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Pentylenetetrazole-Induced Seizures in Wistar Male Albino Rats with Reference to Glutamate Metabolism

Sivaprasad Kanchi, MSc, MPhil, PhD[1], Gurusekhar Meesala, MSc, PhD[2]

Department of Zoology, [1]SKR Government Degree College, Gudur, [2]Government Degree College, Cumbum, India

Background and Purpose: Epilepsy is a common and heterogenous neurological disorder characterized by recurrent spontaneous seizures. Animal models like rats play a crucial role in finding of mechanism of epilepsy in different brain regions. i.e., cerebral cortex, cerebellum, hippocampus, and pons medulla. Glutamate is an important excitatory neurotransmitter in the central nervous system and also glutamate plays a vital role in neuronal development and memory. The process of neuronal death evolved by glutamate receptor activation, has been hypothesized in both acute and chronic degenerative disorders including epilepsy. Considering the multifactorial neurochemical and neurophysiological malfunctions consequent to epileptic seizures, a few antiepileptic drugs are designed, to mitigate the debilitating aspects of epilepsy.

Methods: Rat model, pentylenetetrazole (PTZ), an anticonvulsant drug, was selected for the present study. Induction of epilepsy/convulsions was induced by an intraperitoneal injection of PTZ (60 mg/kg body weight) in saline. Biochemical assays performed through spectrophotometer.

Results: Glutamine and Glutamine synthetase levels were decreased in the epileptic rats brain regions i.e., hippocampus, cerebellum, cerebral cortex, and pons medulla; glutamate dehydrogenase and glutaminase levels were increased in all the regions of epilepsy induced rats. Highest values are recorded in hippocampus when compared to other brain regions.

Conclusion: PTZ suppresses the function of Glutamine and Glutamine synthetase activities in selected brain regions of rat and enhances the activities of the glutaminase and glutamate dehydrogenase when compared to control rats. **(2024;14:21-28)**

Key words: Pentylenetetrazole, Epilepsy, Rats, GABA, Convulsions, Seizures

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Corresponding author:
Sivaprasad Kanchi, MSc, MPhil, PhD
Department of Zoology, SKR Government Degree College, Gudur, Andhra Pradesh 524101, India
Tel. +91-9676841978
Fax. +91-9491311355
E-mail; kanchi1976@gmail.com

Introduction

Epilepsy is a convulsive episode and is the most frequent neurodegenerative disorder affecting about 50 million people world-wide. Pentylenetetrazole (PTZ) is a gamma aminobutyric acid-A (GABA) receptor antagonist. The epileptogenesis is a dynamic process with a tendency to develop hyperexcitability in one or other regions of the central nervous system. Aberrant reorganization of neuronal circuitry, alterations in synaptic plasticity, in inter-neuron number and function result in the development and propagation of an epileptic seizure.[1] The severity of the seizures can differ from person to person. Some people will just experience a trance-like state for a few seconds or minutes, whereas others will lose consciousness and have convulsions (uncontrollable shaking of the body). Epilepsy is not normally life-threatening, although physical injury can occur as a result of seizures. In rare cases, epilepsy can cause sudden, unexplained death, which kills 500 people in the United Kingdom every year.

Potential causes of epilepsy include brain tumors, central nervous system infections, traumatic brain injury, stroke, chemical imbalance, toxic chemical drugs, prenatal insults, degenerative disorders like Alzhemer's, Parkinsonism, and cerebrovascular diseases.[2] The common ictogenesis related characteristics of epilepsy are imbalance between excitatory and inhibitory neurotransmission, alterations in neurotransmitter expression and function, development of "epileptic ion channels" (channelopathies), morphological, and physiological changes in neurons such as hippocampal sclerosis and axonal sprouting leading to aberrant neuronal synchronization.[3]

GABA-ergic activity and exaggerated activity of glutamatergic neurotransmission are thought to contribute to the various types of epilepsies.[4] There are two types of GABA receptors that are involved

in epilepsy, namely GABA-A and GABA-B receptors. The GABA-A receptor belongs to a super family of ligand-gated ion channels mediate rapid inhibitory pre-synaptic potentials by increasing influx of chloride and GABA-B receptors are G protein-coupled receptors, also called metabotropic receptors. GABA-B receptors mediate slow inhibitory pre-synaptic potentials by increasing the potassium conductance and decreasing the calcium entry. It is hypothesized that reduction or loss of GABA ergic inhibition may increase the possibility of generating excitatory postsynaptic potentials and synchronizing burst discharges and therefore induce epileptogenesis. The GABA-ergic mechanisms that have been proposed include impairment of GABA release, changes in GABA receptors, impairment of GABA synthesis, and neuronal loss.[5] Despite the varied primary pathology of epileptic seizures, the mechanisms involving in generating and spreading epileptic seizures converge on a common cellular pathology in which the excitatory glutamatergic system plays a key role. This research paper reveals the PTZ induced epileptic seizures in selected regions of rat brain. These experimental results may be useful to provide some insights hypothesized mechanisms of epileptogenesis.

Mechanism of epilepsy

The N-methyl-D-aspartate (NMDA) and non-NMDA receptors play an important role in seizure induced brain damage. Sommer[6] discovered an area of the hippocampus that is vulnerable to injury in patients suffering from prolonged seizures (status epilepticus) which consists of the pyramidal cell fields in CA1 region. It was reported that hippocampal CA1 region contains a very high density of NMDA receptors[7] and also in the entire brain (Fig. 1).[8]

Reduced cellular energy metabolism during ischemia and epilepsy causes increased release and decreased re-uptake of glutamate, as well as increased extracellular K+ concentrations due to inhibition of the Na+/K+ -ATPase.[9] Intracellular Ca^{2+} influx and NMDA receptor antagonists can attenuate the neuronal damage and death.[10] Persistent glutamate activation of NMDA receptors with simultaneous membrane depolarization leads to a prolonged opening of NMDA receptor channels, permitting massive Ca^{2+} influx across the membrane (glutamate-calcium neurotoxicity hypothesis). Depolarization is also thought to cause additional Ca^{2+} entry into the cell through voltage-operated Ca^{2+} channels.[11] Elevation in intracellular Ca^{2+} levels activate a variety of Ca^{2+} dependent processes, including specific proteases and endonucleases that can breakdown the cellular DNA;[12,13] phospholipase A2 stimulates the release of arachidonic acid which in turn leads to the formation of free radicals and subsequent oxidative cell damage Nitric oxide synthase catalyzes the formation of nitric oxide can also participate in the generation of harmful free radicals[14] and ornithine decarboxylase resulting in tissue injury. Scatton[15] reported that excess accumulation of Ca^{2+} in the mitochondria can also lead to severe damage to brain regions.

In recent years, *in vitro* electrophysiological models such as cultured spinal cord neurons, hippocampal and cortical slices have been developed to study the mechanism of epileptogenesis. GABA receptor agonist, bacuculline, picrotixin or penicillin in hippocampal slice cultures or potassium channel blockers such as tetraethyl ammonium induces *in vitro* epileptogenesis. By lowering of extracellular Mg^{2+} environments can also induce *in vitro* epileptiform events in adult slices.[16] Low Ca^{2+} induced seizure-like events,[17] high K^{+} induced seizure-like events 4-aminopyridine induced recurrent seizures[18] cause *in-vitro* epileptogenesis. Interruption of α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) and NMDA-mediated transmissions causes repeated high-frequency excitation[19] and increased extracellular glutamate (glutamate injury induced epileptogenesis) may also lead to epileptogenesis in *in vitro* models.[20] From the earlier reports, it has been understood that impairment of

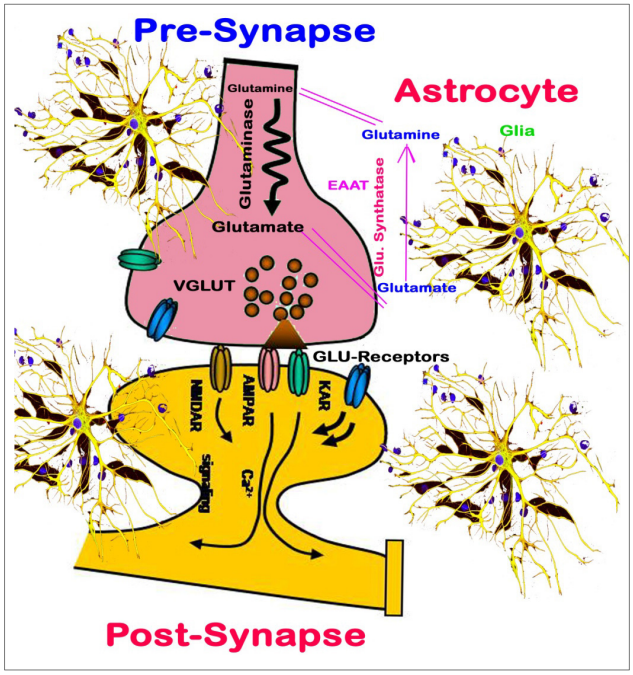

Figure 1. Synaptic transmission mechanism shows that Glutamate metabolism in relation to the glial cells and regulation of convulsions. EAATs, excitatory amino acid transporters; VGLUT, vesicular glutamate transporter; GLU, glutamate; KAR, kainate receptors, or kainic acid receptors; AMPAR, the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; NMDAR, N-methyl-D-aspartate receptor.

ion-channel function and GABA mediated transmission or reciprocal increase in excitation due to glutamatergic and cholinergic influences may lead to epileptic characteristics in experimental animals.[21]

Methods

Procurement and maintenance of experimental animals

Male adult Wistar rats weighing 150±25 g were used as the experimental animals in the present investigation. The rats were purchased from the Indian Institute of Science Bangalore, maintained in the animal house of the department in polypropylene cages under laboratory conditions of 28±2℃ temperature with photoperiod of 12 hours light and 12 hours dark and 75% relative humidity. The rats were fed with standard pellet diet (Hindustan Lever Ltd., Mumbai, Maharastra, India) and water ad libitum. The rats were maintained according to the ethical guidelines for animal protection and welfare bearing the CPCSEA (438/01/a/cpcsea/dt:17.07.2006). This animal study was approved by the resolution (No:09/(i)/a/CPCSCA/IAEC/SVU/Dept.of Zoology/Dt. 04.03.2016).

Selection of drug

PTZ, an anticonvulsant drug, was selected for the present study. It was obtained as commercial grade chemical from Sigma Chemicals (Merck Group., Burlington, MA, USA). Diazepam 2 mg/kg body weight as reference control.

Induction of epilepsy

Convulsions were induced by an intraperitoneal injection of Pentylenetetrazole (60 mg/kg body weight) in saline.[22-25]

Experimental design for screening of PTZ-induced seizures

The rats were divided into three groups, each consisted of six rats and used for studying effects of PTZ. Group 1 consit of normal saline treated control rats, group 2 is rats treated with PTZ and group 3 is epileptic rats pretreated with diazepam ([DP]+PTZ).

Isolation of tissues

After observed the seizures activity falling down on its back, rushing and jumping in all PTZ treated rats within an hour, the animals were sacrificed by cervical dislocation and different brain regions such as cerebral cortex (CC), cerebellum (CB), hippocampus (HC), and pons medulla (PM) were immediately isolated, frozen in liquid nitrogen and were stored at -80℃ until analysis.

Procurement of chemicals

All chemicals used in the present study were analar grade and obtained from the following scientific companies: Sigma (Sigma-Aldrich, St. Louis, MO, USA), Fisher (Fisher Scientific, Pittsburg, PA, USA), Merck (Merck Specialities Private Limited, Mumbai, India), Ranbaxy (Ranbaxy Laboratories, New Delhi, India), and Qualigens (Qualigens Pharma, Mumbai, India). In the present investigation Barnstead Thermoline (Thermo Fisher Scientific, Lake Balboa, CA, USA) water purification plant for nanopure water, Kubota KR (Kubota Corporation, Osaka, Japan) centrifuge and Hitachi U-2000 Spectrophotometer (Hitachi High-Technologies Corporation, Tokyo, Japan) and other standard equipments were used for biochemical/physiological analyses.

Biochemical assays

Glutamine content

Glutamine was estimated by acid hydrolysis method as described by Colowick and Kaplan (1967).[26] 5% homogenates of different tissue homogenates were prepared in cold distilled water and the homogenates were centrifuged at 1,000 g for 15 minutes. To required mL of the supernatant, 0.2 mL of the 10% H_2SO_4 was added and the tubes were kept in boiling water-bath for 10 minutes and then cooled. The contents were centrifuged and to the supernatant 0.3 mL of 10% NaOH was added and the mixture was made upto 2.0 mL with distilled water. The ammonia formed was estimated by Nesslerization. The Glutamine content was expressed as µ moles of ammonia/g wet weight of tissue.

Glutamate dehydrogenase (E.C. 1.4.1.3), (L-glutamate; nicotinamide adenine dinucleotide [NAD] oxidoreductase)

Glutamate dehydrogenase (GDH) activity was assayed by the method of Lee and Lardy (1965).[27] 5% tissue homogenates were prepared in ice cold sucrose (0.25 M) solution and the contents were centrifuged at 1,000×g for 15 minutes. The supernatant was used as an enzyme source. The reaction mixture in a total volume of 2.0 mL contained 100 µm of phosphate buffer (pH 7.4), 40 µm of sodium glutamate, 0.1 µm of NAD, 2 µm of Iodo nitro tetrazolium and 10 mg of tissue as an enzyme source. The reaction mixture was incubated at 37℃ for 30 minutes. The

reaction was arrested by adding 5 mL of glacial acetic acid and the formazan formed was extracted into 5 mL of toluene. The intensity of the colour was read at 495 nm against toluene blank. The enzyme activity was expressed as μ moles of formazan formed/mg protein/hours.

Glutaminase (E.C. 3.5.1.2), (L-Glutamine aminohydrolase)

Glutaminase activity was assayed by the method of Paul and Nathan (1971).[28] 5% tissue homogenates were prepared in cold double distilled water and centrifuged at 2,000×g for 15 minutes. The supernatants were collected for enzyme assay. The reaction mixture contains 50 μ moles of L-Glutamine freshly prepared in sodium acetate buffer (pH 4.9) and required amount of tissue in mg as an enzyme source. The contents were incubated at 37℃ for 30 minutes and the reaction was arrested by adding 1.0 mL of 10% trichloroacetic acid. The contents were centrifuged and to the supernatant 1.0 mL of 15% NaOH and 1.0 mL Nessler's reagent were added. The intensity of the colour developed was read at 490 nm against the reagent blank in a spectrophotometer. The enzyme activity was expressed in μ moles of ammonia released/mg protein/hour.

Glutamine synthetase (E.C. 6.3.12), (L-Glutamine: Adenosine triphosphate [ATP] ammonia ligase)

Glutamine synthetase activity was assayed by the method of Wu (1963).[29] 10% tissue homogenates were prepared in distilled water and centrifuged at 1,000×g for 10 minutes. The supernatant was used as an enzyme source. The reaction mixture in a total volume of 1.0 mL contained 80 μ moles of imidazole buffer (pH 7.2), 20 μ moles of magnesium chloride, 0.1 mL of 2-mercaptoethanol, 50 μ moles of glutamate (pH 7.2), 10 μ moles of ATP, and 100 μ moles of hydroxylamine (pH 7.2) and 0.1 mL of enzyme source.

The reaction mixture was incubated at 37℃ for 15 minutes and the reaction was arrested by adding 1.5 mL of ferric chloride. The contents were centrifuged at 1,000×g for 5 minutes. The clear supernatant was read at 535 nm against the reagent blank. The Glutamine synthetase activity was expressed as μ moles of γ-glutamyl hydroximate formed/mg protein/hour.

Statistical treatment of data

All assays were carried out with six separate replicates from each group. The mean, standard error and analysis of variance (ANOVA) were done using SPSS statistical software version 25 (SPSS Inc., Chicago, IL, USA) for different parameters. Difference between control and experimental assays was considered as significant at $p<0.01$.

Results

The results clearly showed that the Glutamine content have been significantly decreased in all the regions of the rat brain in PTZ epileptic condition when compared to the saline treated control rats and rats pre-treated with diazepam. All the mean values are significant at $p<0.01$ in one-way ANOVA in SPSS version 25 (SPSS Inc.) (Fig. 2). When compared to control rats PTZ induced CC region showed 0.921 decrease and rats pre-treated with diazepam showed 0.356 decrease (n=18), 5.29; $p<0.01$, one-way ANOVA. PTZ induced CB region showed 1.052 decrease and rats pre-treated with diazepam showed 0.21 decrease (n=18), 1.768; $p<0.01$, one-way ANOVA. PTZ induced HC region showed 1.484, decrease and rats pre-treated with diazepam showed 0.614 decrease (n=18), 1.587; $p<0.01$, one-way ANOVA. PTZ induced PM region showed 0.89 decrease and rats pre-treated with diazepam showed 0.723 decrease (n=18), 2.779; $p<0.01$, one-way ANOVA. The highest Glutamine values recorded in the hippocampus region of rat brain (Fig. 2).

The results clearly showed that the GDH levels have been significantly

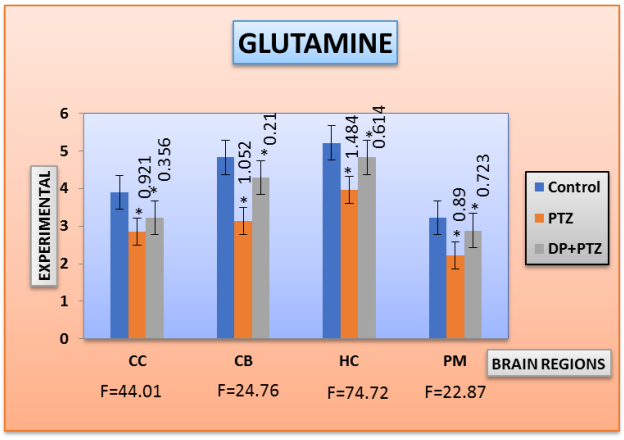

Figure 2. Experimental values are expressed in μg of Glutamine/g wet weight of tissue. Values are showed on the bars mean difference between controls and experimental. F=brain area wise comparisons (control vs. PTZ and DP+PTZ). Hippocampus (HC)=3.964 μ moles of ammonia/g wet weight of tissue. Cerebral cortex (CC)=2.848 μ moles of ammonia/g wet weight of tissue. Cerebellum (CB)=3.128 μ moles of ammonia/g wet weight of tissue. And pons medulla (PM)=2.224 μ moles of ammonia/g wet weight of tissue. PTZ, pentylenetetrazole; DP, diazepam; ANOVA, analysis of variance. *p-value ≤0.01 is considered significant in one way ANOVA.

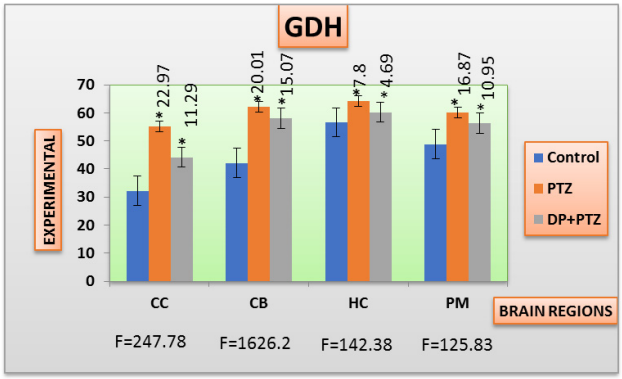

Figure 3. Experimental values are expressed in μ moles formazan formed/mg protein/hour. Values are showed on the bars mean difference between controls and experimental. F=brain area wise comparisons (control vs. PTZ and DP+PTZ). Hippocampus (HC)=64.214 μ moles of formazan formed/mg protein/hour. Cerebellum (CB)=62.245 μ moles of formazan formed/mg protein/hour. Pons medulla (PM)=60.21 μ moles of formazan formed/mg protein/hour. Cerebral cortex (CC)=55.2 and μ moles of formazan formed/mg protein/hour. GDH, glutamate dehydrogenase; PTZ, pentylenetetrazole; DP, diazepam; ANOVA, analysis of variance. *p-value ≤0.01 is considered significant in one way ANOVA.

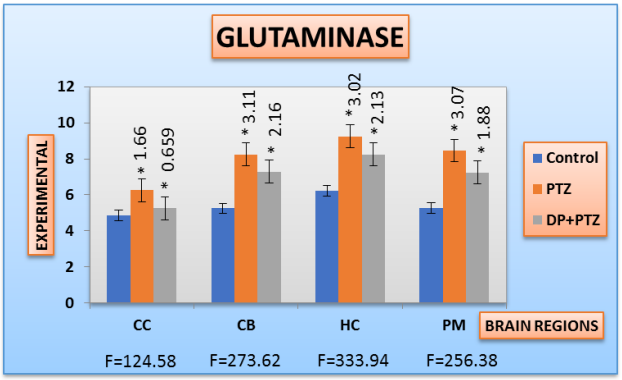

Figure 4. Experimental values are expressed in μ moles of ammonia formed/mg protein/hour. Values are showed on the bars mean difference between controls and experimental. F=brain area wise comparisons (control vs. PTZ and DP+PTZ). Hippocampus (HC)=9.234 μ moles of ammonia released/mg protein/hour. Cerebellum (CB)=8.245 μ moles of ammonia released/mg protein/ hour. Pons medulla (PM)=8.457 μ moles of ammonia released/mg protein/ hour and cerebral cortex (CC)=6.248 μ moles of ammonia released/mg protein/ hour. PTZ, pentylenetetrazole; DP, diazepam; ANOVA, analysis of variance. *p-value ≤0.05 is considered significant in one way ANOVA.

increased in all the regions of the rat brain in PTZ epileptic condition when compared to the saline treated control rats and rats pretreated with diazepam. All the mean values are significant at p<0.01 in one-way ANOVA in SPSS version 25 (SPSS Inc.) (Fig. 3). When compared to control rats PTZ induced CC region showed 22.97 increase and rats pre-treated with diazepam showed 11.29 increase (n=18), 3.238; p<0.01, one-way ANOVA. PTZ induced CB region showed 20.014 increase and rats pre-treated with diazepam showed 15.076 increase (n=18), 2.911; p<0.01, one-way ANOVA. PTZ induced HC region showed 7.807 increase and rats pre-treated with diazepam showed 4.698 increase (n=18), 1.756; p<0.01, one-way ANOVA. PTZ induced PM region showed 16.874 increase and rats pre-treated with diazepam showed 10.95 increase (n=18), 4.225; p<0.01, one-way ANOVA. The highest Glutamate dehydrogenase values recorded in the hippocampus region of rat brain (Fig. 3).

The results showed that the glutaminase levels have been significantly increased in all the regions of the rat brain in PTZ epileptic condition when compared to the saline treated control rats and rats pretreated with diazepam. All the mean values are significant at p<0.01 in one-way ANOVA in SPSS version 25 (SPSS Inc.) (Fig. 4). When compared to control rats PTZ induced CC region showed 1.66 increase and rats pre-treated with diazepam showed 0.659 increase (n=18), 4.534; p<0.01, one-way ANOVA. PTZ induced CB region showed 3.11 increase and rats pre-treated with diazepam showed 2.16 increase (n=18), 1.571; p<0.01, one-way ANOVA. PTZ induced HC region showed 3.02 increase and rats pre-treated with diazepam showed 2.13 increase (n=18), 3.656; p<0.01, one-way ANOVA. PTZ induced PM region showed 3.07 increase and rats pre-treated with diazepam showed 1.88 increase (n=18), 2.525; p<0.01, one-way ANOVA. The highest Glutaminase values recorded in the hippocampus region of rat brain (Fig. 4).

The results clearly showed that the Glutamine synthatase levels have been significantly decreased in all the regions of the rat brain in PTZ epileptic condition when compared to the saline treated control rats and rats pretreated with diazepam. All the mean values are significant at p<0.01 in one-way ANOVA in SPSS version 25 (SPSS Inc.) (Fig. 5). When compared to control rats PTZ induced CC region showed 0.201 decrease and rats pre-treated with diazepam showed 0.118 decrease (n=18), 3.636; p<0.01, one-way ANOVA. PTZ induced CB region showed 0.205 decrease and rats pre-treated with diazepam showed 0.121 decrease (n=18), 1.338; p<0.01, one-way ANOVA. PTZ induced HC region showed 0.27 decrease and rats pre-treated with diazepam showed 0.226 decrease (n=18), 9.648; p<0.01, one-way ANOVA. PTZ induced PM region showed 0.1 decrease and rats pre-treated with diazepam showed 0.04 decrease (n=18), 4.47; p<0.01, one-way ANOVA.

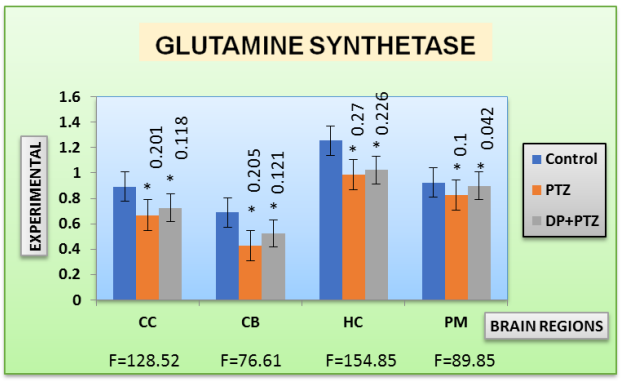

Figure 5. Experimental values are expressed in μmoles of γ-glutamyl hydroxymate formed/mg protein/hour. Values are showed on the bars mean difference between controls and experimental. F=brain area wise comparisons (control vs. PTZ and DP+PTZ). Hippocampus (HC)=0.986 μ moles of γ-glutamyl hydroximate formed/mg protein/hour. Pons medulla (PM)=0.825 μ moles of γ-glutamyl hydroximate formed/mg protein/hour. Cerebral cortex (CC)=0.668 μ moles of γ-glutamyl hydroximate formed/mg protein/hour. Cerebellum (CB)=0.425 μ moles of γ-glutamyl hydroximate formed/mg protein/hour. PTZ, pentylenetetrazole; DP, diazepam; ANOVA, analysis of variance. *p-value ≤0.01 is considered significant in one way ANOVA.

The highest Glutamine synthatase values recorded in the hippocampus region of rat brain (Fig. 5).

Discussion

The present study is mainly focused on estimating different parameters related to glutamate metabolism, i.e., glutamine content, GDH, glutamine synthetase (GS), and glutaminase activities in different areas of rat brain, with the aim of elucidating the metabolic changes in parameters released to glutamate under PTZ induced epilepsy and epileptic rats pretreated with diazepam. Glutamate is the predominant excitatory aminoacid neurotransmitter in the mammalian central nervous system acting on NMDA, AMPA, kainite, and metabotropic receptor-mediated mechanisms in epileptic seizures.[30-32] Excitatory glutamatergic mechanisms are involved during acute and chronic epilepsy models rats with spontaneous, recurring seizures after an early episode of induced status epilepticus.[33] Excitatory glutamatergic transmission plays a key role in learning and memory, generating and spreading of epileptic seizures and pathophysiology of neuronal death after brain injury.[31,33] Glutamate and aspartate or other excitatory endogenous compounds such as quinolinate or some sulfur-containing amino acids, as well as potent selective agonists including NMDA, AMPA, kainite, ibotenic acid, and domoic acid can cause convulsions when administered focally or systemically.[33] During spontaneous seizures in ambulatory patients, there is a marked, bilateral, transient increase in extracellular hippocampal glutamate levels, and which is largest in the epileptic hemisphere.[34,35] Similar increases in glutamate release can be observed associated with evoked seizures during surgery in epileptic patients.[36] Neuronal glutamate is lost during glutamate transmitter release and is taken up by glia, where it is recycled by glutamine synthetase.[37] With increased excitatory activity, the rate of neuronal glutamate loss would be greater. Glutamate lost from the neuron is replaced through phosphate activated glutaminase acting on glutamine synthesized in the glia.[38] Glutamate levels are increased in epileptic human brain.[39] Elevated levels of cerebrospinal fluid glutamate measured in epileptic patient's further support the role of slowed glutamate clearance in the development of epilepsy.[40] The excitatory system mainly consisting of Glutamine was affected by PTZ injection in rats. The GDH activity was found to increase in all the brain areas following the administration of PTZ. Alterations in the activity of key enzymes of amino acid metabolism like GDH signify modulation in the turnover rates of amino acids, glutamate in particular. The endogenous production of glutamate through metabolic pathways is very important in the brain, since the transport of circulating glutamate to brain normally plays only a minor role in regulating the brain glutamate levels.[41] The general decrease of NAD-GDH in different brain regions indicates that metabolism of glutamate by this reaction presumably increases the levels of glutamate during PTZ impact.

Supporting the present results the increase in GDH during seizures is supported by the results of Pitkänen,[42] studies showed decreased glutamate levels and increased GDH activity in cerebrospinal fluid of chronic epileptic patients. The epileptic focus is suggested to release excessive amounts of glutamate, which is reflected as lowered levels of glutamate and increased activity of GDH in the focal area of human epileptic brain.[43] Present results agree with the finding of elevated levels of excitatory amino acids in epileptic brain regions. In the present study, GDH activity increased in epileptic treated rats when compare to control in all areas of the brain. The general increase of NAD-GDH in different brain areas indicates that metabolism of aglutamate by this reaction presumably alters the levels of glutamate during antiepileptic treatment. Increased depolarization of glutamatergic terminals would result in greater glutamate release, greater uptake of glutamate into astrocytes, and greater flux through glycolysis to yield pyruvate.[44] Although glutamate levels were not estimated under PTZ administration and antiepileptic treatment in the

present investigation, the observed increase in GDH activity seem to have their own part to play in regulating the glutamate levels during the seizure threshold and recovery.

The activity of GS recorded decrease with PTZ administration. GS is predominantly localized in astrocytes of intact brain and plays a vital role in the ω-amidation of glutamate to form glutamine.[45] The declined activity of glutamine synthetase ingeneral depicts lesser mobilization of glutamate for the synthesis of glutamine. In other words ω -amidation of glutamate was not favoured during PTZ administration. The deficit of glutamine levels in brain may signify the failure of the brain to opt a protective measure for maintaining low concentrations of ammonia in tissues. Yudkoff et al.[44] also showed the decreased glutamine levels in PTZ treated brain. Taking into consideration the role of glutamine synthetase in ammonia detoxification, the inhibitory effect of the PTZ on the same may tend to induce "ammonia" stress on account of lesser conversion of glutamate to glutamine. In support of this glutamine levels were decreased in different regions of brain. Furthermore, the enhanced activity of glutaminase during the PTZ administration causes the hydrolytic removal of ammonia from the glutamine. Such enhanced activity of glutaminase coupled with reduced glutamine synthetase activity might have improved glutamate levels in brain during PTZ administration. The present investigation demonstrates significant changes in the levels of different parameters related to glutamate metabolism in different areas of the brain of rat following epileptic and antiepileptic treatment.

The parameters related to the glutamate metabolism in the present study showed differential changes upon the administration of PTZ and diazepam. GDH and glutaminase showed increase with PTZ administration in all brain regions. Glutamine and glutaminase synthetase were decreased in induced epileptic condition when compared to the control and epileptic rats pretreated with diazepam. The changes pertaining to the aspects of glutamate metabolism probably result from or at least associated with alterations in the levels of other parameters related to this metabolism. These results were found to have a challenging role in extinguish PTZ-induced glutamate metabolism in different brain regions of rat.

Conflicts of Interest

None.

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