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HYPOGLOSSAL NERVE;

NEURONS CONTRIBUTING THE SENSORY FIBERS IN ALBINO RATS- HORSERADISH PEROXIDASE (HRP) STUDY

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ABSTRACT... rao_sabirdr@yahoo.com **Objective:** To study the neurons contributing sensory fibers to the hypoglossal nerve. **Setting:** Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi. **Period:** From 1992 to 1995. **Study Design:** Experimental animal study. **Material & Methods:** Under general anaesthesia the required hypoglossal nerves of twenty four albino rats were exposed and cut in the neck. Horse radish 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 upper cervical Dorsal Root Ganglia (DRG) were made on a freezing microtome, treated with Tetramethylbenzidine (TMB) and counterstained by 1% neutral red. The number, size and segmental distribution of HRP labeled neurons were observed with the help of light microscope. **Results and Observations:** In most of the animals, the HRP labeled sensory neurons forming the right and the left hypoglossal nerves and their branches were localized ipsilaterally in (Cervical) C1 DRG (more than 90%) whereas in remaining cases labeled sensory neurons were observed in C2 DRG. Size spectrum for sensory neurons of the hypoglossal nerve and its branches ranged from 9 to 52µm, but more than 75% were less than 40 microns. **Conclusion:** Neurons of DRG of C1&2 contribute sensory fibers to the hypoglossal nerve of the same side.

Key words: Hypoglossal Nerve, Horse Radish Peroxidase (HRP), Dorsal Root Ganglia (DRG), Sensory Neurons, Cranial Nerves.

INTRODUCTION

Hypoglossal, the twelfth cranial nerve is generally regarded as a general somatic efferent (pure motor) nerve. Bahar & his assistants described morphologically the hypoglossal dorsal root and its ganglia. Since the histological findings showed that each ganglion placed on the dorsal root of XII nerve had the general features of a spinal ganglion, it can be said that the XII nerve has the

intra-cranial peripheral (sensory) fibers[1]. Stimulation of hypoglossal nerve excites neurons in the trigeminal spinal nucleus, giving clue of presence of afferent fibers that travel within the distal trunk of XII nerve to supply the deep tongue muscles[2,3]. Hypoglossal nerve communicates with vagus, first and second cervical and lingual nerves that may add sensory fibers. Miyoshi and his colleagues gave HRP injection into hypoglossal nerve and observed labeling in the superior ganglia of the glossopharyngeal and vagal nerves ipsilaterally, an evidence of presence of sensory fibers in the hypoglossal nerve[4]. Almost similar results were obtained by Takeuchi and co-workers in Japan[5]. Despite many physiological and morphological investigations of the existence of afferent fibers in the hypoglossal nerve and their source of origin, much controversy still exits. Therefore the present investigation is designed to probe the matter with a reliable neurons tracing technique using Horseradish Peroxidase (HRP).

Purpose of study

Confirmation of existence of afferent fibers and localization and morphology of the neurons contributing sensory fibers to the Hypoglossal nerve.

METHODS AND MATERIALS

In this experimental investigation twenty four adult albino rats were used. These were divided into three equal groups A, B and C based on use of right, left and branches of hypoglossal nerves respectively. General anaesthesia 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 hypoglossal nerves or their branches were cut 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 half an hour. Operated animals were kept alive for a period of 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 was performed according to protocol-II of Rosene and Mesulam[7]. Dorsal Root ganglia (DRG) of C. 1 to 4 (cervical) spinal segments 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 TMB (Tetramethyl Benzedrine) for the 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 a light microscope. To measure the somal size all the sections from one animal of each group were projected on sheet of paper with Leitz microprojector and size was measured and averaged according to method described by Burke et al[9].

RESULTS AND OBSERVATIONS

a. Morphology and labeling characteristics

Application of HRP to the central stump of axotomized hypoglossal nerves of either side or its branches, and its subsequent retrograde intra-axonal transport in the peripheral processes resulted into intense labeling of their perikarya in the respective spinal DRG. In some cases the labeling was so heavy that the HRP-TMB deposits concealed the nuclei. (Fig-1) Labeled sensory perikarya were spheroidal and oval with smooth outline without any clustering. (Fig-2).

b. Segmental distribution

1. Right Hypoglossal Nerve (RHGN).

The sensory somata whose peripheral processes run in the RHN were located in DRG of ipsilateral spinal segments of C1 only in 62.5% cases and C1& C2 in 37.5% animals. Maximum labeling was observed in C1 (92.79) DRG. (Fig-3) Mean number of labeled neurons of DRG supplying the RHN was 35.75 as shown in Table-I.

2. Left Hypoglossal Nerve (LHGN).

HRP labeling was seen in C1& C2 DRG of the same side with 95.54% in C1 DRG. (Fig-3) Mean number of labeled neurons in DRG was 33.63. (Table-I).

3. Medial Branch of Hypoglossal Nerve (MB-HGN)

The DRG cells were distributed in both C1&C2 spinal segments (50%) cases. C1 DRG harbored 90.38% labeled neurons as revealed in fig-3. Maximum number of labeled neurons in one animal were 28. (Table-I).

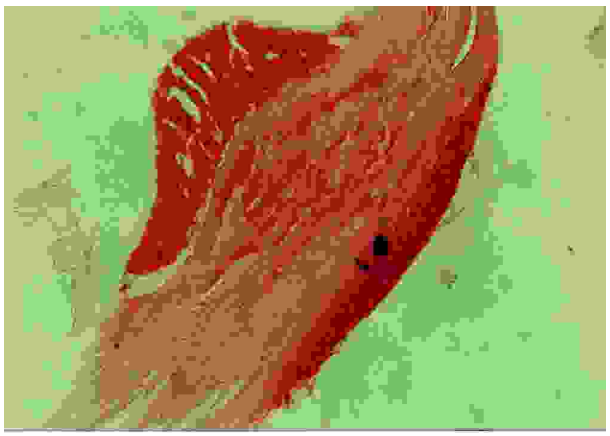


Fig-1. Photomicrograph of 40µm thick longitudinal section of right C2 dorsal root ganglion showing heavily labeled HRP-TMB product in perikaryon which concealed the nucleus following application of the HRP to the transected proximal end of right hypoglossal nerve. Section is counterstained with neutral red. (X 100).

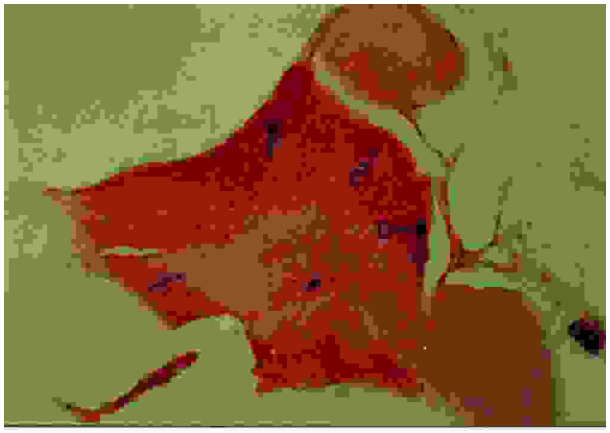
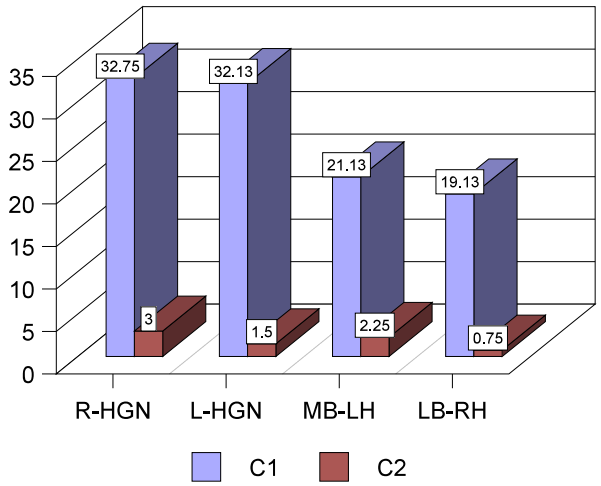


Fig-2. Photomicrograph of 40µm thick longitudinal section of right C1 dorsal root ganglion showing large (↑) and small (√), spherical (♂) and oval (0), HRP-labeled dorsal root ganglion cells following application of HRP to the transected proximal end of right hypoglossal nerve. Section is counterstained with neutral red. (X 100).

Fig-3. Histogram showing mean segmental distribution of HRP-labeled sensory neurons contributing to the hypoglossal nerve of either of the side and its branches in albino rats.



Key:

n* = Total number of animals in each group
R-HGN = Right hypoglossal nerve
L-HGN = Left hypoglossal nerve
MB-LH = Medial branch of left hypoglossal nerve
LB-RHN = Lateral branch of right hypoglossal nerve
⊥ = Standard Error

Table-I. Showing total number of HRP-TMB labeled neurons located in the dorsal root ganglia of either side that contribute fibers to the hypoglossal nerves or their branches.

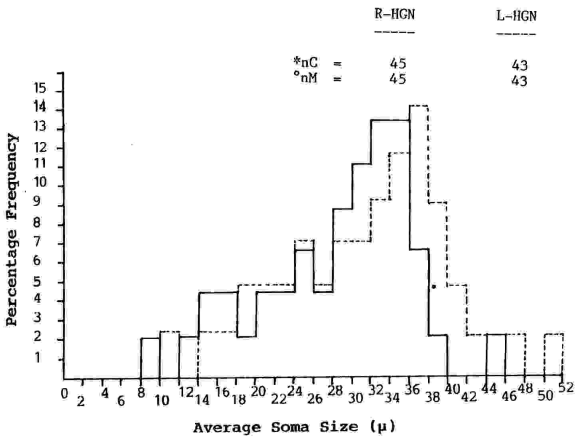
Nerved used	Total No of DRG Neurons	Standard Error
Right Hypoglossal nerve	35.75	2.34
Left Hypoglossal nerve	33.63	2.71
Medial branch of left Hypoglossal nerve	23.38	1.39
Lateral branch of right Hypoglossal nerve	19.88	1.39

4. Lateral Branch of Hypoglossal Nerve (LB-HGN)

Figure-3 shows that DRG cells were distributed in C1 segment up to 96.23%.

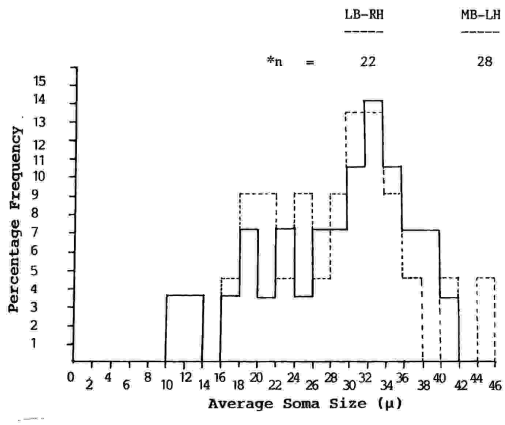
It was observed that neurons contributing to the MB-HGN (54.05%) are more than to the lateral branch (45.95%). (Table-I).

Fig-4. Histogram showing percentage frequency distribution of various sizes of sensory (DRG) cells forming the right and left hypoglossal nerves.



Key:
Right hypoglossal nerve (R-HGN) = Continuous line
Left hypoglossal nerve (L-HGN) = Dashed line
***nC = Total number of neurons counted**
^{0}nM = Total number of neurons measured
DRG= Dorsal root ganglion

Fig-5. Histogram showing percentage frequency distribution of various sizes of sensory (DRG) cells forming the medial and lateral branches of right and left hypoglossal nerves.



Key:
Lateral branch of Right hypoglossal nerve (LB-RHN) = Continuous line
Medial branch of Left hypoglossal nerve (MB-LHN) = Interrupted line
***n = Total number of neurons counted**
DRG = Dorsal root ganglion

c. Topography of DRG cells.

Labeled DRG cells were distributed randomly but more towards periphery of the dorsal root ganglia. (Fig-2).

d. Somal size and Percentage frequency distribution

1. RHN.

The size spectrum of DRG neurons ranged between 9 and 46µm, maximum fall in range 21 to 40µm as shown in Fig 4 continuous line.

2. LHN.

Labeled neurons ranged from 11 to 52µm, whereas the maximum neurons ranged between 19 and 42µm. (Fig 4, interrupted line).

3. Figure-5, continuous and interrupted lines represent the somal size of DRG neurons contributing sensory fibers to MB-HN and LB-HN respectively.

DISCUSSION

Our observation on the intense labeling of the neuronal bodies of ipsilateral DRG with HRP is consistent with the findings of Neuhuber and Niederle[10]. We have reported the random distribution of sensory neurons in the DRG without definite clustering that can be compared with the results of Taylor et al[11]. The present study revealed the HRP labeled neurons in C1 and C2 DRG (more than 90% in C1 DRG). Chibuzo and Cummings found labeled neurons only in DRG of C1 segment, while Nazruddin and his colleagues located the afferent fibers from the hypoglossal nerve of the cat in first three cervical spinal ganglia[12,13]. Anderson and Nishikawa located the cell bodies of hypoglossal afferents in the DRG of third cervical spinal nerve[14].

Partial differences in the segmental distribution of sensory neurons of DRG of cervical spinal nerves may be attributed to the use of different species of animals.

Wild from New Zealand showed labeled neurons in first 4 to 6 cervical DRG bilaterally, which is totally different from all other investigators and thus needs to be reviewed[15]. In addition to motor neurons in the hypoglossal nucleus, a small number of sensory neurons were also found in DRG. This result is comparable to that of Takeuchi and co-workers[16]. The Average somal diameter (ASD) of neurons of DRG whose axons run in the right and left hypoglossal nerves and their medial and lateral branches were 9-46, 11-52, 11-42 and 17-46µm respectively. These observations of the present investigation closely correspond to those reported by Gottschall et all who observed ASD of 15-56µm for the sensory neurons in cervical DRG, and Taylor and colleagues who reported ASD range of 15-50µm[17,18]. Aldes and Boone reported ASD of sensory neurons to be 30-50µm, similar to our observation[19]. Labeling of small sized neurons were also revealed by Sherif and friends[20].

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