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# EXPRESSION OF CALCITONIN GENE RELATED PEPTIDE (CGRP) IN SMALL NEURONS OF TRIGEMINAL GANGLION AND ITS IMPLICATIONS IN MIGRAINE

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## ABSTRACT

CGRP is a neuropeptide present in the central and peripheral nervous system that has diverse functions as primary afferent neurotransmitter which is important in nociception. Migraine is a neurovascular disorder involving trigeminal ganglion characterized by recurrent episodic headache and a rise in levels of CGRP in plasma. In this study expression of CGRP was studied in neurons of trigeminal ganglion in male wistar albino rats. CGRP is expressed in cytoplasm of neurons mainly in the small sized neurons indicating that small sized neurons are involved in nociception.

**KEYWORD:** neuropeptide, migraine, small sized neurons.

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## INTRODUCTION

Neuropeptides like CGRP[1] and substance P[2] are expressed and released by neurons, which mediate or modulate adjacent neuronal communication by acting on the cell surface receptors. Calcitonin gene-related peptide (CGRP) is a widely distributed neuropeptide in central and peripheral nervous system that has diverse functions as a primary afferent neurotransmitter[3]. This neuropeptide (CGRP) is involved in nociception by producing a slow action potential in the dorsal root ganglion neurons[4]. Any inflammation or injury of peripheral tissue will upregulate the production of CGRP in the neurons innervated by type Aδ and type C-fibres indicating CGRP are an important neuropeptide in nociception[5]. The trigeminal ganglion (TG) is located at the base of the brain in the middle cranial fossa, anterior to the superior border of the petrous temporal bone within Meckel's cave. It comprises of satellite glial cells and sensory neurons from the ophthalmic (V1), maxillary (V2), and mandibular (V3) divisions. Ophthalmic division carries sensations from forehead, scalp, upper eyelids, root of nose, eye and conjunctiva. Maxillary division carries sensations from mid-face, lower eyelid, nasal cavity, para nasal air sinuses, upper lip, maxillary teeth and part of external ear. Mandibular division carries sensations from lower face, lower part of posterior scalp, tongue and floor of the mouth, mandibular teeth and part of external ear[6]. Based on size, the pseudounipolar neurons in the trigeminal ganglion can be divided into small(<22µ), medium(22-29µ) and large size(>29µ)[7]. The small sized neurons of trigeminal ganglion are innervated by type Aδ and type C-fibres, which mainly carry nociception from face. Migraine a neurovascular disorder involving meningeal tissues, trigeminal ganglion, and trigeminal nuclei in brain stem, is characterized by recurrent episodic headache with elevated levels of CGRP in plasma during such episodes[8]. Migraine is very common among females as compared to males; nearly 18% of women suffer from migraine[9]. Despite the prevalence, social and economic burden of migraine, the exact pathophysiological mechanisms of migraine involving CGRP are not known. So this study was done to see the expression of CGRP in neurons of the trigeminal ganglion, so that the neurons associated with nociception can be identified.

## MATERIALS AND METHODS

Male albino Wistar rats (n=6) of weight ranging from 200g to 250g were used for immunohistochemical localization of CGRP in the present study. The rats were obtained from the experimental animal facility of All India Institute of Medical Sciences after prior approval of the experimental procedure by Institutional Animal Ethics Committee (IAEC). The animals were kept in cages with not more than three animals in one cage. They were maintained at 12hr: 12hr light/dark cycles with water and food available *ad libitum*.

***Immunohistochemical localization***

Fixation was done using 500ml of 4% paraformaldehyde in 0.1 M phosphate buffered saline, through transcardiac perfusion for a period of 1 hr. Then the skull was cut open and trigeminal ganglion was identified and removed. The ganglion was placed in chuck embedded with OCT medium and sectioned using cryostat (20µm). For each tissue the sections were collected separately in the multivial culture plates and labelled. For free floating immunohistochemical localization the antibodies for **CGRP** was obtained from Sigma laboratories (USA). The standard dilution ratio for CGRP (1:1000) was determined after repeated histochemical localization at various dilution ratios.

***Morphometric analysis of CGRP stained neurons***

The maximum diameter of neurons stained for CGRP is measured using ProgRes image analysis software. The neurons were captured by ProgRes image capture using JENOPTIK ProgRes Capture Pro 2.7 (Germany) in 20 X objective in an E-600 Nikon compound light microscope. Then the staining pattern of CGRP in each sized neurons was studied based on Sankaran et al 2012[7].

## RESULTS

The CGRP is localised in cytoplasm of all sized neurons of trigeminal ganglion(Fig 1). Based on staining patern of neurons, they can be classified into type A – fine, less dense, lightly stained large neurons(Fig 1d blue arrow) and type B - dense, coarse, darkly stained small neurons(Fig 1d yellow arrow). The maximum diameter of coarse, darkly stained CGRP neurons of trigeminal ganglion showed small and medium sized groups. The primary afferent nerve fiber also showed staining for CGRP (Fig 1c white arrow).

**IMMUNOLOCALIZATION OF CGRP**

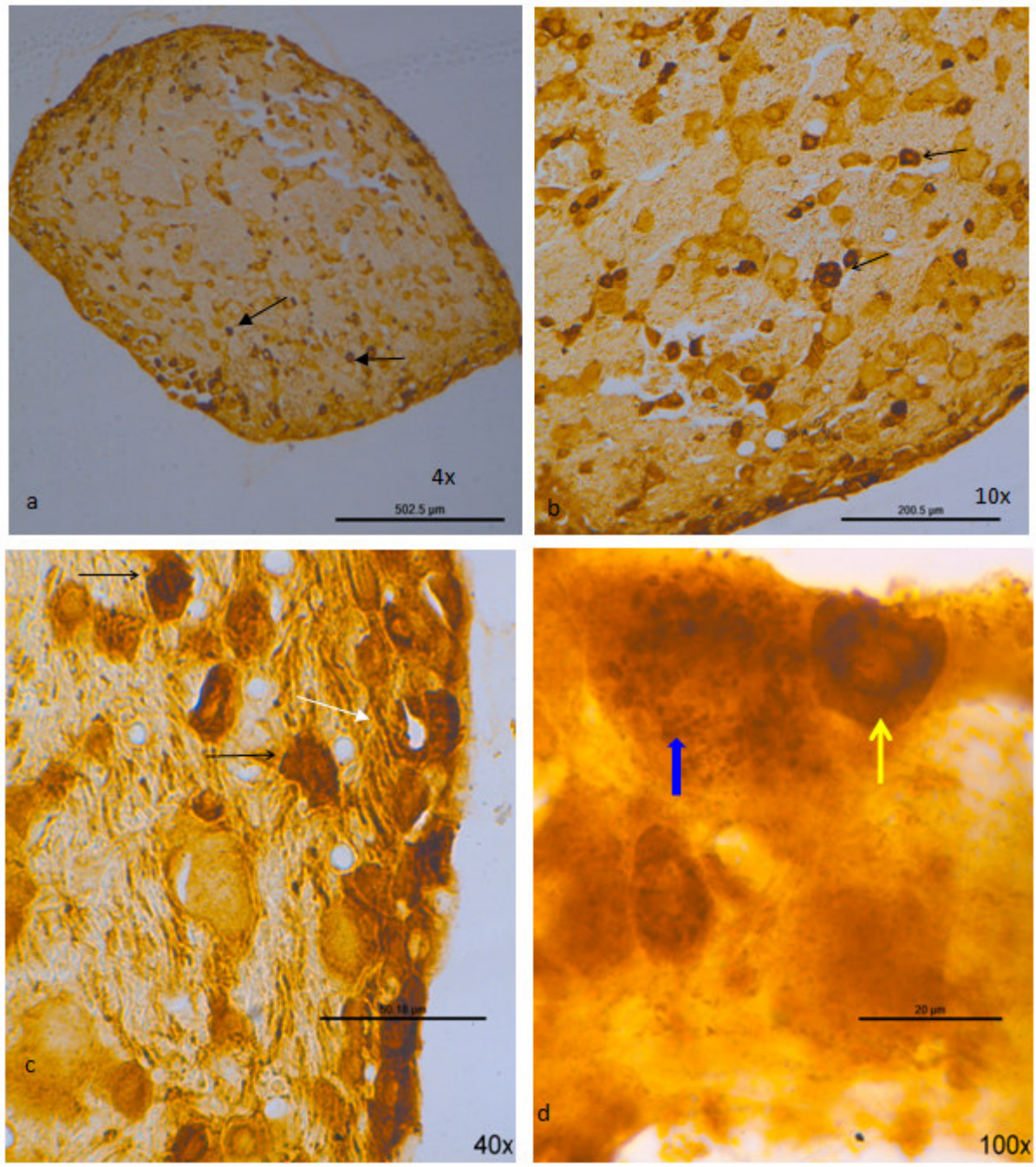


***Figure 1 a, b, c and d***
***Immunolocalization for CGRP in sections of trigeminal ganglion***
***Black arrows: CGRP localization in neurons of TG***
***Blue arrow: CGRP localization in large neuron***
***Yellow arrow: CGRP localization in small neuron***
***White arrow: CGRP localization in afferent nerve fibre***

## DISCUSSION

In the present study there was intense localization of CGRP in the smaller and medium sized neurons and also there was weak localization of CGRP in the large sized neurons. The small sized neurons are innervated by type Aδ and type C-fibres which receive pain perception is carried by the small sized neurons of trigeminal ganglion[5]. Thus calcitonin gene-related peptide appears to have an important role in nociception[6]. The rat and human CGRP gene expression is stimulated in response to cAMP, a secondary messenger generated in response to CGRP receptor activation. Thalakoti *et al* 2007[11] confirmed that activation of trigeminal neurons leads to changes in adjacent glial cells through gap junctions[12] and paracrine signalling by activating one branch of trigeminal nerve resulted in activation other branches[13]. Based on their findings, it is likely that neuronal–glial communication via gap junctions and paracrine signalling i.e., by release of CGRP and substance P are involved in the development of

peripheral sensitization within the trigeminal ganglion and thus, are likely to play an important role in the initiation of migraine. Calcitonin gene-related peptide, which is expressed in most nociceptive neurons in the trigeminal ganglion, is released from the cell body of stimulated neurons and can cause excitation of other neuronal cells, as well as satellite glial cells. Thus CGRP release from neuronal cell bodies would be expected to function as an autocrine signal and potentially increase the synthesis and further release of CGRP. Clinical studies indicate that CGRP is elevated in plasma during migraine episodes[8]. Thalakoti *et al*., 2007[11] also showed that activation of a few neurons within a particular ganglion could release CGRP not only from their cell bodies but also from their processes. In this way, CGRP could function as a paracrine factor to stimulate nearby neuronal and glial cells within the cluster and also cause excitation of more distant neurons and glia located in other clusters, thus propagating an inflammatory signal across the entire ganglion.

## CONCLUSION

This study concludes that pseudounipolar neurons and nerve fibers in trigeminal ganglion express CGRP in the cytoplasm as granules. The small neurons express coarse, darkly stained CGRP indicating that these neurons were mainly concerned with pain sensation.

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