Role of Enzymes in Causing Neurological Disorders

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ABSTRACT

Diseases of the nervous system are always associated with poor prognosis and limited treatment options. The fragile nature of the neurons and their inability to replicate means that neurological disorders are associated with a permanent disability. Pharmacotherapy of neurological diseases requires understanding the molecular mechanisms involved in the disease pathology. In most of the cases a faulty cellular biochemical pathway is involved, resulting from a defective enzyme. This article focusses on role of enzymes in various neurological disorders. To review pertinent literature and summarise the role of enzymes in the underlying pathology of various neurological disorders. A comprehensive literature search was conducted using PubMed, SCOPUS, J-GATE and Google Scholar and relevant papers were collected using the keywords enzymes, Alzheimer's disease, redox, thiamine, depression, neurotransmitters, epileptogenesis. The literature review highlighted the role of enzymes in major neurological disorders and their potential to be used as drug targets and biomarkers. Identifying defective enzymes gives us new molecular targets to focus on for developing more effective pharmacotherapeutic options. They can be also considered as potential biomarkers. An abnormal enzyme is most often a direct result of an underlying genetic abnormality. Identifying and screening for these genetic abnormalities can be used in early identification and prevention of disease in individuals who have a genetic predisposition. The modern advances in genetic engineering shows a lot of promise in correcting these abnormalities and development of revolutionary cures although ethical concerns remain.

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INTRODUCTION

Enzymes are very important protein molecules which are responsible for catalysing almost all reactions in the human body. No biochemical pathway in the human body is complete without enzymes. They are very efficient biocatalysts which ensure all metabolic and biochemical pathways in the human body operate efficiently and smoothly. Since enzymes are crucial in all pathways of the body any defects in their function can lead to disorders. Most of these disorders are of genetic origin since enzymes are products of gene expression. Enzymes are also used as markers for various diseases. From a diagnostic point of view increase or decrease in certain enzyme levels can indicate a disease condition. The focus of this article is on the role of enzymes in causing neurological disorders.

A number of enzymes have been isolated from the cerebrospinal fluid and brain tissues. Studies

indicate that these enzymes have a good association with various neurological disorders. Enzymes play a protective as well as detrimental role in various neurological diseases. Some enzymes are involved in a protective function while aberrations in enzyme expression or protein structure are known to worsen the disease. An example of the protective function is peroredoxin, an enzyme involved in keeping the highly reactive peroxide levels under control. The enzyme comes under sulfhydryl dependent peroxidases. They reduce organic peroxides and hydrogen peroxides thereby providing protection from neurological diseases under oxidative and inflammatory stress. The significance of protective enzymes in diseases is that their decrease or dysfunction can lead to neurological disorders. Apart from protective function many enzymes are known to play a detrimental role in a variety of diseases like brain tumours, Alzheimer's diseases, Parkinsonism etc.

Enzymes and Their Involvement in Various Neurological Disorders

Role of enzymes in Alzheimer's disease

Alzheimer's disease is a condition which affects memory and cognition in patients. It is a neurodegenerative condition in which the patients eventually experience memory deficits, impaired cognition and poor functional status. The underlying pathology in Alzheimer's disease is thought to be the development of Amyloid plaques and Neurofibrillary tangles (NFT). These lesions occur more prominently in the temporal lobe structures and corticomedullary regions of the brain. The amyloid plaque formation is explained by the amyloid cascade hypothesis. As per this hypothesis, amyloid plaques occur as a result of the accumulation of an abnormal protein called Aβ. Aβ is a 36-43 amino acid protein produced from the processing of a much larger protein called Amyloid precursor protein (APP). The imbalance between the production and clearance of Aβ is thought to result in Aβ accumulation. β secretase is a key enzyme involved in Aβ production. It cleaves the amyloid precursor protein (APP) to β Amyloid whose deposition may lead to Alzheimer's disease. β secretase specifically known as BACE1 is elevated in sporadic Alzheimer's disease indicating the involvement of this enzyme in the condition (Chen *et al.*, 2012). The elevated enzyme produces Aβ at a higher rate far exceeding its normal clearance mechanism resulting in Aβ accumulation and plaque formation. Another enzyme that works along with β secretase in cleaving APP is γ secretase which also play a role in the disease pathology.

Apart from β Amyloid production this enzyme can also cause Alzheimer's disease by another mechanism. It is thought to interfere with the cAMP dependent signalling pathway involved in long term memory. cAMP response element binding protein(CREB) phosphorylation is a crucial step in the pathway which result in the transcription of CREB target genes (Lonze and Ginty, 2002). This is necessary for maintaining the plasticity of synapses essential for long term memory. The CREB activation is done by cAMP dependent protein kinase A (PKA) which is in turn activated by cAMP. The BACE1 interacts the transmembrane domain of adenylyl cyclase which result in reduced cAMP production. This result in PKA inactivation leading to reduced phosphorylation of CREB leading to the cognitive and memory defects in Alzheimer's disease (Chen *et al.*, 2012).Both the enzymes β and γ secretase can be targeted to develop novel therapeutic agents to treat alzheimers disease.

Current medications in treating the symptoms of Alzheimer's disease include targeting the cholinergic pathway to increase the concentration of acetylcholine in the synaptic cleft. It was observed that the loss of cholinergic neurons leading to a cholinergic deficit is one of the major causes of disease symptoms. The loss of cholinergic activity is directly proportional to Alzheimer's disease severity. The current medications in Alzheimer's treatment namely donepezil, galantamine and rivastigimine inhibit the enzyme acetylcholinesterase. The enzyme is involved in degrading the acetylcholine to inactive products. This enzyme is strategically placed in the synaptic cleft to degrade the excess acetylcholine at the synaptic cleft. Inhibition of the acetylcholine esterase transiently elevates the acetylcholine levels alleviating the symptoms in the early stages of the disease.

Cyclin dependent kinase 5 (CDK5) is a proline directed serine threonine kinase that comes under the family of cyclin dependent kinases (Dhavan and Tsai, 2001; Liu *et al.*, 2017). CDK5 unlike other members of the CDK family is not directly involved in the regulation of cell cycle. The kinase activity of CDK5 is active only when the enzyme associates with either of its two activator proteins p35 or p39 (Dhavan and Tsai, 2001; Liu *et al.*, 2017). CD5/p35 complex is the predominant active form under healthy conditions. The CDK5 enzyme is abundantly found in post mitotic cells, especially the neurons (Liu *et al.*, 2017). Restricted expression of the CDK5 activators p35 and p39 is the reason for localisation of the CDK5 activity to the post mitotic cells, predominantly to the central nervous system (Liu *et al.*, 2016). In healthy neurons the activated CDK5 performs various important func-

tions like neuronal migration and differentiation, maintenance of synaptic plasticity, memory consolidation, gliogenesis, development of the cerebellum and cortex layer formation (Liu *et al.*, 2016). The tight regulation of CDK5 is disrupted in many pathological conditions especially when the neurons undergo various stresses. Neurotoxic insults cause an excess of calcium influx into the cells resulting in the activation of a group of proteases called calpains. Calpains cleave the protein p35 into two components p10 and p25. p25 has a longer half-life that p35 and has the ability to pathologically activate CDK5 into a hyperactive kinase for a longer period of time (Liu *et al.*, 2017). The resultant hyperactive CDK5/p25complex hyper phosphorylates various substrates resulting in neuropathology. In Alzheimer's disease, the hyperactivity of CDK5 is closely linked to the formation of amyloid plaques and neurofibrillary tangles. Studies in transgenic mice showed that aberrant CDK5 phosphorylates the Thr668 amino acid residue of APP. This results in increased processing of APP to Aβ and its deposition as plaques (Liu *et al.*, 2016). Neurofibrillary tangle formation is also a result of hyperphosphorylation of substrates by the CDK5. The tau protein contains high amount of serine threonine residues which are excellent sites for phosphorylation by the CDK5/p25 complex (Liu *et al.*, 2016). Tau is a microtubule binding protein involved in the proper assembly of microtubules and in connecting the microtubules with other cytoskeletal structures. Hyperphosphorylation of tau at the serine threonine residues by the CDK5/p25complex results in formation of paired helical filaments (PHF) which in turn is a component of the neurofibrillary tangles. The loss of function of tau protein results in synaptic loss and cell death. Apart from being a direct part of Alzheimer's disease pathology CDK5/p25complex also damages the central nervous system by causing mitochondrial dysfunction, cell cycle re-entry, cell apoptosis and synaptic dysfunction (Liu *et al.*, 2016).

Glycogen synthase kinase 3 (GSK3) is another serine threonine kinase which has been implicated in the pathogenesis of Alzheimer's disease. GSK3 is named as such due to its ability to phosphorylate and inactivate glycogen synthase, the enzyme responsible for storage of glucose as glycogen. Two forms of GSK3 has been identified, GSK3α and GSK3β coded by gsk3α and gsk3β genes respectively. Both of these genes are located on different chromosomes. GSK3 is involved in both Aβ and neurofibrillary tangle formation. GSK3 is known to modulate Aβ formation by interaction with the protein prensilin. Prensilin is produced by the genes PSEN1 and PSEN2 and has a major role in γ secretase activity. GSK3 interacts with PSEN1 thereby modifying its localisation and function, leading to senile plaque formation (Avila *et al.*, 2010). GSK3 also phosphorylates tau at the serine threonine residues leading to PHF formation, resulting in neurofibrillary tangles. GSK3 requires preactivation of its substrates by other kinases for efficient phosphorylation. This preactivation is facilitated by the kinases CDK5, Protein kinase C (pkC), Protein Kinase A (pkA), casein kinase 1 (CK1) and PAR1. This links GSK3 mediated tau hyperphosphorylation with other kinases in the neurons. GSK3 has been studied as a potential drug target for treatment of Alzheimer's disease. Lithium has been shown to inhibit GSK3 in both in vitro and in mice models of the disease (Alam and Sharma, 2019). The models have shown a decline in senile plaque as well as neurofibrillary tangle formation due to the enzyme inhibition (Hampel *et al.*, 2019). But the use of lithium as a GS3 inhibitor is limited by the ability of GSK3 to inhibit other kinases and increasing the potential for toxicities (Avila *et al.*, 2010). NP12, a thiazolidinediaone has been studied as a GS3 inhibitor and was observed check the plaque formation and tau pathology in transgenic mice models of Alzheimer's disease (Serenó *et al.*, 2009). GS3 inhibition is a promising path to develop a novel treatment strategy for Alzheimer's disease.

Microtubule affinity regulating kinases (MARK) are a group of enzymes under the calcium calmodulin dependent protein kinase family involved in the tau pathology of Alzheimer's disease. Physiologically these enzymes are involved in regulating the crucial microtubule dynamics necessary for the proper functioning of cells. In the central nervous system, they play important roles such as maintenance of synaptic plasticity, axonal growth, regulation of cell cycle and also in intracellular signal transduction (Annadurai *et al.*, 2017). Four MARK enzymes have been isolated in humans, namely MARK1, MARK2, MARK3 and MARK4 (Annadurai *et al.*, 2017). The ability of MARK enzymes to phosphorylate tau protein at the Ser262 site on the microtubule binding domain is the reason for tau pathology associated with it (Annadurai *et al.*, 2017). Phosphorylation of the Ser262 makes the tau protein susceptible for further phosphorylation by other kinases such as CDK5, GSK3 and MAPK, resulting in further hyperphosphorylation of tau and neurofibrillary tangle formation (Annadurai *et al.*, 2017). The kinase activity of MARK is activated by other upstream kinases namely MARK kinase (MARKK) and Liver Serine/Threonine kinase B1 (LKB1). The activation happens by the phosphorylation of MARK at Thr208 residue by these upstream kinases (Annadurai *et al.*, 2017). MARK 2 and 4

have been highly implicated with the pathogenesis of Alzheimer's disease (Annadurai *et al.*, 2017). Direct inhibition of MARK enzymes as a target for treating Alzheimer's disease have been investigated with Drosophila models as well as animal models and showed positive results (Annadurai *et al.*, 2017). Indirect inhibition of MARK enzymes by inhibiting the upstream kinases were also studied. Although MARK enzyme activity was reduced by inhibiting the upstream kinases, deleterious effects were also observed (Ozcan *et al.*, 2015).

Catecholamine-O-methyl transferase (COMT) is an important enzyme involved in the metabolism of catecholamines. Due to its participation in dopamine degradation, the enzyme is also involved in cognitive functions. The enzyme is coded by the COMT gene located on chromosome 22 (Martínez *et al.*, 2009). A single nucleotide polymorphism (SNP) of the gene rs4680 SNP; G>A is shown to be a non-independent risk factor in Alzheimer's disease (Alam and Sharma, 2019). The polymorphism is of pathological significance when it is expressed in patients with Apolipoprotein ε4 (APOE ε4) allele (Lanni *et al.*, 2012). The G allele which results in a valine substitution instead of methionine at codon 158 is associated with higher enzyme activity and greater cognitive decline when co-expressed with APOE ε4. Hence rs4680 SNP; G>A polymorphism along with the presence of APOE ε4 can be used as a biomarker for predicting cognitive decline later in life (Lanni *et al.*, 2012). However rs4680 SNP; G>A polymorphism is not an independent predictor of cognitive decline as statistical significance could not be attained when the variable was assessed independently in longitudinal studies (Lanni *et al.*, 2012).

Phospholipase A2 (PLA2) is an enzyme responsible for hydrolysis of phospholipids, specifically at the sn2 ester bond (Gentile *et al.*, 2012). Physiologically they perform important tasks like signal transduction, eicosanoid synthesis, cell differentiation, proliferation and membrane trafficking (Gentile *et al.*, 2012). In humans 25 different PLA2 isoforms have been identified, of which larger cytosolic calcium dependent PLA2 (cPLA2) is associated with the inflammatory processes involved in Alzheimer's disease (Gentile *et al.*, 2012; Alam and Sharma, 2019). cPLA2 is involved in cleaving membrane phospholipids into arachidonic acid, a precursor of prostaglandins necessary for causing inflammation (Gentile *et al.*, 2012). cPLA2 is extensively located in the hippocampus, substantia nigra (SG), caudate nuclei, corpus callosum and subthalamic nucleus and is responsible for neuroinflammation and maintenance of membrane plasticity (Gentile *et al.*, 2012). cPLA2 is activated by the increase in intracellular calcium concentration as well as phosphorylation by mitogen activated protein kinase (MAPK). Aβ peptide is found to have the ability to alter the intracellular calcium concentration, resulting in cPLA2 activation leading to arachidonic acid production and neuroinflammation (Gentile *et al.*, 2012). The arachidonic acid is also susceptible to lipid peroxidation by NADPH oxidase, causing oxidative stress, further exacerbating the damage (Gentile *et al.*, 2012). Several PLA2 inhibitors such as natural extracts from the plant Withania somnifera are being studied as potential strategies for blocking the inflammatory processes (Gentile *et al.*, 2012; Alam and Sharma, 2019). Synthetic molecules such as arachidonyl trifluoromethyl ketone (AACOCF3), bromoenol lactone also show promising PLA2 inhibition and reduced inflammation (Gentile *et al.*, 2012).

Tissue transaminase (tTG) an enzyme involved in protein cross linking has been found to play a role in the process of Aβ and NFT formation. The main function of this enzyme is protein cross linking through the formation of ε-(γ-glutamyl)lysine isopeptide bonds, (γ-glutamyl)polyamine bonds and deamidation of protein substrates. The enzyme catalyses both intra and inter-protein cross linking. In Alzheimer's disease the enzyme is involved in cross linking of both Aβ monomers to oligomers as well as cross linking of tau proteins to form NFT. tTG can cross link both phosphorylated as well as unphosphorylated tau. Also the enzyme polyaminates the tau protein making it resistant to degradation by calpases thereby reducing tau clearance (Wilhelmus *et al.*, 2014). Autopsies of Alzheimer's disease showed increased expression of tTG in their brain cortex compared to controls (Wilhelmus *et al.*, 2014). Also increased levels of ε-(γ-glutamyl) lysine isopeptides were detected in the CSF suggesting increased activity of tTG in Alzheimer's disease patients (Wilhelmus *et al.*, 2014). Therefore tTG can be explored as a potential biomarker and drug target in Alzheimer's disease (Alam and Sharma, 2019).

Monoamine oxidases (MAO) are enzymes located in the outer membrane of mitochondria responsible for oxidation of monoamine neurotransmitters such as dopamine, noradrenaline and serotonin. This oxidation produces hydrogen peroxide as a byproduct which is converted into water by free radical scavenging mechanisms. But overexpression of this enzyme results in increased production of free radicals, overwhelming the normal free radical scavenging mechanisms. The excess free radicals cause lipid peroxidation and neurodegeneration.

MAO- B enzyme is overexpressed in patients with Alzheimer's disease (Schedin-Weiss *et al.*, 2017). This has been observed on post mortem studies of Alzheimer's disease brains (Schedin-Weiss *et al.*, 2017). The enzyme is also found to be interlinked with γ secretase function involved in Aβ formation. (Schedin-Weiss *et al.*, 2017). Taking into consideration the role of MAO-B in free radical induced neuronal damage, current MAO-B inhibitors can be studied as a potential treatment option in Alzheimer's disease.

Sirtuins are a group of enzymes responsible for deacetylation and ADP ribosylation of various protein substrates (Alam and Sharma, 2019). Sirtuins performs various essential roles in the survival of cells such as cell cycle control, chromatin regulation, mitochondrial function and DNA repair (Cacabelos *et al.*, 2019). There are 7 sirtuins (SIRT 1-7) identified in humans and they are all dependent on Nicotinaminde adenine dinucleotide (NAD$^+$) for catalysing reactions (Cacabelos *et al.*, 2019). Of all the SIRT enzymes, SIRT 1 is the most extensively studied enzyme in Alzheimer's disease pathology. SIRT 1 up regulates the enzyme ADAM10, via the activation of retinoic acid receptor beta (RARB). (Cacabelos *et al.*, 2019). ADAM10 is an alpha secretase necessary for converting APP to soluble APPα peptides thereby preventing Aβ plaque formation (Cacabelos *et al.*, 2019). SIRT 1 also reduces NF-κB signalling thereby keeping inflammatory processes responsible for neuronal damage in check (Cacabelos *et al.*, 2019). Pharmacological up regulation of SIRT1 could prove beneficial in treating Alzheimer's disease (Dai *et al.*, 2018; Alam and Sharma, 2019). Other SIRT enzymes namely SIRT 2 and 3 were also found to be up regulated in Alzheimer's disease and could be explored as biomarkers through future studies.

Thiamine Dependent Enzymes and Their Role in Neurological Disorders

The water soluble vitamin thiamine plays an important role in the function of many enzymes. Its deficiency has shown a good correlation with several neurological diseases and manifestations. In the brain, thiamine pyrophosphokinase converts thiamine to thiamine diphosphate (TDP) which serves as a cofactor for enzymes involved in glucose metabolisms such as α keto glutarate dehydrogenase, pyruvate dehydrogenase and transketolase enzymes. Of these α keto glutarate dehydrogenase is a part of the citric acid cycle through which oxidation of acetyl-coA to produce energy takes place. α keto glutarate dehydrogenase is a rate limiting enzyme of this pathway and any irregularities in the

function of this enzyme can have significant ramifications in cellular energy metabolism. A reduction in the levels of thiamine will cause a reduction in the TDP levels. This would lead to a reduction in the activitiy of TDP dependendent enzymes. Decrease in the activity of α keto glutarate dehydrogenase results in neuronal cell death which can lead to pathological manifestations like Wernicke Korsakoff syndrome characterised by loss of neurons in the thalamus, cerebellum and midbrain. The disease will show a variety of symptoms including mental impairment, confusion, ataxia and rapid involuntary movement of the eyes. Acute thiamine deficiency affects mitochondrial function resulting in oxidative stress in certain areas of the brain. Oxidative stress initially starts in areas of high metabolic activity. α keto glutarate dehydrogenase is also shown to play a role in Alzheimer's disease. β Amyloid peptide and oxidative stress was shown to reduce to reduce the activity of α keto glutarate dehydrogenase causing death of neurons. Ethanol is shown to inhibit the thiamine pyrophosphokinase which could precipitate neuronal cell death in alcoholics. Also, in other neurodegenerative disorders like progressive cerebral palsy and Parkinson's disease reduced activity of α keto glutarate dehydrogenase has been observed.

Role of DNA Repair Enzymes in Neurological Disorders

Uncorrected DNA damage can result in various neurological manifestations due to the long life and high transcriptional activity of neurons. These damages can occur due to reactive oxygen species, errors in replication and transcription or due to external factors like radiation. Normally there are DNA repair mechanisms in place which will repair the damage and prevent any pathological manifestations. Single stranded DNA breaks (SSB) are a frequently observed DNA damage and can result from the action of Reactive oxygen species. If uncorrected it can cause neurodegeneration. SSB are repaired using XRCC1 based single strand break repair (SSBR) /base excision repair (BER) pathway. Unrepaired double stranded breaks (DSB) due to defective double strand break repair (DSBR) can also lead to neurological problems.

Polynucleotide kinase phosphate (PKNP) is an important enzyme that participates in both DSBR and SSBR pathway. In DNA repair the PKNP uses its 3' Phosphatase and 5' kinase activity to make the ends of the DNA breaks compatible for ligation. Various mutations in this enzyme result in neurological disorders including microencephaly with seizures, neurodegeneration, occulomotor apraxia and dystonia due to defective DNA repair. Microencephaly

occur congenitally. Other manifestations like cerebellar ataxia is a result of neuronal degeneration and can occur after birth.

Ataxia telangiectasia mutated (ATM) is a serine threonine kinase activated in response to DSB (Choy and Watters, 2018). It is a member of the phosphatidylinositol-3 kinase-like protein kinase (PIKK) family. Defects in the ATM gene located on the long arm of chromosome 11 is responsible for the rare autosomal recessive disease ataxia telangiectasia (Choy and Watters, 2018). Clinical presentation of this disease is complex and varies from individual to individual. The major distinguishing features are cerebellar degeneration and ataxia, immunodeficiency, telangiectasia, radiation sensitivity and increased risk of cancers of lymphoid origin. The kinase activity of ATM is involved in various crucial processes like DNA repair, cell cycle regulation, response to oxidative stress, autophagy, apoptosis and mitochondrial function. Defects in the enzyme causes impairment of these crucial processes, especially DNA repair and cell cycle regulation resulting in reduced cell proliferation and cell death. This is the reason for neurodegeneration. Studies in mice with knocked out ATM gene showed neurodegenerative changes in the cerebellum (Choy and Watters, 2018). Also, markers of increased oxidative stress such as lipid peroxidation products and reduced levels of antioxidants were observed in AT patients (Choy and Watters, 2018).

Role of Enzymes in Epilepsy

A number of enzyme related aberrations are known to be a factor in causing myoclonic epilepsies and seizures. Diseases like Tay Sachs disease, Sandhoff disease, GM2 Gangliosidosis are some of the causes of progressive myoclonic epilepsy. Also, metabolic disorders like hypoglycaemia, mitochondrial disorders, biotin deficiency are also known to cause myoclonic epilepsies.

Accumulation of gangliosides especially in the lysosomes are the cause of GM2 Gangliosidosis. Defects in the enzymes involved in the catabolism of gangliosides causes the condition. β hexosaminidase A and B coded by genes HEXA and HEXB are two enzymes involved in this catabolism, whose defects can lead to accumulation of gangliosides. Tay Sachs disease is a result of mutations in the HEXA gene. Defects in the HEXB gene can result in Sandhoff disease. Also a very rare kind of gangliosidosis known as AB variant have been identified due to mutation a protein known as GM2A activator protein (Kochumon et al., 2017).

Kinases such as CDK5, Casein kinase 2 (CK2), TrKB kinase, Adenosine kinase (ADK) and kinases involved in the m-TOR pathway were found to be aberrantly expressed in animal models of epilepsy (Dixit et al., 2016).

From an epileptogenic point of view CDK5 is involved in synaptic transmission and synaptic plasticity as well as maintenance of neuronal excitability. CDK5 keeps neurotransmitter release in check and its inhibition has resulted in neurotransmitter release and unmasking of silent synapses (Dixit et al., 2017). Hence this enzyme is crucial for maintaining the balance between inhibitory and excitatory neurotransmission. Deregulation of CDK5 could lead to an excess neurotransmitter release and hyper excitability of neurons leading to epileptogenesis (Dixit et al., 2017). CDK5 is also thought to cause imbalances in dopamine homeostasis under conditions of elevated glutamate leading to excitotoxicity. CDK5 is found to both inhibit and induce epileptogenesis (Dixit et al., 2017). Under normal physiological conditions CDK5 inhibits epileptiform activity (Dixit et al., 2017). But under stressful conditions, due to the high influx of calcium into the cytosol, CDK5 plays the role of inducer of epileptogenesis (Dixit et al., 2017). This is due to the glutamine mediated calcium influx via activation of NMDA and AMPA receptor under conditions of stress leading to aberrations in the excitatory feedback circuit (Dixit et al., 2017). In the case of intractable epilepsy CDK5 was found to be aberrantly expressed at the transcriptome level (Dixit et al., 2017). Hence CDK5 has the potential to be used as a biomarker for epilepsy, especially in drug resistant cases (Dixit et al., 2017).

Casein kinase 2 (CK2) is a serine threonine kinase involved in regulation of circadian rhythm, cell cycle control and DNA repair. It has also been shown to regulate the slow after hyperpolarising potential, a major mechanism for inhibition of neurotransmission (Dixit et al., 2016). Chronic inhibiton of CK2 in rat pilocarpine model showed augmentation of slow hyperpolarising potential. Hence CK2 is a feasible target in the treatment of drug resistant epilepsy (Dixit et al., 2016).

The purine nucleotide adenosine is observed to act as a strong endogenous anticonvulsant and has the ability to cease seizure activity (Weltha et al., 2019). Levels of adenosine in the brain is regulated by astrocytes. Adenosine is released into the synaptic cleft by astrocytes in the form of Adenosine triphosphate (ATP). The ATP is then cleaved by various ectonucleotidases to generate adenosine which in turn exerts anticonvulsant activity by binding to adenosine receptors (Boison, 2016). Adenosine kinase (ADK) is the enzyme responsible for the inactivation of adenosine in the synaptic

cleft by converting it to adenosine monophosphate (AMP) (Weltha *et al.*, 2019). Since adenosine has no natural reuptake mechanisms, ADK takes up the role of preventing excess adenosine from building up. Overexpression of ADK is linked to the pathogenesis of epilepsy by reducing the amount of adenosine in the synaptic cleft (Weltha *et al.*, 2019). In astrogiosis, overexpression of ADK is the underlying pathological mechanism behind epileptogenesis (Dixit *et al.*, 2016). This has been observed in animal models (Dixit *et al.*, 2016). Also studies in animal models have shown reduction in seizures upon inhibition of ADK as well as adenosine receptors (Boison, 2016). Hence adenosine kinase can be used as a future therapeutic target as well as biomarker in epilepsy (Dixit *et al.*, 2016). Adenosine supplementation is also a feasible option in combating epilepsy as a result of astrogliosis (Boison, 2010).

Tropomyosin related kinase B (TrkB) the receptor of BDNF is known to play a role in the development of epilepsy following status epilepticus (SE) and traumatic brain injury (Lin *et al.*, 2020). Bindng of BDNF to its receptor Trkb promotes neuronal differentiation, neurite outgrowth and survival. Neurite outgrowth and formation of new neural connections in response to SE and traumatic brain injury is one of the reasons for development of chronic recurrent seizures (Lin *et al.*, 2020). TrkB is also known as receptor tyrosine kinase B and is a member of the tyrosine kinase family of receptors. TrKB has been specifically implicated in the pathogenesis of temporal lobe epilepsies (TLE) based on studies done on mice models (Lin *et al.*, 2020). Transient inhibition of TrkB in animal models prevented TLE after SE (Dixit *et al.*, 2016). Hence TrkB is a potential target for therapeutic intervention, specifically in drug resistant TLE (Lin *et al.*, 2020).

Traumatic brain injury and SE also causes epileptic changes by the activation of Janus kinase (JAK) - Signal transducer and activator of transcription proteins (STAT) pathway (Clossen and Reddy, 2017). JAK-STAT activation is found to de regulate inhibitory neurotransmission and promote hyper excitability (Clossen and Reddy, 2017). This happens through the loss of Gamma amino butyric acid A receptors (GABAAR) (Clossen and Reddy, 2017). Phosphorylation of the STAT3 molecule in traumatic brain injury leads to the loss of $\alpha 1$ subunit of GABAAR, leading to hyper excitability and seizures (Clossen and Reddy, 2017). BDNF also plays a role in JAK-STAT activation and modulation of this process (Clossen and Reddy, 2017). Inhibition of the STAT3 protein by an inhibitor WP1066 in animal models leads to an increase in GABAAR $\alpha 1$

subunit (Clossen and Reddy, 2017). Further studies in JAK-STAT inhibition could pave way for the development of novel strategies to reverse epileptiform changes.

Hyperactivation of the PI3K-AKT-mTOR pathway has been implicated in the development of structural lesions that could lead to epilepsy. The pathway has been associated with hypertrophic disorders such as focal cortical dysplasia, tuberous sclerosis complex (TSC) and Cowden disease. The pathway is a promoter of cell growth, differentiation and anabolism and inhibition of various components in the pathway could have therapeutic benefit. mTOR (mammalian target of rapamycin) has been extensively studied as a therapeutic target in epilepsy. Inhibition of mTOR via rapamycin administration reduced the progression of hypertrophic lesions in animal models of the disease (Dixit *et al.*, 2016). The two main upstream enzymes in the pathway namely the phosphatidylinositol-3-kinase (PI3K) and Protein kinase B (AKT) could also be potential targets in blocking this process. PI3K is activated by the receptor tyrosine kinase in response to various growth signals, which in turn would activate AKT and mTOR. Combined inhibition of PI3K and AKT inhibit mTOR signalling and thus reduced seizure activity in rat hippocampal model of post traumatic epilepsy (Berdichevsky *et al.*, 2013). Post traumatic epileptic changes could be decreased or alleviated by blocking the mTOR pathway (Berdichevsky *et al.*, 2013). The short and long term memory deficits due to seizure is also attributed to the overexpression of PI3K-AKT-mTOR pathway. In rat models, it was observed that Wortmannin, a PI3K inhibitor could offer protection against seizure induced changes and memory deficits (Carter *et al.*, 2017).

Enzymes in the Pathogenesis of Parkinson's disease

Reduced dopaminergic signalling in the Substantia nigra pars compacta (SN-PC) and nigrostriatal tract is the primary pathology in Parkinson's disease. Due to the rapid inactivation of dopamine by enzymes involved in catecholamine metabolism dopamine has a very short half-life. Reduced dopaminergic signalling is the reason for characteristic symptoms of Parkinsonism namely, tremor, rigidity, bradykinesia and postural instability. Enzymes MAO and COMT are involved in the metabolism of dopamine into inactive products. Of the two isoenzyme forms of MAO, MAO-A is expressed primarily in the peripheral structures and MAO-B is located in the CNS. COMT and MAO convert dopamine into 3-methoxytyramine (3-MT) and 3,4-dihydroxyphenylacetaldehyde (DOPAL) respec-

tively. Both these metabolites are further acted upon by various enzymes to form the inactive product homovanillic acid. Apart from administration of L-DOPA, inhibition of these enzymes are an indispensable part of current Parkinson disease pharmacotherapy. COMT inhibitors entacapone and tolcapone as well as MAO-B inhibitors seligiline and rasagiline as administered as adjuvant to levodopa to increase the half-life of dopamine. MAO-B inhibition is preferred over MAO-A inhibition due to less interference with the metabolism of peripheral amines. Dopamine is also hydroxylated into Noradrenaline by the enzyme Dopamine-β-hydroxylase which could also contribute to the reduction in dopamine half-life.

Dopaminergic neurons in the substantia nigra are in increased risk of oxidative stress from the free radical byproducts of catecholamine metabolism. Unchecked oxidative stress can cause neuronal cell death, resulting in reduced dopamine signalling leading to Parkinson disease. Overexpression of the enzyme Leucine rich repeat kinase 2 (LRRK2) has been identified as a key factor in causing oxidative stress (Johnson *et al.*, 2015). G2019S mutation increases the LRRK2 activity and causes excess free radical production thereby resulting oxidative stress to neurons (Johnson *et al.*, 2015). Several treatment strategies are being investigated to inhibit this enzyme and prevent neuronal damage. LRRK-IN-1 a compound with highly potent inhibitory activity of LRRK2 was found to be neuroprotective in cell cultures and C elegans model of parkinsonism (Yao *et al.*, 2013). But LRRK-IN-1 does not cross the blood brain barrier hence does not have therapeutic value. But two new inhibitors GNE-0877 and GNE-9605 which is blood brain barrier permeable and can make its way into clinical trials in the future (Estrada *et al.*, 2014).

Glutaredoxin (Grx1), a mammalian thioltransferase has also been identified to play a role in the pathogenesis of Parkinsons disease. The function of glutaredoxin is to deglutathionylate the cysteine residues of proteins causing them to regain their normal function and restore steady state functions (Gravina and Mieyal, 1993; Allen and Mieyal, 2012). Evidence for the protective role of Grx1 was first suspected when mice treated with a Parkinson's disease inducing compound 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was found to overexpress the Grx1 enzymes (Kenchappa and Ravindranath, 2003). Also, female mice were less susceptible to the effects of MPTP due to the high levels of Grx1 in their brains compared to that of males (Kenchappa *et al.*, 2004). Studies in the SH –SY5Y cells treated with the pro oxidant drug

L-DOPA showed that cell death due to L-DOPA is a consequence of covalent bonding of L-DOPA to the Grx1 enzymes (Sabens *et al.*, 2010). Postmortem studies of humans with Parkinson's disease showed the diminution of the Grx1 enzyme compared to healthy humans. Dopaminergic neuronal cell death was found to be more severe in Grx1 deficient patients if they also have hyperfunctional LRRK1 mutations like G2019S. Currently treatment of Parkinson disease is focussed on increasing the dopamine levels in the brain. It does not treat the underlying cause and there is still no way to halt the disease progression. By targeting the LRRK1, Grx1 enzymes and other redox proteins, treatment strategies can be developed prevent the dopaminergic neuronal loss and halt the disease in its tracks.

Role of Enzymes in Brain Malignancies

Tumours of the brain are always associated with poor prognosis and survival. Such tumors arise as a result of the uncontrolled proliferation of glial cells. Glioblastoma multiforme is a highly invasive grade 4 astrocytoma which is extremely difficult to treat and is associated with worse prognosis. The aggressive and invasive nature of the tumour means that even if fully treated the median survival is extended by just 2-3 months to 1 year. Surgical approach improves the symptoms but the disease will not be cured and the likelihood of recurrence is very high. Studies show that expression of the enzyme kallikrein 6 is associated with treatment resistance, aggressive tumor invasion and poor prognosis (Drucker *et al.*, 2013). Kallikrein 6 (KLK6) is a serine protease under the family of enzymes Kallikreins. Kallikreins are encoded by genes on the chromosome locus 19q. A study conducted by Drucker et al. showed kallirein 6 overexpression in astrocytoma tumour samples taken from patients (Drucker *et al.*, 2013). Further, the study also indicated a worse prognosis for patients who overexpressed the kallikrein 6 enzyme and cell line studies showed resistance to cytotoxic agents in KLK6 overexpressed cells Drucker *et al.* (2013). The mechanism of action of KLK 6 in tumour cell survival and treatment resistance is by the activation of the Protease activated receptor 1 (PAR1) (Drucker *et al.*, 2013). This was also confirmed by Druker et al. on cell lines (Drucker *et al.*, 2013). Cleavage of the extracellular domain of PAR1 by KLK6 or other KLKs reveals a tethered ligand. Intramolecular binding of this ligand result in cell signalling promoting tumour growth (Adams *et al.*, 2011). KLK6 being a protease is also thought to weaken the extracellular matrix facilitating tumour invasion.

A tumor in the brain is associated with ischaemia at its location which can further damage the nearby neurons through the development of oxidative stress. Ischaemic cell injury is thought to release iron from the cells which would further form an iron-ascorbate salt mixture causing lipid peroxidation and free radical formation. The hydrophilic free radicals cause local damage but some of the more lipophilic ones diffuse to distant sites spreading the damage. The damage can cause oedema and vascular compression in the brain tissue further worsening the situation. The body has a mechanism to prevent or to an extent reduce the ongoing damage through free radical scavenging mechanisms. A number of enzymes such as superoxide dismutase(SOD), Glutathione peroxidase (GPx), Gluthathione reductase (Grx) are involved in the free radical scavenging mechanism. Any deficiency of these enzymes compromises the ability to protect cells from oxidative stress. In the case of a tumour this situation is a factor in aggravating brain damage.

CONCLUSION

Enzymes are indeed the molecular machines that runs all the biochemical pathways of our body and keeps our cells alive. Enzymes are a product of gene expression and any aberration in genes coding for an enzyme can lead to defective enzymes, compromising the pathways and functions the enzyme normally performs. Hence genetics and enzymes are interlinked. Modern knowledge in genetics and genetic engineering are so advanced many of these genetic aberrations can be targeted to prevent and possibly cure diseases like Alzheimer's disease, Parkinsonism, depression and various other psychiatric disorders. With the advent of gene editing technologies like CRISPR there is scope for early detection and prevention of individuals with genetic predisposition though ethical concerns remain. Enzymes itself can also be a target of treatment. Drugs can be developed to target the enzymes involved in the neurobiology of a disease. Enzymes are also of diagnostic value. Detection of defective enzymes is a way to identify people who are the risk of a disease and take preventive measures early on. In other words, enzymes involved in neuropathology of disorders can be used as biomarkers. Considering the significant role enzymes play in the neuropathology of various neurological disorders, understanding them is key to developing better treatment modalities and screening techniques for the same.

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Conflict of Interest

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