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EFFECT OF UNILATERAL INTRACEREBROVENTRICUAR INJECTION OF KAINIC ACID ON THE DENDRITIC INTERSECTIONS OF CA3 NEURONS OF HIPPOCAMPUS AT THE END OF 04 WEEKS

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ABSTRACT

Temporal lobe epilepsy (TLE), a chronic brain disorder characterized by recurrent seizures with temporal lobe origin is the most frequent type of epilepsy in humans. Mesial temporal sclerosis is seen in TLE, in addition to the complex partial seizures that might spread to cause convulsive seizures (1). Kainate model of temporal lobe epilepsy (TLE) is a well known model known to mimic hippocampal sclerosis, as seen in a patient with temporal lobe epilepsy. In this study, kainic acid was injected into the right lateral ventricle of 04 months Wistar rats to create the model of TLE. At the end of 04 weeks post intracerebroventricular injection of kainic acid, the changes in the number of dendritic intersections were quantified, both in the apical and basal dendrites, for the understanding of the dendritic modifications in TLE. There was a significant decrease seen in the apical dendritic intersections on the ipsilateral side and the basal dendritic intersections showed a decrease both on the ipsilateral and the contralateral sides. The experimental procedures and the results have been discussed.

KEY WORDS

Temporal lobe epilepsy, kainic acid

INTRODUCTION

Epilepsy is a chronic brain disorder, characterized by recurrent seizures and it affects about 1-2% of the population worldwide (2). Epileptic seizures may cause long-lasting structural, physiological and pathomorphological changes in the brain. Of epilepsy, the complex partial seizure having a temporal lobe origin is termed temporal lobe epilepsy (TLE), the most frequent type of epilepsy in human.

Hippocampal sclerosis, characterized by selective loss of CA3 and hilar neurons and gliosis, is the most common neuropathology seen in human TLE. Mossy fiber sprouting (MFS), seen as the axonal sprouting in the axons of dentate granule cells, is frequently reported in animal and human TLE (3-6). Of the MTLE patients, about 70% have hippocampal sclerosis (Ammon's horn sclerosis or mesial temporal sclerosis) including neuronal degeneration, astrogliosis (7) and aberrant mossy fiber sprouting in the dentate gyrus of hippocampus (inner molecular layer).

Among the regions of cornu ammonis (CA), CA3 in particular in the hippocampus is a prime region of the brain that is related to the processes of learning and memory (8-10). This region is the second in the trisynaptic circuit and receives the mossy fiber inputs from the granule cells of the dentate gyrus. The *schaffer collaterals* from here project on to the neurons in the CA1 region. CA3 cells close to the DG project to CA1 located septally and those CA3

cells close to CA1, project more to those cells of CA1 located temporally (11). These cells are the most kainate responsive of the neurons in the brain as any local or distal injection of kainate readily degenerates them (12). CA3 pyramidal neurons are also shown to be highly vulnerable to network hyperactivity and readily degenerate probably due to repeated release of glutamate, which leads to activation of kainate receptors. It is proved that repetitive high frequency stimulation is toxic to the CA3 neurons as it induces a selective loss of pyramidal cells in this region (13). The Dense network of recurrent collateral glutamatergic axons in the CA3 region interconnects the pyramidal neurons and acts as a centre for generation of synchronized activities. These synchronizing activities of the CA3 region get it the name pacemaker of hippocampus in addition to these generated activities being subsequently propagated to the CA1 region and to the other regions of the brain. In humans and various animal species, mossy fiber synaptic regions are enriched with high affinity kainate receptors and initiation of these high affinity receptors has been suggested for the epileptogenic effects of kainate in CA3. These receptors get activated even by a small concentration of kainate capable of crossing the blood-brain barrier in a model created by systemic injection of the neurotoxin (14).

In the present study, post-unilateral intracerebroventricular injection of kainic acid, there was a loss of neurons observed in the CA3 region of the hippocampus along with the loss of cells seen in the dentate hilus and the CA1 regions. The apical dendritic intersections were significantly decreased in the ipsilateral side but there was no significant change on the contralateral side. The basal dendrites showed a significant decrease on the ipsilateral side as well as the contralateral side.

MATERIALS AND METHODS

Male albino rats of Wistar strain aged 4 months were used in the present study. Research was conducted in compliance with policies and principles contained in institutional animal ethical committee guidelines. Approvals from the institutional animal ethics committee (IAEC, MAHE) (IAEC approval number: IAEC/KMC/07/2007-2008) was obtained for all the experiments.

All animals were maintained under 12:12 dark: light environment in institutional animal house. Animals were housed in polypropylene cages with paddy husk as bedding. Animals were given water *ad libitum* and standard pellet (Hindustan lever) as feed.

In this model of temporal lobe epilepsy (15), an insult or injury was created on hippocampus by injecting kainic acid into the right lateral ventricle.

Male Wistar rats of 4 months age were anaesthetized with a cocktail of ketamine (50 mg/ml) and xylazine (4.5 mg/ml) at a dose of 0.70 ml/kg body weight. Anaesthetized rats were fixed in the stereotaxic apparatus, with the incisor bar located 3.7mm below the interaural plane. An incision was made on the scalp in the midline to expose the skull. The injection of kainic acid was done into the right lateral ventricle. Injection into the right ventricle made it the ipsilateral side and left side was the contralateral side. The location for the burr hole for the injection of kainic acid was marked with the following coordinates: Anteroposterior: 3.7 mm behind the bregma; 4.1 mm lateral to mid line. A burr hole was drilled into the located point. After drilling the burr hole, a Hamilton syringe needle filled with kainic acid (0.5 µg/µl) was lowered by 4.5mm from the surface to reach the lateral ventricle. One µl of kainic acid was injected slowly over a period of 20 minutes. Once the kainic acid was injected completely, the

needle of the Hamilton syringe was removed slowly. After withdrawing the needle, the skin was sutured and the animal was kept warm until it recovered from anesthesia. Lesioned animals were housed and maintained individually until they were sacrificed for further experimental procedures at specific durations.

Rats were anaesthetized with anesthetic ether and brain was removed by rapid dissection and fixed in Golgi fixative.

Preparation of Golgi fixative:

Stock solutions:

Potassium dichromate – 5% solution in distilled water

Mercuric chloride – 5% solution in distilled water

Potassium chromate – 5% solution in distilled water

Solutions:

Solution A – 5 volumes of potassium dichromate

+

5 volumes of mercuric chloride

Solution B – 4 volumes of potassium chromate

+

10 volumes of distilled water

Stain:

Solution A was slowly poured into solution B while slowly stirring the solution B continuously. A dark reddish/yellow precipitate (mercuric chloride) is formed. Then the mixture was allowed undisturbed for approximately more than 2 hours until no more precipitate is formed. Now this final solution is filtered using a Whatmann filter paper and the solution obtained after filtration is used for staining brains. The brains dissected are immediately immersed into this Golgi fixative and left undisturbed at room temperature in dark for 2 days. After two days, the fixative was changed with freshly prepared fixative. Now the brains were left undisturbed for a period of 6 to 10 weeks before taking sections.

These brains were then taken out and dehydrated using different concentrations of ethanol as follows:

50% ethanol: 1 hour

70% ethanol:1 hour

95% ethanol: 2 hours

100% ethanol: 1 hour

After the above-mentioned procedures, the brains were taken out and 120µ thick sections were taken using a base sledge microtome. These sections were then mounted on slides using DPX.

The dark and consistent impregnation throughout the extent of dendrites, and relative isolation from the neighboring impregnated cells were the criteria for the selection of neurons.

Golgi stained neurons from CA3 region were traced using camera lucida. Dendritic intersections were quantified by concentric circle method of Sholl (16). Using camera lucida, circles with radius of 20µ (using stage micrometer) were drawn on a transparent sheet. This sheet was placed over the camera lucida drawings of neurons and the dendritic intersections were quantified at 20, 40, 60, 80, 100 µ distances from the soma. These individual numbers were then tabulated and used for analysis.

RESULTS

The quantification of dendritic intersections was done at different distances starting from 20 to 100 µ using the concentric method of Scholl. Here the CA3 neurons of both the sides were analysed for the results.

Ipsilateral apical dendrites: There was a significant ($p<0.001$) reduction in the number of intersections at 100. The normal control had 6.02 whereas the experimental group had 2.75 dendritic intersections. At 80, the number of intersections in normal control was 4.67 and the experimental group was 3.96, which had a significant ($p<0.01$) level of reduction in the

number of branching points. At other distances, there was no significant change in the number of intersections between the normal control and the experimental groups.

Contralateral apical dendrites: On the contralateral side, there was no significant change in the number of intersections between the normal control and the experimental groups.

Ipsilateral basal dendrites: There was a significant ($p<0.001$) reduction in the number of intersections at distances 60, 80, 100. The normal control had 7.71, 6.25, 4.52, whereas the experimental group had 5.42, 4.79, 2.88 dendritic intersections respectively. At 40, the number of intersection in normal control was 6.73 and the experimental group was 5.54, which had a significant ($p<0.01$) level of reduction in the number of dendritic intersections. At other distances, there was no significant change in the number of intersections between the normal control and the experimental groups.

Contralateral basal dendrites: There was a significant ($p<0.001$) reduction in the number of intersections at distance 80. The normal control had 6.38, whereas the experimental group had 5.38 dendritic intersections. At 100, the number of intersections in normal control was 4.69 and the experimental group was 3.92, which had a significant ($p<0.01$) level of reduction in the number of branching points. At other distances, there was no significant change in the number of intersections between the normal control and the experimental groups.

TABLES:

GROUPS	SIDE	Distance from soma (microns µ)				
		20	40	60	80	100
NORMAL CONTROL	IPSILATERAL	1.29±0.06	2.42±0.19	4.08±0.23	4.67±0.31	6.02±0.70
	CONTRALATERAL	1.33±0.06	2.77±0.15	4.23±0.18	4.85±0.12	6.04±0.51
SHAM CONTROL	IPSILATERAL	1.45±0.10	2.44±0.47	4.44±0.53	5.94±0.13	6.73±0.30
	CONTRALATERAL	1.35±0.09	2.44±0.39	4.19±0.45	5.73±0.20	6.38±0.54
EXPERIMENTAL GROUP	IPSILATERAL	1.30±0.13	2.21±0.41	3.58±0.22	**3.96±0.46****	**2.75±0.40*****
	CONTRALATERAL	1.48±0.09	3.00±0.25	4.69±0.50	5.31±0.25	5.63±0.92

Table 1. Number of apical dendritic intersections of the hippocampal CA3 neurons at various distances from the soma in normal control sham control and experimental groups, 04 weeks post intracerebroventricular injection of kainic acid

Values are expressed as Mean ± S.D. n = 06 in all groups.
*** Statistical values shown for Normal control compared with experimental group.**
(* = p<0.05, ** = p<0.01, *** = p<0.001)

GROUPS	SIDE	Distance from soma (microns μ)				
		20	40	60	80	100
NORMAL CONTROL	IPSILATERAL SIDE	3.77±0.22	6.73±1.09	7.71±0.47	6.25±0.45	4.52±0.39
	CONTRALATERAL SIDE	3.68±0.41	6.79±0.45	7.42±0.36	6.38±0.19	4.69±0.31
SHAM CONTROL	IPSILATERAL SIDE	3.54±0.35	6.48±0.80	7.23±0.33	5.70±0.49	4.23±0.65
	CONTRALATERAL SIDE	3.60±0.41	6.17±0.46	7.33±0.19	5.98±0.57	4.52±0.38
EXPERIMENTAL GROUP	IPSILATERAL SIDE	3.15±0.28	**5.54±0.30****	**5.42±0.38*****	**4.79±0.68*****	**2.88±0.62*****
	CONTRALATERAL SIDE	3.42±0.20	6.48±0.28	**6.71±0.28***	**5.38±0.37*****	**3.92±0.61****

Table 2. Number of basal dendritic intersections of the hippocampal CA3 neurons at various distances from the soma in normal control, sham control and experimental groups, 04 weeks post intracerebroventricular injection of kainic acid.

Values are expressed as Mean ± S.D. n = 06 in all groups.
*** Statistical values shown for Normal control compared with experimental group.**
(* = p<0.05, ** = p<0.01, *** = p<0.001)

Figure – 1 :Normal control: ipsilateral and contralateral side

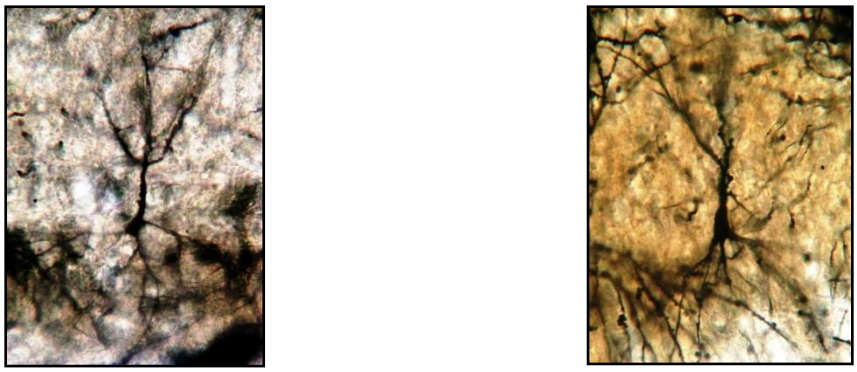

Figure – 2: Experimental group: ipsilateral and contralateral

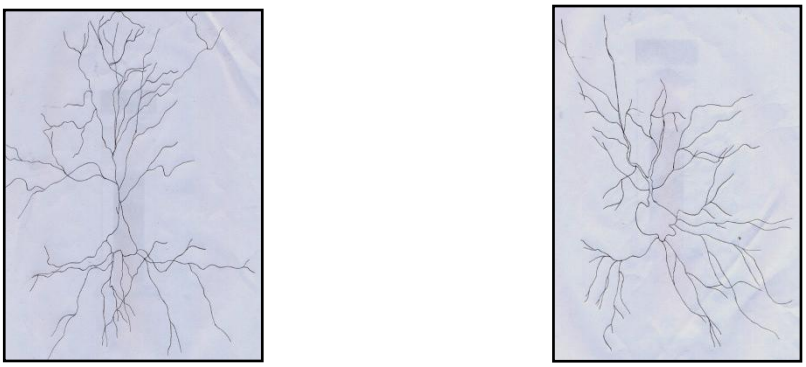

Figure – 3: Normal control: ipsilateral and contralateral side

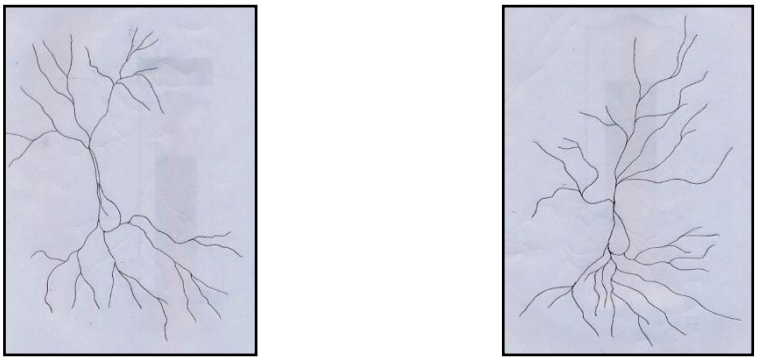

Figure – 4: Experimental group: ipsilateral and contralateral side

DISCUSSION

In chronic models of epilepsy, stimuli produced by the use of agonists at glutamate receptors are known to induce changes resulting in an epileptic brain (17, 18). KA, an agonist of glutamate has been well studied in particular. During the period, post injection of kainate, the sclerotic death undergone by the principal cells is reported (19). The pathological synchronous discharges have been attributed to the long-term reactive changes in the cell excitability (20, 21), synaptic function (22, 23) and connectivity (24, 25).

The injection of kainic acid leads to the loss of neurons of the hippocampus, particularly in the CA3 and the hilar regions. The surviving neurons undergo modifications in their dendritic morphology to accommodate the afferents from the dentate granule cells, the main afferents for the CA3 cells. Kainic acid also leads to the retraction of dendrites when applied iontophoretically.

The powerful convulsant effect of kainic acid has been known for several decades now (26). Kainic acid, originally was identified by the Japanese, was found to be a constituent of a seaweed Digenea simplex. This was also found to possess more potent excitatory activity than glutamate and this is a rigid analog of glutamate that readily binds with a subset of glutamate receptors. The areas damaged due to the administration of kainic acid include several amygdaloid and thalamic nuclei, insular and sensorimotor cortices, the piriform, entorhinal cortices and especially the hippocampus (27). When kainic acid is administered, damage to the neurons in the CA3, dentate hilus and CA1 regions of the hippocampus is well reported and this damage caused supports the promotion of synaptic reorganization along with mossy fibre sprouting of the dentate granule cells (28). These changes seen in the kainic acid model resemble to that observed in the human hippocampal sclerosis (5, 29).

One of the most extensively studied seizure models of SE is the kainate model. As this model mimics most of the characteristic features of human temporal lobe epilepsy (TLE) (30), it is a regularly used type to induce SE. Kainate when injected spares the glia and the axons passing through the injection site, although it acts as a lesioning agent and kills the cells bodies of the neurons at the site of injection (31-33). Additional sites, distant from the site injection were also found to be damaged and seen in the brains from kainic acid injected animals (17). Thus, it is revealed that the action of kainic acid is by two mechanisms, direct and indirect. Direct is damage to the neurons owing to its excitotoxic effect and the indirect by way of seizure-induced damage away from the injection site.

A change in the internal environment either due to an internal stimulus or an external stimulus is said to alter the neuronal network. The sudden loss of the cells in the hippocampus (especially the CA3 neurons), results in the reduction in the number of these cells present within this region of the hippocampus. The surviving neurons in this region react by reducing their length of dendrites that results in the decrease in the number of dendritic intersections as seen in this study. The possible reason behind this decrease in the number of dendritic intersections could be the direct neurotoxic effect of kainic acid. This change in turn could be a short term change undergone by the neurons which survive the excitotoxic effect of kainic acid. Studies on the dendritic intersections of CA3 neurons immediately and longer durations, post intracerebroventricular injection of kainic acid would throw light on the nature of adaptations. Isolated neurons in a culture environment, if treated with kainic acid could probably provide a better understanding. The reduction in the dendritic intersection could be better explained in this method, if it is due to the direct action of kainic acid or due to the drastic loss of CA3 neurons that result in the change in the internal network.

CONCLUSION

The neuroexcitotoxic effect of kainic acid is dedicated to its agonist nature to glutamate and its efficiency in the increase of the glutamatergic response when injected. This action of kainic acid has lead to its extensive use in the temporal lobe epilepsy models. Injection of kainic acid

results in hippocampal sclerosis and this mimics the loss of cells in the hippocampus, as seen in temporal lobe epilepsy patients. The reduction in the dendritic intersections could possibly be the effect of kainic acid action or an adaptive mechanism involved in the reorganization of the hippocampal circuitry. The sudden loss of cells in the hippocampus changes the normal internal environment and this could be a stimulus in the activation of the repair process of the surviving or damaged neurons. Further studies at shorter durations and longer durations along with the results presented in this study could project some of the important changes in a step by step manner for our understanding.

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