Research Article

Actions of riluzole on GluR5 subunit of kainate receptors in rat migraine model

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ABSTRACT

Introduction: Migraine a major public health problem occurring due to the consequences of serial multi-pathophysiological changes in the trigeminal nerve ganglion leading to imbalance in the excitation and inhibition. The glutamate is the major excitatory neurotransmitter in the central nervous system causing excitotoxicity to the sensory neurons leading to sensitization and nociception. **Materials and Methods:** The present study was done to determine the effects of riluzole on the GluR5 subunits of kainate receptors after inducing migraine. The rats were treated with riluzole (6 mg/kg) after inducing migraine with nitroglycerin 10 mg/kg. **Results and Conclusions:** The nitroglycerin treated rats showed intense staining for GluR5 subunits and there was a decrease in its expression after riluzole treatment. This study concludes that the GluR5 subunits are upregulated during migraine and riluzole can be used to control those upregulation by its neuroprotectant property.

KEY WORDS: GluR5, Glutamate, Headache, Kainate, Migraine, Riluzole

INTRODUCTION

Migraine is a common neurological disorder prevalent among 15% of the population causing disability to the individual and society.[1,2] Migraine involves combination of headache with other sensory disturbances such as hearing and visual modifications or deficit.[3] The main manifestation of migraine is the activation or the perception of activation in the trigeminal afferents mainly ophthalmic division of the trigeminal nerve innervating the dura mater.[4] The activation of afferents leads to vasodilatation of the vessels supplying dura mater producing key transmitters such as calcitonin gene-related peptide and substance P inducing migraine.[5,6] All these pathological changes lead to imbalance in the excitation and inhibition cycle leading to degeneration of neurons.

Glutamate is a major excitatory neurotransmitter in the central nervous system participating in all functions of the nervous system like migration, differentiation, and death. It has been showed in many studies involving neuropathic pain and developing antagonist for pain relief. Glutamate exerts its action by acting on glutaminergic receptors which are of two types ionotropic and metabotropic. Ionotrophic receptors such as N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, and kainate receptors which are ligand-gated ion channels activated by glutamate neurotransmitter. Metabotropic receptors made active by indirect metabotropic process involving G-protein coupled receptors.[7] There are various studies suggesting involvement of glutamate receptors in nociception and its upregulation. Studies had shown the elevated levels of glutamate in spinal cord following inflammation[8] and nerve injury[9] leading to neuropathic pain. Since migraine is caused due to triggering of neurons either in the trigeminal ganglion or trigeminal complex in the brain stem, the glutamatergic system could play a vital role in exciting these neurons.

Riluzole is a neuroprotective drug acts by blocking glutamatergic cell transmission and controls the neuroexcitotoxic damage. It is thought that the neuroprotective action is done by non-competitive blockade of the NMDA receptors and G protein-dependent signal transduction, thus controlling the excitotoxicity.[10] It was also proved that any

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neuropathic pain is due to imbalance between the inhibitory and excitatory synaptic functions followed by any nerve injuries.[11] Hence, this study was done to prove the changes in the kainate receptors and its counterregulatory actions of riluzole on kainate receptors in rat migraine model.

MATERIALS AND METHODS

After approval from the Institute Animal Ethics Committee, the male Wistar rats were obtained from experimental animal lab of Saveetha Medical College and Hospital, Chennai. The rats of 18 numbers weighing from 200 g to 250 g were used and kept in cages with not more than three in single cage. They were maintained at 12 h12 h light/dark cycles with water and food available *ad libitum*.

Methodology

The rats were divided into three groups:

- Group 1 Control rats ($n = 6$) – saline treated rats
- Group 2 Migraine model rats ($n = 6$) – nitroglycerin (10 mg/kg subcutaneous back of neck) induced rat migraine model for 7 days
- Group 3 Riluzole treated rats ($n = 6$) – riluzole (6 mg/kg i.p) treated nitroglycerin induced rat migraine model for 7 days. Riluzole was given 1 h before the administration of nitroglycerin.

Tissue Collection

After 7 days, the rats of experiment the rats were sacrificed and fixed. The fixation was done thoroughly using 4% paraformaldehyde perfused through transcardiac approach for 1 h. Dissection was done to open the skull and brain was lifted to identify trigeminal ganglion. The trigeminal ganglion of all groups was collected separately and labeled then kept for overnight fixation. The tissues were sectioned (40 µ thick) using optimum cutting temperature medium with cryostat and collected in multivial culture plates separately for each group.

Immunohistochemistry

The antibodies for GluR6 subunits were obtained from sigma laboratories and the standard dilution ratio was determined after repeated histochemical localization at various dilution ratios. The free-floating sections of trigeminal ganglion were localized for GluR6 subunits, focused by JENOPTIK ProgRes Capture Pro 2.7 (Germany) captured using ProgRes image capture software.

Measurement of Small Neurons

The neurons in the images of immunostained trigeminal ganglion will be measured for the maximum diameter using Image J software. The neurons of diameter <22 µ[12] were small neurons and the staining pattern of those neurons was studied.

RESULTS

The GluR5 subunits were localized in the cytoplasm of trigeminal ganglion neurons in the control rats [Figure 1] and there was upregulation in migraine model rats, especially in the small neurons [Figure 2]. The GluR5 subunits expression in the small neurons was decreased after treatment of riluzole [Figure 3]. The GluR5 expression was also seen in satellite glial cells surrounding the neurons [Figure 1], but there was no difference in the intensity of expression in all three groups.

The maximum diameters of the neurons were measured and the staining pattern was studied in those neurons of diameter <22 µ. These small neurons expressed coarse granular [Figure 2] stain in Group 2 (migraine model rats) and in other neurons, it was fine thin stain.

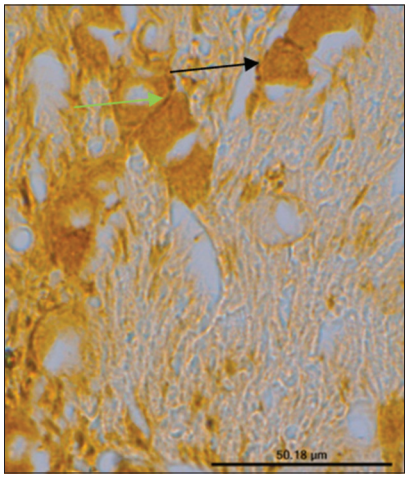

Figure 1: Immunolocalization of GluR5 subunits in the neurons of the trigeminal ganglion of control rats (black arrow) and in the satellite glial cells (green arrow)

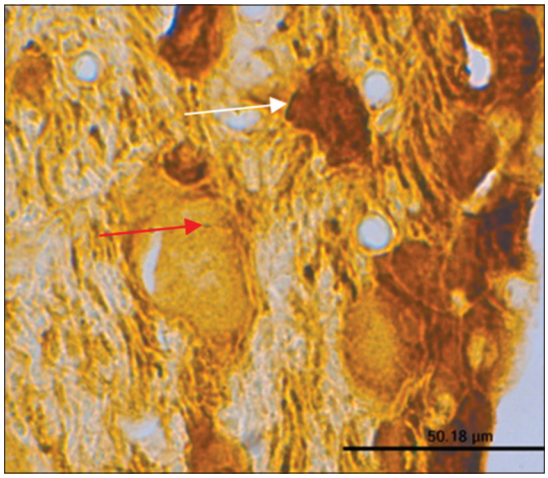

Figure 2: Localization of GluR5 is seen more intense in the small neurons (white arrow) than the large neurons (red arrow) in migraine model rats

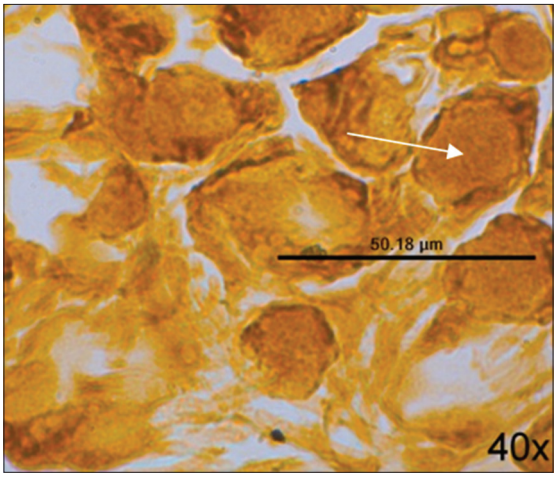

Figure 3: Localization of GluR5 intensity has reduced compared to migraine model rats in the small neurons (white arrow)

DISCUSSION

In the present study, the GluR6 was localized in the small neurons of the trigeminal ganglion which are mainly concerned with nociception. The kainate system is seemed to be selective for slowly conducting C-fibers as they depolarize them early. This can be confirmed by the results which showed kainite selectively depolarized neurons of size small and intermediate type innervated by Aδ and C-fibers in the dorsal root ganglion. The northern blot test showed there were all types of kainite receptors in the dorsal root ganglion, but the GluR5 predominated. The GluR5 subunits were increased after nitroglycerin treatment, especially in the small neurons. These results provide evidence that the glutamate receptors are expressed in the sensory ganglion innervating head-face and get upregulated during orofacial pain processing.[13] Activation of GluR5 receptors leads to rapid influx of calcium increasing intracellular calcium levels inside the pseudounipolar neurons causing primary afferent depolarization and release of neuropeptides.[14]

Peripheral stimulus leads to activation of GluR5 receptors which causes a rapid influx of calcium inside the neurons. Followed by activation of protein kinases and transcription factors leading to changes in the membrane excitability of dendrites and alteration in cytoskeletal architecture of neurons. Until the calcium influx is prevented this process is about to continue leading to excitotoxicity.[15]

In this study, after treatment with riluzole, there is decrease in expression of GluR5 subunits compared to migraine induced rats. Riluzole a neuroprotectant and with antiglutaminergic activity modulates the neurons and protects from excitotoxicity. Administration of riluzole increases the uptake of glutamate by increasing the glutamate transporters and reduces the neuropathic pain. Its antiglutaminergic action is exerted mainly by blocking the sodium channels by inhibiting the alpha activity and stabilizing the voltage-gated calcium channels[16] and also by blocking the postsynaptic glutamate receptor without a direct receptor interaction.[17] Studies have also shown that riluzole decreases the molecular markers of injury such as reactive oxygen species and immune cells reducing the inflammatory process thus having neuroprotection and modulatory actions.[18]

CONCLUSIONS

This study concludes that the neurons and satellite glial cells express GluR5 subunits of Kainate receptors and it gets upregulated during migraine. Riluzole with antiglutamatergic properties reduces the upregulation in the neurons indicating glutamate excitotoxicity the main cause for migraine. Furthermore, riluzole can be used in treatment of migraine due to its neuroprotectant and neuromodulatory actions.

REFERENCES

1. Steiner TJ, Scher AI, Stewart WF, Kolodner K, Liberman J, Lipton RB, *et al*. The prevalence and disability burden of adult migraine in england and their relationships to age, gender and ethnicity. Cephalalgia 2003;23:519-27.
2. Lipton RB, Bigal ME, Diamond M, Freitag F, Reed ML, Stewart WF, *et al*. Migraine prevalence, disease burden, and the need for preventive therapy. Neurology 2007;68:343-9.
3. Goadsby PJ, Lipton RB, Ferrari MD. Migraine current understanding and treatment. N Engl J Med 2002;346:257-70.
4. Goadsby PJ. Migraine pathophysiology. Headache 2005;45 Suppl 1:S14-24.
5. Akerman S, Kaube H, Goadsby PJ. Vanilloid receptor 1 (VR1) evoked CGRP release plays a minor role in causing dural vessel dilation via the trigeminovascular system. Cephalalgia 2002;22:572.
6. Akerman S, Williamson DJ, Kaube H, Goadsby PJ. Nitric oxide synthase inhibitors can antagonize neurogenic and calcitonin gene-related peptide induced dilation of dural meningeal vessels. Br J Pharmacol 2002;137:62-8.
7. Bonsi P, Cuomo D, De Persis C, Centonze D, Bernardi G, Calabresi P, *et al*. Modulatory action of metabotropic glutamate receptor (mGluR) 5 on mGluR1 function in striatal cholinergic interneurons. Neuropharmacology 2005;49:104-13.
8. Pitcher MH, Ribeiro-da-Silva A, Coderre TJ. Effects of inflammation on the ultrastructural localization of spinal cord dorsal horn group I metabotropic glutamate receptors. J Comp Neurol 2007;505:412-23.
9. Hudson LJ, Bevan S, McNair K, Gentry C, Fox A, Kuhn R, *et al*. Metabotropic glutamate receptor 5 upregulation in A-fibers after spinal nerve injury: 2-methyl-6-(phenylethynyl)-pyridine (MPEP) reverses the induced thermal hyperalgesia. J Neurosci 2002;22:2660-8.
10. Doble A. The pharmacology and mechanism of action of riluzole. Neurology 1996;47:S233-41.
11. Kawasaki Y, Zhang L, Cheng JK, Ji RR. Cytokine mechanisms of central sensitization: Distinct and overlapping role of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in regulating synaptic and neuronal activity in the superficial spinal cord. J Neurosci 2008;28:5189-94.
12. Sankaran PK, Ramar S, Al Dajah SB. Morphological study of

nociceptive neurons in the trigeminal ganglion. Int J Health Rehabil Sci 2017;5:1-4.
13. Dong XD, Mann MK, Kumar U, Svensson P, Arendt-Nielsen L, Hu JW, *et al*. Sex-related differences in NMDA-evoked rat masseter muscle afferent discharge result from estrogen-mediated modulation of peripheral NMDA receptor activity. Neuroscience 2007;146:822-32.
14. Li J, McRoberts JA, Nie J, Ennes HS, Mayer EA. Electrophysiological characterization of N-methyl-D-aspartate receptors in rat dorsal root ganglia neurons. Pain 2004;109:443-52.
15. Lu C, Wang Y, Furukawa K, Fu W, Ouyang X, Mattson MP, *et al*. Evidence that caspase-1 is a negative regulator of AMPA receptor-mediated long-term potentiation at hippocampal synapses. J Neurochem 2006;97:1104-10.
16. Cifra A, Mazzone GL, Nistri A. Riluzole: What it does to spinal and brainstem neurons and how it does it. Neuroscientist 2013;19:137-44.
17. Carlton SM, Hargett GL. Colocalization of metabotropic glutamate receptors in rat dorsal root ganglion cells. J Comp Neurol 2007;501:780-9.
18. Wu Y, Satkunendrarajah K, Teng Y, Chow DS, Buttigieg J, Fehlings MG, *et al*. Delayed post-injury administration of riluzole is neuroprotective in a preclinical rodent model of cervical spinal cord injury. J Neurotrauma 2013;30:441-52.

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