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AMPA receptor localization in trigeminal ganglion and its upregulation in migraine

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ABSTRACT

Migraine is characterized by headache due to imbalance between excitation and inhibition of neurons disabling normal day to day activities. The excitations of neurons are done by excitatory neurotransmitter glutamate which plays the key role in creating any pathology related to neurons. This study was done to identify GluR1 a subunit of AMPA glutamate receptor in the cells of trigeminal ganglion after inducing migraine and compare it with control rats. The GluR1 subunits were localized in the cytoplasm of neurons, and these subunits were up-regulated following a migraine. The GluR1 was also localized in satellite glial cells and nerve fibers, indicating these subunits expressed in neurons and migrate during nociceptive sensitization. This GluR1 expression in the cells of trigeminal ganglion may be crucial in nociceptive sensitization leading to migraine and other painful conditions like trigeminal neuralgia.

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INTRODUCTION

Glutamate is an excitatory neurotransmitter in the neurons which are responsible for degeneration leading to pathogenesis. Glutamate acts on two kinds of receptors Ionotrophic and Metabotrophic, which have a different pathway of activation. Ionotropic receptors are N-Methyl D-Aspartate (NMDA) receptors, α-Amino-3-hydroxy-5-Methyl-4-isoxazolePropionic Acid (AMPA) and Kainate receptors, which acts through voltage-gated sodium and calcium channels (Bonsi *et al.*, 2005). The metabotropic receptors are of three groups (I, II, III) which consist of mGluR1-8 acts by G protein-coupled receptor mechanism. Blocking these ionotrophic glutamate receptors in the peripheral tissues decreases pain behaviour and nociception in inflammatory models (Julio-Pieper *et al.*, 2011).

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.

Studies have shown that glutamate expression increased in the dorsal root ganglion after periph-

eral nerve injury indicating glutamate involvement in nociception (Kung *et al.*, 2013; Lee and Ro, 2007). Based on those studies, the AMPA receptors acting by voltage-gated calcium channel could play a major role in pathological excitations leading to nociception. The AMPA receptors are of 4 types GluR 1-4 out of which GluR1 is present in all neurons innervated by both myelinated and unmyelinated fibers (Lee *et al.*, 2002). The glutamate receptors from the cell body can be exported to the nerve endings in the skin, muscles and joints (Lee and Ro, 2007) and followed by any inflammation these receptors get sensitized and increase its expression (Du *et al.*, 2006). This study was done to identify the GluR1 receptors in the cells of trigeminal ganglion and its changes after nociceptive stimulation.

METHODOLOGY

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 12 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.

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 multivial culture plates separately for each group.

Immunohistochemistry

The antibodies for GluR1 subunits of AMPA receptors were obtained from sigma laboratories, and the standard dilution ratio (1:250) was determined after repeated histochemical localization at various dilution ratios. The free-floating sections of trigeminal ganglion were localized for GluR1 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 and Ramar, 2017) were small neurons, and the staining pattern of those neurons was studied.

RESULTS AND DISCUSSION

The GluR1 subunits was seen in the cytoplasm of neurons in the trigeminal ganglion (Figure 1). In migraine model rats, there was up-regulation of GluR1 subunits, and there was intense staining (Figure 2). The control rat staining was fine dots, and in migraine model rats, the staining was coarse and granular. The most of the intense staining neurons the maximum diameter was less than 22μ proving most of them are small neurons. The GluR1 was also localized in satellite glial cells and nerve fibers, but there was no change in the expression in the migraine model rats (Figure 1).

Peripheral stimulus leads to activation of AMPA receptors, especially GluR1 subunits, 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 (Lu *et al.*, 2006), causing damage to neurons and creating pathogenesis for various disorders. Previous studies had shown the presence of glutamate receptors in primary afferent neurons, indicating their involvement in the regulation of sensory processing. In this study, it was identified that the trigeminal ganglion neurons express GluR1, and these receptors increase its expression when there is nociceptive stimulation. Since AMPA receptors are ionotrophic glutamate receptors involved in fast glutaminergic transmission (Lu *et al.*, 2002) may be the reason for up-regulation as found in this study. As mentioned earlier, there are four subtypes of AMPA receptors, and all these receptors are studied in the sensory neurons. The small neurons express GluR1, GluR2 and GluR3 and the GluR 2 and 3 expressions were mild compared to GluR1. The large neurons lack the expression of GluR1 subtype proving GluR1 is involved in nociceptive processing in the neurons which is supported by another study done in unmyelinated cutaneous axons for the

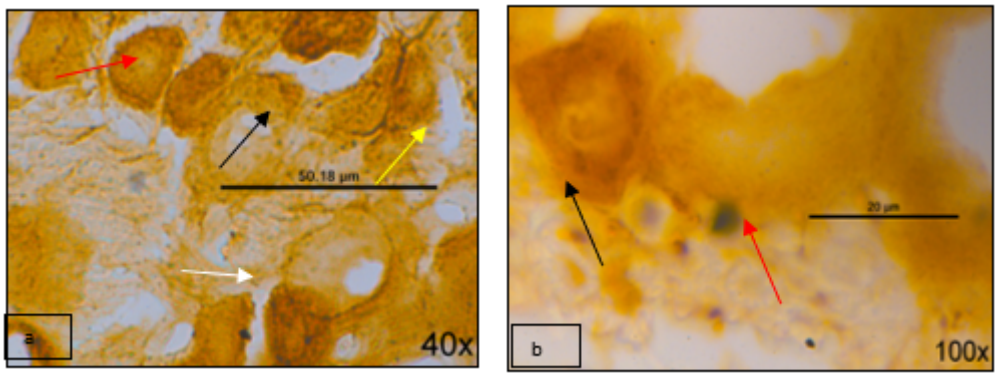

Figure 1: (a & b) : GluR1 localization large neurons (Red arrow), small neurons (Black arraow), satellite glial cell (Yellow arrow) and nerve fibers (White arrow) in control rats

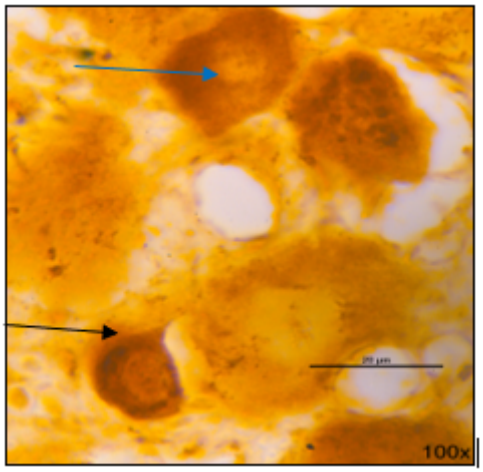

Figure 2: Migraine model rats showing intense localization of GluR1 in cytoplasm of the small neurons (Black arrow) and fine pale staining in large neurons (Blue arrow)

expression of AMPA receptors (Coggeshall and Carlton, 1998).

In the present study, the GluR1 was localized in all type of neurons (Large and Small), but the localization was coarse in small neurons and fine in large neurons. Another study done on various types of glutamate receptors by recording the inward currents flowing through each of them following chronic constrictive injury showed the AMPA receptors are the major channels up-regulated during injury-causing largest inward current flowing proving that the AMPA receptors contribute most significantly to the nociception (Kopach *et al.*, 2011). This regulation can be explained based on calcium permeability through the GluR1during normal and injured state. Uninjured neurons, the GluR1 subunit of AMPA receptors is totally impermeable to calcium ions with little permeability in GluR2 (Isaac *et al.*, 2007). Following any injury, there is a large proportion increase in GluR1 with an increase in permeability to calcium ions increasing plasticity through activation of kinases resulting in early genes expression (Vikman *et al.*, 2008). The more specific activation of AMPA receptors resulted in mechanical allodynia and hyperalgesia and applying antagonist specific for calcium ion permeability reduced pain (Zhou *et al.*, 1996). The specific deletion of GluR1 subunit reduced pain and its sensitization in neuropathic pain models suggesting that GluR1 could exert pro-nociceptive action both in central and peripheral (Gangadharan *et al.*, 2011).

CONCLUSION

This study concludes that the trigeminal ganglion neurons express GluR1 of AMPA receptors and these receptors expression increased following a migraine. This subunit up-regulation may be critical in pathogenesis of migraine since they are the fastest ionotrophic glutamate receptors. The GluR1 subunits were also expressed by satellite glial cells and nerve fibers, concluding they are also involved in the

nociceptive sensitization. By developing antagonist to this subunit the migraine can be treated effectively so that the excitotoxicity can be prevented.

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