NEURONS CONTRIBUTING SYMPATHETIC FIBERS TO THE HYPOGLOSSAL NERVE IN ALBINO RATS- A HORSERADISH PEROXIDASE (HRP) NEURONS TRACING STUDY

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ABSTRACT

Background: Hypoglossal Nerve (HGN) is known to be a pure motor (General somatic efferent) cranial nerve. **Objective:** To confirm the existence, localization and morphology of the neurons contributing sympathetic fibers to the Hypoglossal nerve (HGN). **Material & Methods:** This experimental animal study, was conducted in Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, from 1992 to 1995. Under general anesthesia the required hypoglossal nerves of twenty four albino rats were exposed and cut in the neck. Horseradish Peroxidase (HRP) crystals were applied to the central cut ends of the nerves and allowed to travel for about 48 hours. After transcardial fixation with 1.25% gluteraldehyde and 1% paraformaldehyde solution, serial sections of superior, middle and inferior cervical ganglia of both sides were made on a freezing microtome, treated with Tetramethylbenzidine (TMB) and counterstained by 1% neutral red. Labeled neurons were observed with the help of light microscope. **Results:** In all animals, the HRP labeled neurons contributing sympathetic fibers to the right and left hypoglossal nerves and their branches were located in the ipsilateral superior cervical ganglia only. Neurons were oval and rounded in shape, and randomly distributed in the ganglia. Average soma diameter of the neurons ranged between 6 to 38 microns. **Conclusion:** Hypoglossal nerve also contains sympathetic fibers which are contributed by ipsilateral superior cervical ganglion cells.

Key words: Hypoglossal nerve, Horseradish peroxidase (HRP), Superior cervical ganglion, sympathetic neurons, Cranial nerves.

INTRODUCTION

Hypoglossal nerve (HGN) is known to be a pure motor (General Somatic Efferent) cranial nerve.[1] HGN also contains sensory fibers which are contributed by dorsal root ganglia of Cervical-1segment mainly and Cervical-2 partly.[2] It was noticed that HGN is joined with superior cervical sympathetic ganglion (SCG).[3] Detailed dissection by Hedger and Webber indicated that although the postganglionic sympathetic fibers have three possible routes of access to the tongue; HGN, ansacervicalis and lingual artery, yet the main route is HGN.[4] Cheng et al investigated the ultrastructural changes in the nervous system following intraneural injection of mercuric chloride into the HGN and observed loss of almost all unmyelinated fibers in the HGN and found mercury deposits in superior cervical ganglion (SCG) giving clue of some relation between HGN and SCG.[5] Inspite of many physiological and morphological investigations of the existence of sympathetic fibers in the HGN, much controversy still exits. Therefore the present study is designed to probe the matter with a more reliable neurons tracing technique using Horseradish Peroxidase (HRP). This study was planed for the confirmation of existence of sympathetic fibers in HGN and Localization and morphology of the neurons contributing sympathetic fibers to the HGN.

MATERIALS AND METHODS

This experimental work was carried out at Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre Karachi, Pakistan during 1992 to 1995. In this study twenty four adult male albino rats were used. These were divided into three equal groups A, B and C based on use of right, left HGNs and their branches respectively. General anesthesia was induced by ether and maintained by intraperitoneal injection of 3.5% chloral hydrate solution in a dose of 300 mg/kg body weight.[6] After exposing by a midline incision in the neck, the required HGNs or their branches were severed and then HRP crystals (Sigma Type-IV, Sigma Chemical Co. St. Louis MO, USA) were applied to the proximal cut ends of the nerves at frequent intervals for a period of one hour. Operated animals were kept alive for 48 hours so that HRP may travel to the perikarya of the respective neurons and thus labeling them. Transcardial perfusion fixation with 1.25% gluteraldehyde and 1% paraformaldehyde solution

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was performed according to protocol-II of Rosene and Mesulam.[7] Superior, middle and inferior cervical ganglia of both sides were removed, stored in 30% sucrose at pH 7.4, cut into serial sections on a freezing microtome (American optical company, model-860) and then treated with Tetramethyl Benzedrine (TMB) for histochemical demonstration of HRP according to Mesulam technique and counter-stained with 1% neutral red.[8] Total number of labeled neurons were counted with the help of light microscope. To measure the somal size all the sections from one animal from each group were projected on a sheet of paper with Leitzmicroprojector and size was measured and averaged according to the method described by Burke et al.[9]

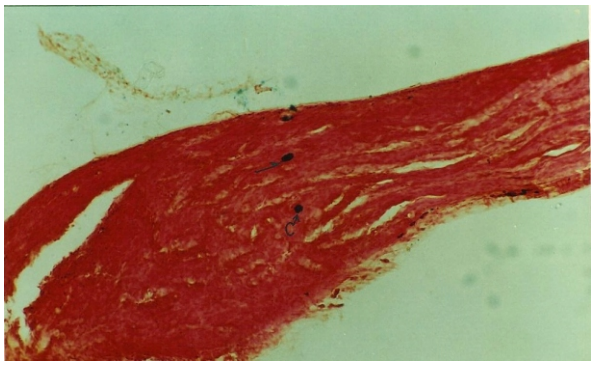
RESULTS

a. Morphology and labeling characteristics

Application of HRP to the central stumps of axontomized HGNs of either side or its branches, and its subsequent retrograde intra-axonal transport in the processes resulted into intense labeling of their perikarya in the respective superior cervical ganglia. Labeled neurons appeared rounded or oval. (Fig-I).

Figure-I:

Photomicrograph of 40 micron thick longitudinal section of left superior cervical sympathetic ganglion showing random distribution of HRP labeled postganglionic sympathetic neurons of oval and rounded (c) shapes following application of HRP to the proximal cut end of medial branch of left hypoglossal nerve. Section counterstained with neutral red. X 100.



b. Distribution and topography of Postganglionic sympathetic (PGS) neurons.

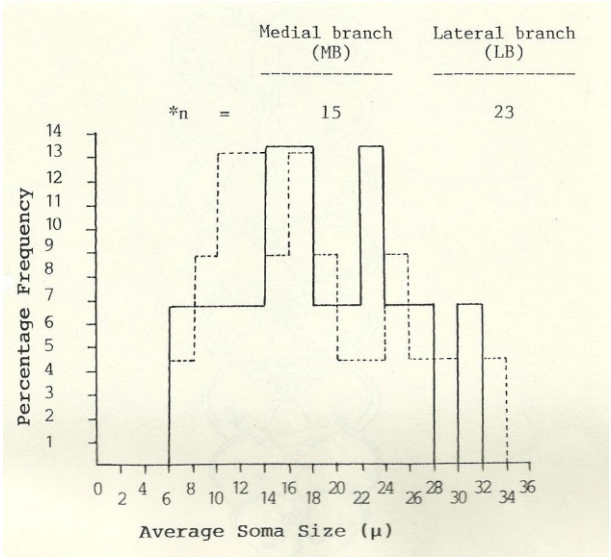
Labeled PGS neurons were distributed only in Superior cervical ganglion (SCG). No labeling was observed in middle or inferior cervical sympathetic ganglia. Neurons were randomly scattered throughout the ganglia showing no grouping or clustering. The number of the labeled PGS neurons was 20.5± 2.11 in right HGN, 19.75±1.24 in left HGN, 11.71±1.04 in medial branch, and 13.75±1.68 in lateral branch of HGNs.

Figure-II:

Histogram showing percentage frequency distribution of various sizes of postganglionic sympathetic (PGS) neurons forming the right and left hypoglossal nerves

Key:

Right hypoglossal nerve (R-HGN) = continuous line
Left hypoglossal nerve (L-HGN) = dashed line
*N = Total number of neurons measured



c. Soma Size Spectrum and frequency distribution

1. Right HGN

Labeled neurons ranged from 7 to 36 µm, whereas the peak frequency distribution was observed between 13 to 28 µm as shown in Figure-II continuous line.

2. Left HGN

Labeled PGS neurons showed diameter measuring between 9 and 38 microns with majority of the somata measuring between 13 and 26 µm. (Figure-II interrupted line)

3. Branches of HGN

Figure-III, continuous and interrupted lines represent the soma size of SCG neurons contributing PGS fibers to medial and lateral branches of HGN respectively.

DISCUSSION

Random distribution of neurons in SCG without definitive groups or clusters which contribute sympathetic fibers to the HGN or its branches,

reported in our investigation is consistent with the conclusion drawn by Tseng and his co-workers who observed random distribution of multipolar neurons in SCG when they used HRP and experimental degeneration technique[10], and Leong et al who reported no distinctive pattern of distribution of sympathetic neurons in stellate and thoracic sympathetic ganglia in monkeys after HRP application to the transected radial and ulnar nerves.[11] Our findings that the labeled sympathetic neurons were found in ipsilateral superior cervical ganglion (SCG) and were oval and rounded in shape is comparable to the results of Fukui et al, and Hayakawa et al.[12,13] Our investigation showed slightly more sympathetic neurons in right SCG. This observation is contrary to the result of Tseng et al who observed more neurons on left side when they used hamsters.[14] This difference may be attributed to the different species used in both studied. Average somal diameter of labeled neurons of SCG giving fibers to the HGN ranged from 6 to 38 μm. Perikarya of neurons of this size are considered to be small to medium sized. This result shows close similarity to the findings of Janjua and Leong[15], and Fukai and his associates. [12] The present investigation revealed that the number of the labeled PGS neurons was 20.5±2.11 in right HGN, 19.75±1.24 in left HGN, 11.71±1.04 in medial branch, and 13.75±1.68 in lateral branch of HGNs. However, on literature search no study is available for comparison.

CONCLUSION

Hypoglossal nerve also contains sympathetic fibers which are contributed by ipsilateral superior cervical ganglion cells.

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