an important role in the neurodegeneration of the brain of Alzheimers's disease (AD) patients. The studies revealed two to three times higher mRNA DSCR1 levels in Alzheimer's disease (AD) patients and in those with extensive neurofibrillary tangles when compared to controls (5,12). Calcipressin, a CaN inhibitor was shown to be over expressed in the pyramidal neurons of the temporal lobe in AD patients (13).

Central nervous system toxicity such as tremors, temporal lobe partial epilepsy or encephalopathy have been observed in few cases upon administration of tacrolimus, a CaN inhibitor in recipients of orthotopic liver transplantation (14). Cyclosporine (CsA), another inhibitor of CaN is known to cause more severe neurotoxic effects such as hallucinations, psychosis, blindness, seizures, cerebellar ataxia, motoric weakness or leukoencephalopathy. Studies reveal that occipital white matter is susceptible to the neurotoxic effects of CsA, which may cause hypoperfusion or ischemia and local secondary toxicity in the white matter (15). Cyclosporin A (CsA) has also been reported as a major cause of secondary hypertension in humans (16). The role of CaN inhibitors in epilepsy was investigated since they are the major modulators of synaptic transmission likely to be involved in molecular and cellular events leading to epileptogenesis. Studies in animal models revealed alterations in the activities of CaN in the rat hippocampus with temporal lobe epilepsy (17) and in the brain tissue of epileptic fowl (18). Studies have shown that CaN activity decreases with increasing age in three brain subcellular fractions and nerve ending enriched fraction from the hippocampus which may alter signal transduction at synapses during the aging process. CaN inhibitor, calcipressin is believed to play a key role in this process (7).

In certain instances, low levels of CaN have proved to be effective in the treatment of some diseases such as Huntington's and noise induced hearing loss. CsA and FK506 have a therapeutic approach for Huntington's disease (HD) patients. HD is caused by an abnormal expanded polyglutamine (poly Q) repeat in the huntingtin protein. CaN dephosphorylates S421 and inhibition of CaN activity by treatment with FK506 favours phosphorylation of S421 and prevents poly Q mediated cell death of striatal neurons (19,20). These immunosuppressants also have a protective effect

against acoustic injury of the cochlea (21) and confer invitro protection to dorsal columns during hypoxic injury at physiological temperatures (22).

The overall studies on calcineurin present evidence for the adverse and therapeutic effects in various diseases. Thus, further studies are warranted to understand the in-depth role of CaN in various pathophysiological conditions.

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