THE TERMINATION OF ULNAR AND RADIAL NERVES IN THE CUNEATE AND EXTERNAL CUNEATE NUCLEI. A TRANS GANGLIONIC HORSERADISH PEROXIDASE (HRP) STUDY

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SUMMARY

This study seeks to extend the observations of previous studies of projection of primary afferent fibers from the forelimb nerves and muscles to the cuneate and external cuneate nuclei of mammals by the method of transganglionic transport of neuronal marker, horseradish (HRP). Following application of horseradish peroxidase to the central cut ends of the two main forelimb nerves, namely the ulnar and radial nerves, labelled terminals of the primary afferent fibers in the cuneate nuclei were studied under light microscope. The results show that the terminals of ulnar nerve were mostly located in the medical parts of the cuneate and external cuneate nuclei while the radial nerve terminals were observed in the dorsal and dorsolateral areas of the cuneate and medial part of the external cuneate nucleus.

INTRODUCTION

The dorsal column nuclei (DCN); nucleus gracilis and nucleus cuneatus are unitary structures which have classically been regarded as the first relay in the dorsal column- medical lemniscus (DCML) pathway, which is one of the major channels for somesthetic information to the ventrobasal complex of the thalamus and ultimately to the somatosensory cortex. Previous anatomical studies based on anterograde degenerative technique have demonstrated the distribution of dorsal root fibers and nerves to the cuneate and external cuneate nuclei.[1-8] However, this technique did not successfully demonstrate the central sensory connections of the individual peripheral nerve due to intervention of sensory ganglia.[9]

On the other hand, the electrophysiological studies have demonstrated a musculo-tropic (somatotopic) organization of the neck, trunk, shoulder, arm, forearm and hand muscles within the external cuneate nucleus (ECN) of albino rat.[10]

Our knowledge is deficient with regard to the projections of afferent fibers in different peripheral nerves due to lack of a suitable neuroanatomical method to trace connections across the sensory ganglia but when it was clearly shown that horseradish peroxidase (HRP) is actively transported from muscles or peripheral nerves across the dorsal root ganglion (DRG) and then aterogradely along its central processes within the spinal cord and medulla,[11,12] the transganglionic transport of HRP became a tool for tracing neuronal connections. Recently, with this technique of transganglionic transport of HRP, a number of studies have been carried out to localize in the dorsal column nuclei the specific site of termination of the forelimb nerves in cat,[13-16] muscle nerves in gerbil,[17] cervical dorsal root ganglia and thoracic nerves in rat.[18,19]

The present study was undertaken to ascertain the projection pattern of the primary afferent fibers present in the ulnar and radial nerves to the cuneate nucleus (CN) and external cuneate nucleus (ECN) in albino rat, as to date there appears to be no data available about the mapping of these nerves in this species of mammals.

MATERIAL AND METHODS

In the present study , which was carried out at the Department of Anatomy, M.S.I, J.P.M.C, Karachi, 24 adult male albino rats, 60-150 days old weighing between 150-200 gm were used. In one half the number of animals, the ulnar nerve while in the other half the radial nerve was investigated. All surgical procedures were carried out under general anaesthesia by intraperitoneal administration of 3.5% solution of chloral hydrate in a dose of 300 mg/kg body weight.[20] The ulnar or radial nerve was exposed in midarm or in axilla respectively, identified and carefully dissected free of the surrounding fasciae, muscles, blood vessels and epineurium and sectioned obliquely. The proxineal cut end was held free of the surrounding tissues by grasping the perineural sheath with finely tipped forceps. 2-3% agar-agar solution was poured onto the exposed tissues. After the agar-agar was set, the proxineal cut end of the nerve was allowed to rest on the surface of the set agar-agar and HRP crystals (Sigma type IV, Sigma Chemical Co, St. Louis, MO, USA) were applied to it at frequent intervals for about two hours. At the end of HRP application the agar-agar was removed, the nerve was rinsed with saline, wound closed and covered with a thin film of an antiseptic plastic spray - on dressing.

After survival period of 72-96 hours, the animals were perfused and fixed transcardially under deep anaesthesia according to the procedure II of Rosene and Mesulum.[21] The medulla and spinal ganglia C4-T2 were removed and kept in specimen bottles containing 30% buffered sucrose, pH 7.4 at 4°C for overnight. Serial sections of the medulla in caudorostral directions were cut transversely, while those of dorsal root ganglia longitudinally at 40 um thickness on a freezing microtome. The sections were processed for histochemical demonstration of HRP according to the protocol of Mesulum.[22] The sections were then transferred to gelatinized slide in serial order and counterstained with neutral red. The labelling of the afferent terminals were mapped using a camera lucida attached to a microscope.

RESULTS

Following application of HRP to the proximal cut end of the ulnar and radial nerves, the reaction product was present in the dorsal root ganglia and dorsal column nuclei in medulla oblongata. The segmental distribution of the dorsal root ganglion (DRG) cells forming the ulnar and radial nerves are shown in the table.

Although the neuronal cell bodies in the dorsal root ganglia were very well labelled in all animals, the brainstem varied greatly and in a few cases no labelling could be detected. The labelled terminals were always observed in the ipsilateral cuneate and external cuneate nuclei. No labelling could be detected in the gracile nucleus either ipsilaterally or constralaterally.

TABLE – I

LABELLED DORSAL ROOT GANGLION CELL

Nerve	Labelled DRG
Ulnar Nerve	C7-T2 *
Radial Nerve	C6-T1 **

* In one animal only one HRP labelled cell was found in **C6** DRG.

** In one animal only one HRP labelled cell was found in **C5** DRG.

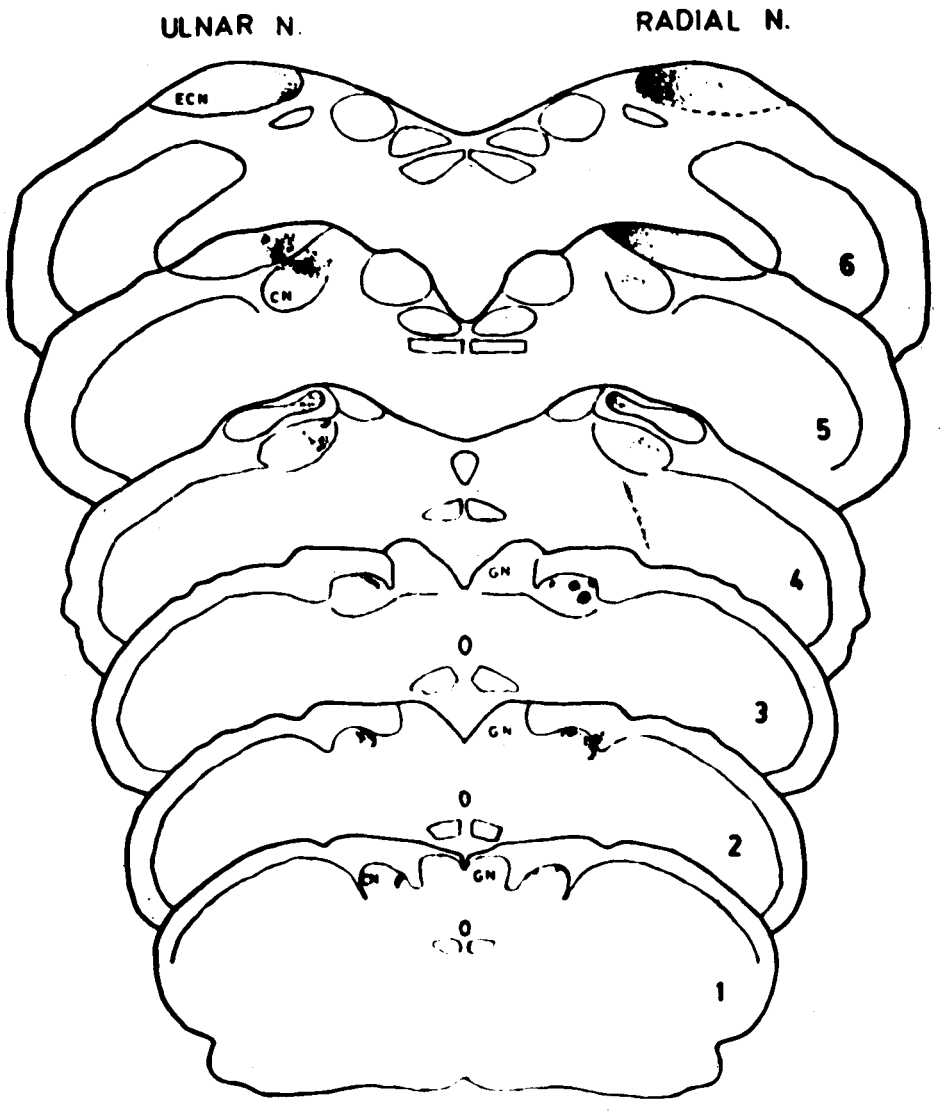

Fig. 1. Photograph of camera lucida drawing of transverse sections through the lower brain stem showing the extent of transganglionic labelling of nerve terminals (dots) following exposure of the ulnar and radial nerves to HRP. Sections 1 to 6 represent progressively more rostral sections. The distance from the obex (= section 4) is 1000 um for section 1 , 680 um for section 2 , 520 um for section 3 , 200 um for section 5 and 440 um for section 6. Key : CN = cuneate nucleus ; ECN = external cuneate nucleus ; GN = gracile nucleus . x 20

Ulnar Nerve (Fig-1).

HRP positive nerve terminals were demonstrable along the whole extent of CN except its most rostral limits. The total rostrocaudal extent of the labelled terminals was 1560 um with 440 um being located rostral and 1120 caudal to obex. Labelling

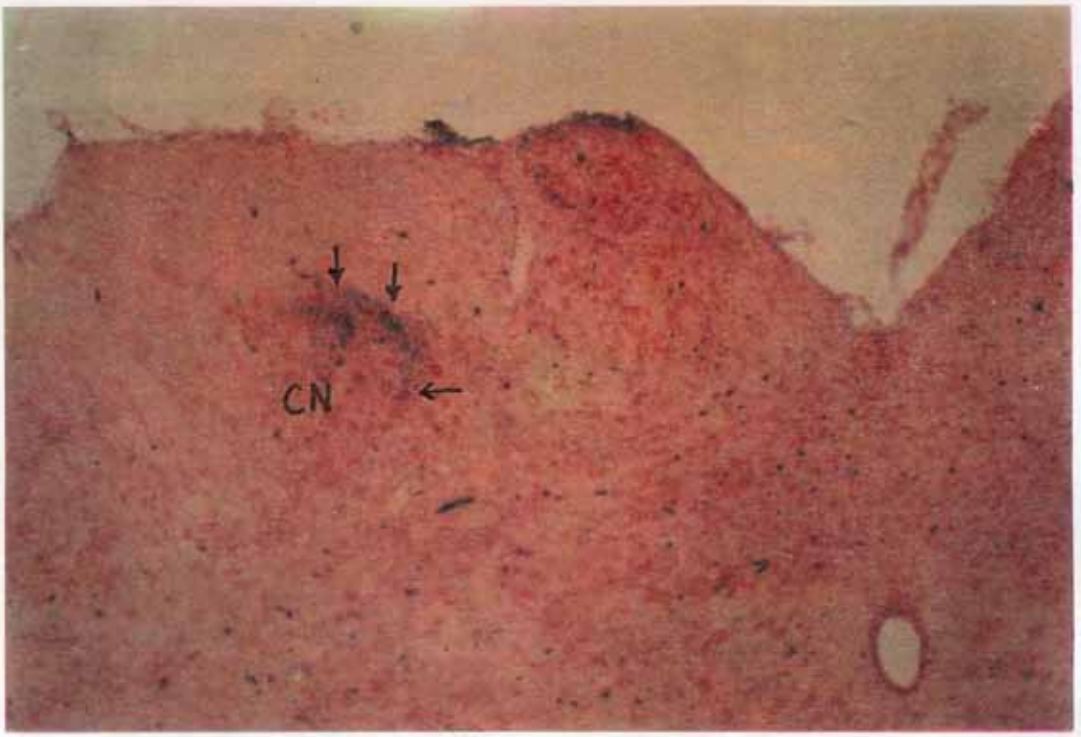

Fig. 2. Photomicrograph of 40 um thick transverse section through medulla oblongata caudal to obex, showing HRP-TMB reaction product demonstrating nerve terminals in the left cuneate nucleus (CN) in its dorsal and medial parts (arrows) following application of HRP to the cut end of left ulnar nerve. Section counterstained with neutral red . x 100

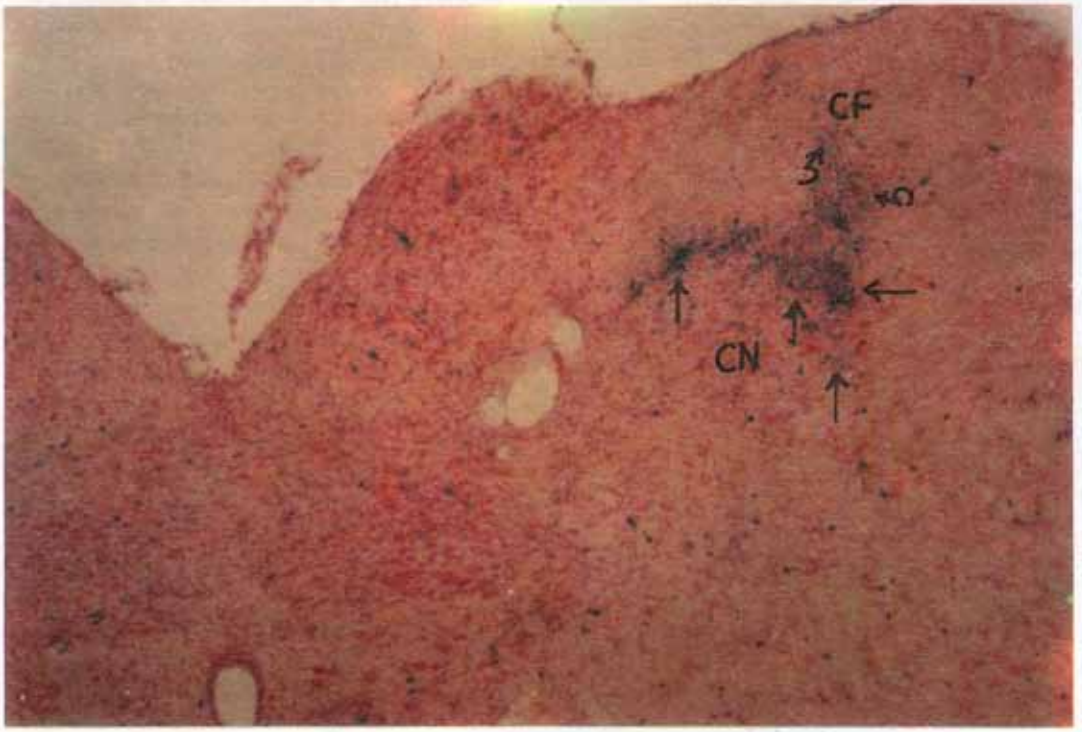

Fig. 3. Photomicrograph of 40 um thick transverse section through medulla oblongata caudal to obex, showing HRP-TMB reaction product demonstrating nerve terminals in the right cuneate nucleus (CN) in its dorsal ventrolateral parts ( ) and sensory fibres ( ) in the cuneate fasciculus (CF). Section counterstained with neutral red. x 100

was quite intense throughout the nucleus except the extreme rostral and caudal parts. At all levels, it terminated mostly in the medial part of the nucleus (Fig-2) ,however, at the level of obex the terminals spread over the whole of the dorsal and ventromedial areas of the CN.

The ECN was also labelled in its medial part.

Radial Nerve (Fig-1).

The afferent terminals of the radial nerve were labelled throughout the extent of CN with the exception of its rostral limits. The total rostrocaudal extent of the labelled terminals was 1720 um with 440 um located rostral and 1280 um caudal to obex. The most intense labelling was detected in sections lying caudal to obex, which showed dense projection in the dorsal half of the nucleus concentrated in the outer marginal zone. The pattern of the terminal labelling form circumscribed oval or circular areas in the dorsocentral, dorsolateral and lateral regions of the CN with sparse distribution in the ventral part of the nucleus (Fig-3). Rostral to obex, a sparse but diffuse projection was observed in the medial and lateral parts of the central region of the nucleus. Incoming labelled dorsal root fibers in fasciculus cuneatus were observed to penetrate the nucleus from its dorsal surface.

Fairly intense labelling was present in the medial part of the ECN. In addition a sparse distribution to dorsal aspect of the nucleus was also evident.

DISCUSSION

The termination of primary afferents of ulnar and radial nerves in the cuneate and external cuneate nuclei was studied by the method of transganglionic transport of HRP. Despite taking rigorous precautions involved in the histochemical procedures of HRP neurohistochemistry, the labelled nerve terminals at the dorsal column nuclei levels in all the animals could not be demonstrated. Leong et al.[23] while exposing the central cut ends of the ulnar and radial nerves to HRP in monkeys observed either scanty or no labelling of nerve terminals at the medullary levels.

The laterality of the nerve terminals in the brain stem was systematically examined as the HRP was applied unilaterally to either ulnar or radial nerve in all animals and studied bilaterally. Labelled terminals were present only ipsilaterally without any labelling on the contralateral side. This conforms to the findings of Nyberb and Blomqvist[14] and Jasmine et al.[13] who observed the contralateral side of medulla always free from peroxidase activity. Rustioni and Macchi[6] utilizing anterograde degenerating method reported a projection to the nucleus reticularis lateralis with sectioning of dorsal roots C7 ,C8 and T1 in cat while the present study could not detect any labelling in the said nucleus in rat which is in conformity with the findings of Jasmine et al[13] who after injecting HRP in C7 ,C8 and T1 dorsal root ganglia did not observe any labelling in the nucleus reticularis lateralis in cat.

The findings of the present study of the ulnar nerve terminations in the medial, ventromedial and dorsal parts of the CN and those of the radial nerve in the dorsal half, ventrolateral and ventral parts of the CN are in agreement with the sum total of the results obtained by Nyberg and Blomqvist[14] and Jasmine et al.[13], who exposed the central cut ends of the cutaneous and muscular branches of the ulnar and radial nerves separately to HRP in the cat. Both these studies reported that the terminals of the ulnar nerve project mainly to the medial part of the CN throughout its extent but also expand over the dorsal part of the nucleus in the region rostral to the obex. These studies also showed that the superficial branch of the radial nerve project to terminate densely in the dorsal part specially in the middle region of the nucleus cuneatus,

while the deep branch projects entirely to its ventral part.

The present study has also demonstrated that the ulnar and radial nerve terminals project to the medial part of the ECN which is in conformity with the findings of the previous studies in cat[13,14] which showed that the cutaneous and muscular branches of ulnar and radial nerve terminate in the medial part of the ECN. Moreover the projection of the ulnar and radial nerves mostly to the medial and slightly to the dorsal part of the ECN could be correlated to the electrophysiological study of Campbell et al.[10] who demonstrated that in the ECN of rat, the arm muscles are represented dorsolaterally in the middle part while the forearm and hand muscles are represented in the most caudo-medical part of the nucleus. The medial projection of the ulnar and radial nerves into the ECN as observed in the present study has also been observed in two very recent studies involving intra neural injection of Ricinus Communis agglutinin (RCA 60) into the radial, ulnar and median nerves of rat[8] and implantation of HRP immersed gelfoam into the forelimb muscles of the gerbil.[17]

The study concludes that the pattern of termination of primary afferents of forelimb nerves in cat, rat and gerbil are almost the same in these species of mammals.

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