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Effects of Riluzole on Nr2B subunits of NMDA receptors in rat migraine model

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ABSTRACT

Migraine, a major public health problem, occurring due to the consequences of serial multi-pathophysiological changes in the trigeminal nerve ganglion leading to an 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. The present study was done to determine the effects of Riluzole on the Nr2B subunits of NMDA receptors after inducing a migraine. The rats were treated with Riluzole after inducing migraine with nitroglycerin 10mg/kg. The nitroglycerin treated rats showed intense staining for NR2B subunits, and there was a decrease in the expression after Riluzole treatment. This study concludes that the NR2B subunits are upregulated during migraine and Riluzole can be used to control those upregulations by its neuroprotectant property.

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INTRODUCTION

Chronic migraine characterized by moderate to severe episodes of headache very much disabling the normal day to day activities and accounts for 20% of all neurology outpatient consultation (Stone *et al.*, 2010). Migraine is one of the neurological conditions listed by the World Health Organization, with a prevalence of 6-8% in male and 15-25% in female (Pietrobon and Striessnig, 2003). The hypothesis of migraine involves both vascular and neuronal components triggering the migraine attacks through trigeminovasular system, which is present in the brain stem.

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 shown in many studies involving neuropathic pain and developing antagonist for pain relief. Glutamate exerts its action by acting on glutamineric receptors, which are of two types Ionotropic and metabotrophic. Ionotrophic receptors like NMDA, AMPA and kinate receptors which are ligand gated ion channels activated by glutamate neurotransmitter. Metabotrophic receptors like mgluR receptors made active by indirect metabotrophic process involving G-protein coupled receptors (Bonsi *et al.*, 2005). There are various studies suggesting the involvement of glutamate receptors in nociception and its upregulation. Studies had shown the elevated levels of glutamate in the spinal cord following inflammation (Pitcher *et al.*,

2007) and nerve injury (Hudson *et al.*, 2002), 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 glutamanergic system could play a vital role in exciting these neurons.

Riluzole is a neuroprotective drug acts by blocking glutamanergic cell transmission and controls the neuroexcito-toxic damage. It is thought that the neuroprotective action is done by noncompetitive blockade of the NMDA receptors and G protein-dependent signal transduction, thus controlling the excitotoxicity (Doble, 1996). It was also proved that any neuropathic pain is due to an imbalance between the inhibitory and excitatory synaptic functions followed by any nerve injuries (Kawasaki *et al.*, 2008). So this study was done to prove the changes in the NMDA receptors and its counter-regulatory actions of Riluzole on NMDA receptors in a rat migraine model.

MATERIALS AND METHODS

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

Experimental Design

The rats were divided into three groups;

Group 1: Control rats (n=6) – Saline treated rats

Group 2: Migraine model rats (n=6) – nitroglycerin (10mg/kg subcutaneous back of neck) induced rat migraine model for 7 days.

Group 3: Riluzole treated rats (n=6) – Riluzole (6mg/kg i.p) treated nitroglycerin induced rat migraine model for 7 days. Riluzole was given 1 hour prior to the administration of nitroglycerin

Tissue collection

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

Immunohistochemistry

The antibodies for NR2B subunits were obtained from sigma laboratories, and the standard dilution ratio (1:500) was determined after repeated histochemical localization at various dilution ratios. The free-floating sections of trigeminal ganglion were localized for NR2B 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 the immunostained trigeminal ganglion will be measured for the maximum diameter using Image J software. The neurons of diameter less than 22μ (Sankaran *et al.*, 2016) were small neurons, and the staining pattern of those neurons was studied.

RESULTS AND DISCUSSION

The Nr2B subunits was localized in the cytoplasm of trigeminal ganglion neurons in the control rats (Figure 1), and there was up-regulation of Nr2B subunits in migraine model rats especially in the small neurons (Figure 2). The Nr2B subunits expression in the small neurons was decreased after treatment of Riluzole (Figure 3). The Nr2B 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 less than 22μ. These small neurons expressed coarse granular (Figure 2) stain in group 2 (Migraine model rats), and in other neurons, it was a fine thin stain.

Nr2B subunits in small neurons

NMDA receptors gate the ion channels present in the presynaptic and postsynaptic regions also in some extra-synaptic locations. The amount of glutamate present in the synaptic cleft is very important in deciding stimulatory and excitatory transmissions. So the maintenance of glutamate concentrations between the neurons is very important so that excessive activation of glutamate receptors can lead to various pathological conditions (Danbolt, 2001).

The present study demonstrates that the NMDA receptor subunit NR2B were expressed in the trigeminal ganglion neurons. The NR2B subunits were increased after nitroglycerin treatment, especially in the small neurons. These results provide

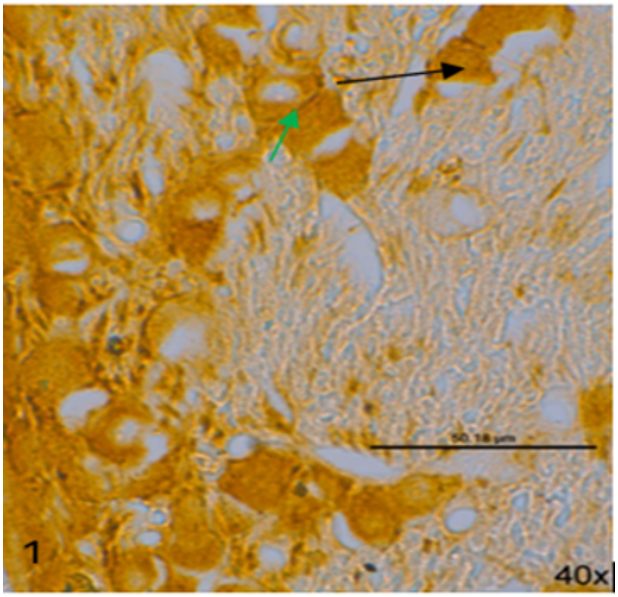

Figure 1: Immunolocalization of NR2B subunits in the neurons of the trigeminal ganglion of control rats (Black arrow) and in the satellite glial cells (green arrow)

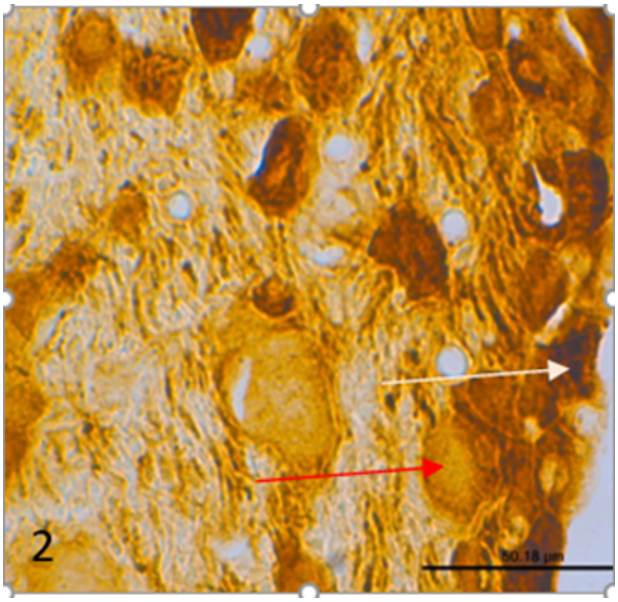

Figure 2: Localization of NR2B is seen more intense in the small neurons (white arrow) than the large neurons (Red arrow) in migraine model rats

evidence that the glutamate receptors are expressed in the sensory ganglion innervating head-face and gets up-regulated during orofacial pain processing (Dong *et al.*, 2007). Another study had shown N2B subunits predominates out of all NMDA receptors in the rat spinal cord and dorsal root ganglion (Hummel *et al.*, 2008). Activation of NMDA receptors leads to a rapid influx of calcium increasing intracellular calcium levels inside the pseu-

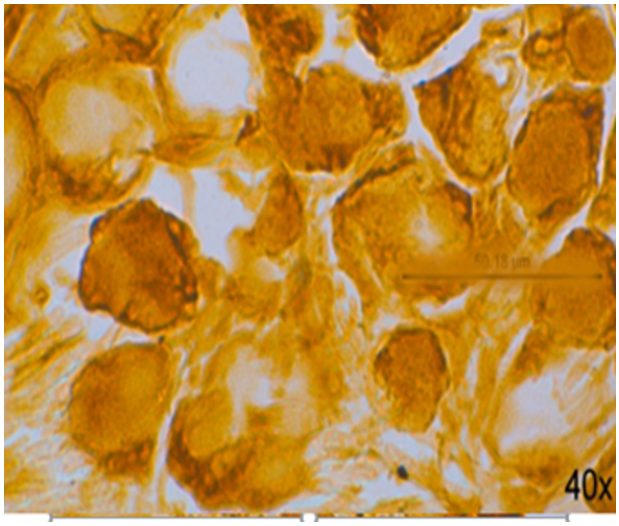

Figure 3: Localization of NR2B intensity has reduced compared to migraine model rats in the small neurons (white arrow)

dounipolar neurons causing primary afferent depolarization and release of neuropeptides (Li *et al.*, 2004).

In the present study, the diameter of the intensely stained neurons in the migraine model rats were measured. The maximum diameter of those neurons were less than 22μ, indicating the NR2B subunits are concerned with nociception. The small neurons are innervated by typr Aδ and type C fibers which receive pain perception. The small neuron, when activated releases CGRP and Substance P, which are the neuropeptides concerned with nociceptive sensitization (Thalakoti *et al.*, 2007).

Mechanism of excitotoxcity

Peripheral stimulus leads to activation of NMDA receptors, especially NR2B subunits, which causes a rapid influx of calcium inside the neurons. Floowed 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 (Lu *et al.*, 2006).

Riluzole – blocking Nr2B subunits

In this study, after treatment with Riluzole, there is a decrease in the expression of NR2B subunits compared to migraine induced rats. Riluzole a neuroprotectant and with antiglutaminergic activity modulate the neurons and protects from excitotoxicity. Administration of Riluzole increases the uptake of glutamate by increasing the glutamate transporters and reduce 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 (Cifra *et al.*, 2013) and also by blocking the postsynaptic glutamate receptor without a direct receptor interaction (Carlton and Hargett, 2007). Studies have also shown that Riluzole decreases the molecular markers of injury like reactive oxygen species and immune cells, reducing the inflammatory process, thus having neuroprotection and modulatory actions (Wu *et al.*, 2013).

CONCLUSION

This study concludes that the neurons and satellite glial cells expresses NR2B subunits of NMDA receptors, and it gets up-regulated during a migraine. Riluzole with anti glutaminergic properties reduces the up-regulation of NR2B subunits in the neurons indicating glutamate excitotoxicity the main cause for migraine. Also, Riluzole can be used in the treatment of migraine due to the neuroprotectant and neuromodulatory actions.

REFERENCES

Bonsi, P., Cuomo, D., Persis, C. D., Centonze, D., Bernardi, G., Calabresi, P., Pisani, A. 2005. Modulatory action of metabotropic glutamate receptor (mGluR) 5 on mGluR1 function in striatal cholinergic interneurons. *Neuropharmacology*, 49:104–113.

Carlton, S. M., Hargett, G. L. 2007. Colocalization of metabotropic glutamate receptors in rat dorsal root ganglion cells. *The Journal of Comparative Neurology*, 501(5):780–789.

Cifra, A., Mazzone, G. L., Nistri, A. 2013. Riluzole: What It Does to Spinal and Brainstem Neurons and How It Does It. *The Neuroscientist*, 19(2):137–144.

Danbolt, N. C. 2001. Glutamate uptake. *Progress in Neurobiology*, 65(1):67–75.

Doble, A. 1996. The pharmacology and mechanism of action of riluzole. *Neurology*, 47(6):233–241. Supplement 4.

Dong, X. D., Mann, M. K., Kumar, U., Svensson, P., Arendt-Nielsen, L., Hu, J. W., Cairns, B. E. 2007. Sex-related differences in NMDA-evoked rat masseter muscle afferent discharge result from estrogen-mediated modulation of peripheral NMDA receptor activity. *Neuroscience*, 146(2):822–832.

Hudson, L. J., Bevan, S., Mcnair, K., Gentry, C., Fox, A., Kuhn, R., Winter, J. 2002. Metabotropic Glutamate Receptor 5 Upregulation in A-Fibers after Spinal Nerve Injury: 2-Methyl-6-(Phenylethynyl)-Pyridine (MPEP) Reverses the Induced Thermal Hyperalgesia. *The Journal of Neuroscience*, 22(7):2660–2668.

Hummel, M., Strassle, B., Miller, S., Kaftan, E., Whiteside, G. 2008. Anatomical localization and expression pattern for the NMDA-2D receptor subunit in a rat model of neuropathic pain. *Neuroscience*, 155(2):492–502.

Kawasaki, Y., Zhang, L., Cheng, J. K., Ji, R. R. 2008. Cytokine Mechanisms of Central Sensitization: Distinct and Overlapping Role of Interleukin-1 , Interleukin-6, and Tumor Necrosis Factor- in Regulating Synaptic and Neuronal Activity in the Superficial Spinal Cord. *Journal of Neuroscience*, 28(20):5189–5194.

Li, J., Mcroberts, J. A., Nie, J., Ennes, H. S., Mayer, E. A. 2004. Electrophysiological characterization of N-methyl-d-aspartate receptors in rat dorsal root ganglia neurons. *Pain*, 109(3):443–452.

Lu, C., Wang, Y., Furukawa, K., Fu, W., Ouyang, X., Mattson, M. P. 2006. Evidence that caspase-1 is a negative regulator of AMPA receptor-mediated long-term potentiation at hippocampal synapses. *Journal of Neurochemistry*, 97(4):1104–1110.

Pietrobon, D., Striessnig, J. 2003. Neurobiology of migraine. *Nature Reviews Neuroscience*, 4(5):386–398.

Pitcher, M. H., Ribeiro-Da-Silva, A., Coderre, T. J. 2007. Effects of inflammation on the ultrastructural localization of spinal cord dorsal horn group I metabotropic glutamate receptors. *The Journal of Comparative Neurology*, 505(4):412–423.

Sankaran, P., Ramar, S., Dajah, S. B. A. 2016. Morphological study of nociceptive neurons in the trigeminal ganglion. *International Journal of Health and Rehabilitation Sciences*, 5(1):1–10.

Stone, J., Carson, A., Duncan, R., Roberts, R., Warlow, C., Hibberd, C., Sharpe, M. 2010. Who is referred to neurology clinics?-The diagnoses made in 3781 new patients. *Clinical Neurology and Neurosurgery*, 112(9):747–751.

Thalakoti, S., Patil, V. V., Damodaram, S., Vause, C. V., Langford, L. E., Freeman, S. E., Durham, P. L. 2007. Neuron?Glia Signaling in Trigeminal Ganglion: Implications for Migraine Pathology. *American Headache Society*, 47(7):1008–1023.

Wu, Y., Satkunendrarajah, K., Teng, Y., Chow, D. S., Buttigieg, L., Fehlings, J. 2013. Delayed Post-Injury Administration of Riluzole Is Neuroprotective in a Preclinical Rodent Model of Cervical Spinal Cord Injury. *Journal of Neurotrauma*, 30(6):441–452.