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RESEARCH ARTICLE

Glutamate decarboxylase and Gamma amino transeferase levels in different regions of rat brain on the onset of Electroshock induced convulsions

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Abstract

The imbalance in the pools of excitatory or inhibitory neurotransmitters at their nerve endings causes depolarization or hyperpolarisation of the postsynaptic membrane. The heightened neuronal activity of the brain results in convulsions. Since Glutamate and γ-Aminobutyric acid (GABA) play an important metabolic role, quantitative changes in the content of this amino acid during altered neuronal activity may be possible. Although different convulsants such as drugs or electroshock may cause convulsions by different mechanisms, the basic change is an increase in neuronal firing. In order to understand the probable changes in the neurotransmitter pools of glutamate and GABA during convulsions, the enzymes involved in the production, liberation and disposal of glutamate and GABA, during pre, post and convulsions caused by electroshock have been studied. Convulsions were induced in white albino rats weighing 150-200 grams with convulsometer of "Techno" India using corneal electrodes. The animals were grouped into four. Group I served as control, whereas Group II-IV was subjected to electroconvulsions. The animals were sacrificed and the cerebral cortex, cerebellum and brainstem were separated from the brain. The tissue homogenates were used to estimate the enzymes such as GAD and GABA-T. The results showed that the activity of Glutamate decarboxylase (GAD) decreases significantly ($p<0.001$) in all the three regions of the brain in all the three phases. The activity of γ-Aminobutyric acid transferase **(**GABA-T) decreases significantly ($p<0.001$) in all the three brain regions in all the three phases of convulsions. Therefore, it is suggested that the concomitant changes of GAD activity and GABA concentration is probably an important factor in the onset of convulsions.

Introduction

The change in neuronal firing is a result of an imbalance in the pools of excitatory or inhibitory neurotransmitters at their respective nerve endings. The transmitters thus released act on the postsynaptic membrane causing either depolarization (excitatory) or hyperpolarisation (inhibitory). Aminoacids such as glutamate and aspartate are excitatory neurotransmitters whereas γ-aminobutyric acid (GABA) and glycine are inhibitory neurotransmitters

(Awapara et al.1950, Roberts, et al.1950, Bazemore, et al. 1956, Florey, 1991). Glutamate is distributed in brain in higher concentration than any other amino acid. It has an important metabolic and functional role in the brain (Krebs, 1948) although its synthesis and metabolism are compartmentalized in a very complex pattern. It also act as a precursor for the neurotransmitter i.e. GABA (Roberts,et al.1950) which is a non protein amino acid, is formed via decarboxylation of L-glutamate by glutamate decarboxylase (GAD).

The heightened neuronal activity of the brain results in convulsions. Seizures may be defined as the clinical or electrocephalographic (EEG) manifestations or both of uncontrolled firing of neurons within the central nervous system (CNS) especially in the cerebral cortex, but often neurons in more deeply placed structures are also involved. There are several biochemical consequences of seizure activity such as drop in ATP and Creatinine phosphate, decrease in intracellular pH, rise in cyclic AMP, release of cellular potassium and release of neurotransmitters.

The choice of appropriate animal models for the initial *invivo* testing of a potential anticonvulsant drug is on the most important steps in the successful treatment of convulsions. In seizures amino acids content of the brain especially glutamate and GABA have been measured by many researchers. The seizures can be induced in animal models by drugs such as Leptazol (Nasreen, et al. 2012), Picrotoxin or by Electroshock. Although different convulsants such as drugs or electroshock may cause convulsions by different mechanisms, the basic change is an increase in neuronal firing. It is only the quantitative changes occurring in the pools of these amino acids present in the synaptosomes will bring about a derangement in the function of the nervous system resulting in convulsions. Since glutamate and GABA plays an important metabolic role, quantitative changes in the content of this amino acid during states of altered neuronal activity may be possible

In order to understand the probable changes in the neurotransmitter pools of glutamate and GABA during convulsions the levels of glutamic acid decarboxylase (present in glutamatergic neurons and nerve endings) (Fonnum 1968) and GABA- aminotransferase (found in the glial cells and nerve endings) (Martin et al 1993) during pre, post and convulsions caused by electroshock have been studied.

Materials and Methods

Ethical Permission

All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (IAEC) and were in accordance with the guidance of IAEC.

Study design

All the experiments were carried out in white albino male rats of local strain, weighing between 150-200 grams. They were placed in polypropylene cages with paddy husk as bedding. Animals were housed at room temperature and relative humidity of 30-70%. A 12:12 h light:dark cycle was followed. They were allowed free access to water and fed with standard commercial pelleted rat chaw before subjecting them for electrical convulsions.

Electroshock

Convulsions were induced with convulsometer of "Techno" India using corneal electrodes. Electrodes were dipped in normal saline and were retained by clamps and current strength of 150 mA for 0.2 second was employed by the standard method (Collins, Albertonic). Before introducing the corneal electrodes, the eyes of the rats were treated with two drops of local anesthetic, benzoximate hydrochloride and one drop of normal saline to anaesthetize the cornea (Swisyatd 1972).

Study plan

The experimental set up consisted of 24 animals grouped into four with 6 animals in each. Group I: served as control for attaining normal values. The remaining three groups (II- IV) were subjected to electroconvulsions. Animals in group II were sacrificed immediately after the application of shock (20-60 seconds) to obtain the activities of the enzymes in the preconvulsive phase. The rats in group III were sacrificed when they went into the tonic phase of convulsions which was approximately 90 seconds after the application of shocks. The animals in group I were sacrificed along with the animals in group III. The animals in group IV were sacrificed after the cessation of the convulsions, i.e 2 minutes after application of shock to obtain values during the post-convulsive phase. The animals were sacrificed by decapitation and the brains were removed and placed immediately in ice cold saline. The cerebral cortex, cerebellum and brainstem were separated as per the procedure described by Sadasivudu and Lajtha (1970). The tissues were homogenized within 2-3 minutes in the media using a Potter-Elvehgem type homogenizer with Teflon pestle for the estimation of the enzymes.

The activity of GAD was done by the method of Stylinsky et al (1975). The content of GABA-T in the homogenate obtained from three regions of brain was determined by using paper chromatography (Sadasivudu, et al. 1978). The amino acid was diluted in 7.5% alcohol (with 0.05% $CuSO_4$) and the optical activity was measured at 515 nm. The activity was expressed as µmole GABA formed/g wet weight of tissue/hr.

Statistics

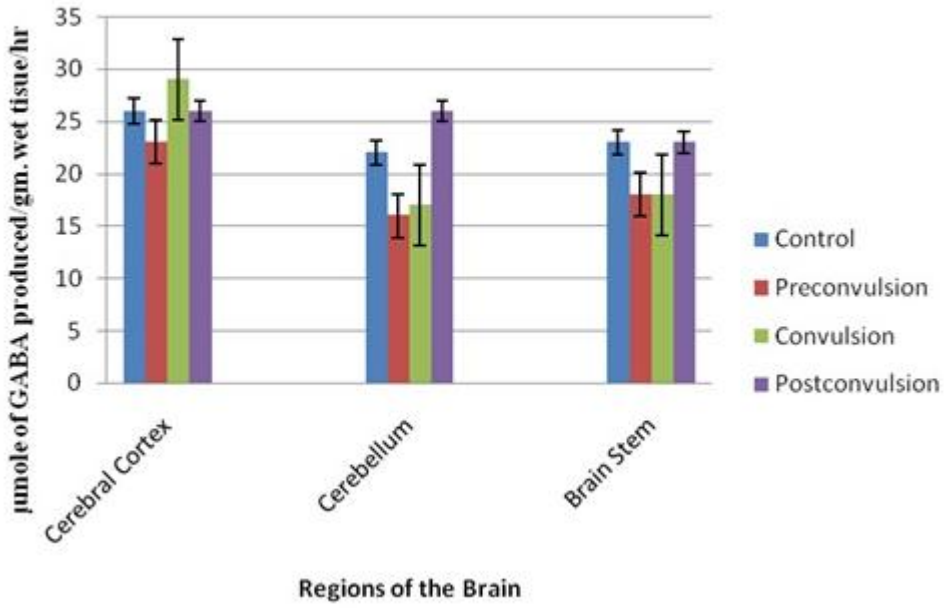
The statistical evaluation was done by one way Anova test. $p < 0.05$ was considered significant.

Results

The activity of GAD decreases significantly ($p<0.001$) (Figure 1) in all the three regions of the brain in the preconvulsive phase 23.7± 0.81 in cerebral cortex, 16.9± 1.28 in cerebrum and 18.6 ± 0.50. In the convulsive phase the activity of this enzyme shows the decreases only in cerebellum (17.9± 0.65, $p<0.001$) and brain stem (18.8± 0.66, $p<0.01$) when compared to control values (23.5± 0.68). A significant increase ($p<0.05$) in activity is observed in postconvulsive phase in cerebral cortex (28.8 ± 1.68) and cerebellum (26.1 ± 1.43). The activity of GAD was measured as µmoles of GABA produced/gm wet weight of tissue/hr.

The activity of γ-Aminobutyric acid transferase **(**GABA-T) decreases significantly ($p<0.001$) (Figure 2) in all the three brain regions in the preconvulsive (17.4± 2.72 in cerebral cortex, 17.7± 1.79 in cerebrum and 22.8 ± 1.93 in the brain stem) and convulsive phase (18.5 ± 1.43 in cerebral cortex, 22.0± 1.28 in cerebrum and 27.4± 4.02 in brain stem) although the activity tended to increase in postconvulsive phase (23.7± 1.42 in cerebral cortex, 26.9 ± 3.0 in cerebrum and 32.6 ± 1.65 in brain stem). The activity was expressed as µmoles of ammonia liberated/gm wet weight of tissue

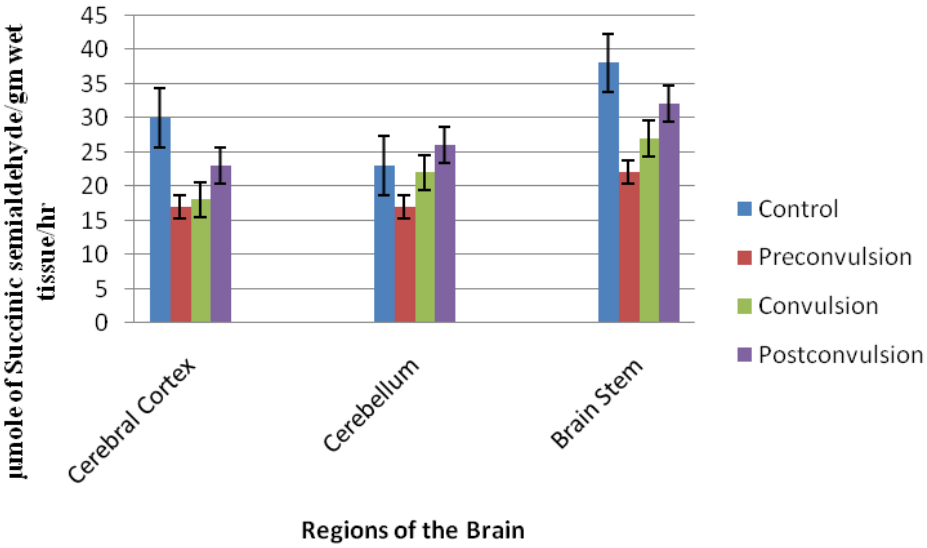
Figure 1: Activity of Glutamate decarboxylase in rat brain in Convulsion by Electroshock



The activity of Glutamate decarboxylase in rat brain in convulsions induced by Electroshock. The animals were sacrificed and the cerebral cortex, cerebellum and brainstem were separated from the brain. The tissue homogenates were used to estimate the enzymes. Group I: served as control for attaining normal values. The remaining three groups (II- IV) were subjected to electroconvulsions. Animals in group II were sacrificed immediately after the application of shock (20-60 seconds) to obtain the activities of the enzymes in the preconvulsive phase. The rats in group III were sacrificed when they went into the tonic phase of convulsions which was approximately 90 seconds after the application of shocks. The animals in group IV were sacrificed after the cessation of the convulsions, i.e 2 minutes after application of shock to obtain values during the post-convulsive phase. The

activity of the glutamate decarboxylase is expressed as µ moles of GABA produced/gm wet weight of tissue/hr.

Figure2 : GABA Aminotransferase acitivity in rat brain in Convulsion by Electroshock



The activity of GABA aminotransferase activity in rat brain in convulsions induced by Electroshock. The animals were sacrificed and the cerebral cortex, cerebellum and brainstem were separated from the brain. The tissue homogenates were used to estimate the enzymes. Group I: served as control for attaining normal values. The remaining three groups (II- IV) were subjected to electroconvulsions. Animals in group II were sacrificed immediately after the application of shock (20-60 seconds) to obtain the activities of the enzymes in the preconvulsive phase. The rats in group III were sacrificed when they went into the tonic phase of convulsions which was approximately 90 seconds after the application of shocks. The animals in group IV were sacrificed after the cessation of the convulsions, i.e 2 minutes after application of shock to obtain values during the post-convulsive phase. The activity of the GABA-T activity is expressed as µ moles of Succinic semialdehyde/gm wet weight of tissue/hr.

Discussion

Glutamate is known to be present in high amounts in brain has been widely studied by a number of worker as regards to its metabolic and physiological role in brain. It is interesting to know that glutamate by itself a neuroexcitatory substance, metabolically give rise to GABA a known inhibitory substance in the brain. The metabolic role of glutamate in brain has been established in that it was shown to serve as an energy source to take part in disposal of ammonia and in protein synthesis.

Seizures or convulsion in experimental animal would occur as a result of overproduction of excitatory neurotransmitters or due to decreased production and liberation of inhibitory neurotransmitters at the various nerve endings within the central nervous system (CNS). It has been stated that 30-40% of synaptic connections in the CNS utilize GABA as their transmitter, and that another 15-20% utilize glutamate as their transmitter. These aminocids therefore constitute the major inhibitory and excitatory transmitters in brain. Glutamate and GABA may have an important role in convulsions although other putative neurotransmitters such as acetylcholine and biogenic amines (Catecholamine and Serotonin) may also have a role in convulsions.

It is only the quantitative changes occurring in the pools of these amino acid present in the synaptosomes will bring about a derangement in the function of the nervous system causing either heightened neuronal activity leading to convulsions or may lead to lower neuronal activity resulting in depression. However changes occurring within the neurotransmitter pool during neuronal or synaptic activity may be low and the measurement of total amount of amino acid in any part of the brain may not reveal the small changes occurring in the quantities of these amino acids occurring at the nerve endings.

Although different convulsants such as drugs or electroshock may cause convulsions by different mechanisms, the basic change is increase neuronal firing as a result of an imbalance in the functional pools of glutamate and GABA

at their respective nerve endings. The processes leading to such an imbalance may begin to occur in the period preceding the convulsions (preconvulsions) and then progressing during convulsions. In states of heightened neuronal activity leading to convulsions, the neurotransmitter pools of glutamate being an excitatory amino acid get depleted (Shank et al 1981). For sustained neuronal activity during convulsion this pool is to be continuously replenished from the various precursors giving rise to glutamate. The enzymes involved in such a process would be glutaminase, transaminases such as aspartate, alanine aminotransferases. The enzymes in GABA production and disposal will be GAD and GABA-T. Changes in the activities of the enzymes present in the nerve endings and related to the production, liberation and disposal of glutamate and GABA in preconvulsive phase may have a casual relationship to convulsions, while the changes in the activities of these enzymes during convulsions may reflect both casual as well as the consequential metabolic changes during increased neuronal activity. Both GAD and GABA-T exhibit same pattern of change in the three regions of brain in all the three phases of convulsion caused by electroshock (Sadasivudu et al. 1977). GAD was found to decrease in preconvulsions rising maximum during convulsions followed once again by a decline during post convulsions tending to the significantly more than the control values. The decrease in the preconvulsive phase may contribute to a fall in the content of GABA tilting the balance of the neuronal activity towards excitation thereby triggering increase neuronal firing. However the increase activity of GAD during convulsions and preconvulsions may contribute to the increase formation of GABA as compensatory process o build up neuronal inhibition and thereby causing termination of convulsions. The decrease activity of GABA-T observed during preconvulsions and its gradual rise during convulsions and postconvulsions although significantly less than the control activity point out that whatever GABA that is available or produce during convulsions would be preserved to built up neuronal inhibition in an attempt to overcome the neuronal excitation and to cause the termination of the convulsions.

Conclusion

The decrease in the activity of glutamate decarboxylase in preconvulsive phase may help in the initiation of convulsions. The subsequent increase in the activities of this enzyme and also the decrease in the activities of GABA-T during the different phases of convulsions may be compensatory in that they help in building up of a state of neuronal inhibition facilitating termination of convulsions.

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