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Short Report

Location of the phrenic nucleus in the human spinal cord

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ABSTRACT

Eight normal human spinal cords were studied. Spinal segments were identified and embedded in paraffin wax. Serial cross sections were cut at 25 μm and stained by cresyl violet. Motor columns were reconstructed adapting Elliott's (1942) methods. Motor columns were classified into the medial and lateral divisions and were numbered sequentially from medial to lateral at the level of Cl. In the cervical cord, 8 motor columns were traced. Column 1, corresponding to the medial column, presented 3 subdivisions designated as 1a, 1b and 1c with ventral, dorsal and lateral positions respectively. Columns 1a and 1b extended throughout the cervical region while 1c was confined to 3rd, 4th and 5th cervical segments. At the level of C3, 1c was a discrete column situated lateral to 1a and 1b but at C4 and C5 it became displaced medially close to the medial margin of the ventral horn. In cross section, it presented smaller medial and large lateral part. With the help of clinical and developmental evidence an attempt was made to correlate column 1c with the phrenic nucleus.

Key words: Diaphragm; motoneurons; cervical spinal cord.

INTRODUCTION

In the human cervical spinal cord the phrenic nucleus is an area of considerable interest, but opinions have differed as to its vertical extent and its position within the ventral horn. The most recent report on the human phrenic nucleus by Keswani & Hollinshead (1956) mentioned it as a part of the ventromedial column of the spinal cord between 3rd and 5th cervical segments. In our previous study (Routal & Pal, 1999) an attempt was made to trace the motor columns of the complete human spinal cord. We observed that column 1 corresponded to the medial column. In the cervical cord, column 1 was composed of 3 subdivisions designated 1a, 1b and 1c. The third subdivision, 1c, was identified at the level of C3, C4 and C5. The present study is an attempt to correlate the position of 1c with the phrenic nucleus.

MATERIALS AND METHODS

Eight normal human spinal cords were collected from male cadavers. Cervical spinal segments (C1 to C8) were identified with the help of spinal nerves. Thus a line above the attachment of the 1st ventral rootlet of the 1st cervical nerve is a demarcation line between lower end of the medulla and the cranial end of C1. The caudal end of C1 was determined by a line between the attachment of the lowest ventral rootlet of the 1st cervical nerve and uppermost rootlet of the 2nd cervical nerve. Segments were embedded routinely in paraffin wax; serial cross sections were cut at 25 μm and stained with cresyl violet.

Motor columns were reconstructed adopting Elliott's (1942) method (Routal & Pal, 1999). Columns were numbered sequentially from medial to lateral. Column 1 was allotted to the most medial column at C1, succeeding numbers were allotted to the columns of the lateral division in the order of their appearance in a craniocaudal direction.

RESULTS

Eight motor columns were traced in the cervical spinal cord. They were classified into medial and lateral divisions; column 1 corresponded to the medial column and was the only member of the medial

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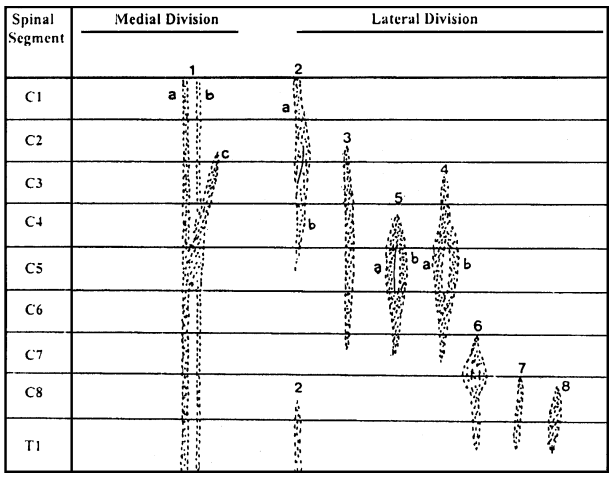

Fig. 1. Segmental extent of motor columns of the human cervical spinal cord.

division. Columns 2 to 8 belonged to the lateral division (Fig. 1).

In the cervical region, column 1 comprised 3 subdivisions, found in ventral, dorsal and lateral positions and were designated as 1a, 1b and 1c respectively. Columns 1a and 1b extended throughout the cervical cord. Column 1c was the shortest, confined to the 3rd, 4th and 5th cervical segments (Fig. 1). At C3, 1c was a discrete column situated lateral to 1a and 1b, overlapping the adjacent margins of these columns at its ventral and dorsal ends (Fig. 2*B*). At the level of C4 and C5 the medial part of this column shifted more medially between columns 1a and 1b and was very close to the medial margin of the ventral horn. Its large lateral part overlapped the lateral margin of 1a and 1b (Fig. 2*B*).

In cross sections 1c was distinct and the largest subgroup at the level of C3. At this level it was situated away from the medial margin of the ventral horn, (Fig. 2*A*). Caudal to C3, 1c extended medially bridging the gap between 1a and 1b. Thus its medial part was wedged between 1a and 1b. The larger lateral part of 1c extended dorsally to the dorsal end of 1b whereas ventrally it extended to the ventral end of 1a.

Extensions of 1c in a ventral, dorsal and medial direction encircled the other 2 subdivisions of column 1 and together they formed a large mass where its small ventromedial area was formed by 1a, the smallest dorsomedial area was formed by 1b. The major bulk of column 1 was formed by 1c (Fig. 2*A*). Caudal to mid C5, 1c reduced in size and in the caudal third of this segment it disappeared completely. At this point column 1 was represented by columns 1a and 1b, where 1a was the prominent subdivision. In the lower cervical segments 1b was represented by very few neurons. Thus in the cervical cord column 1

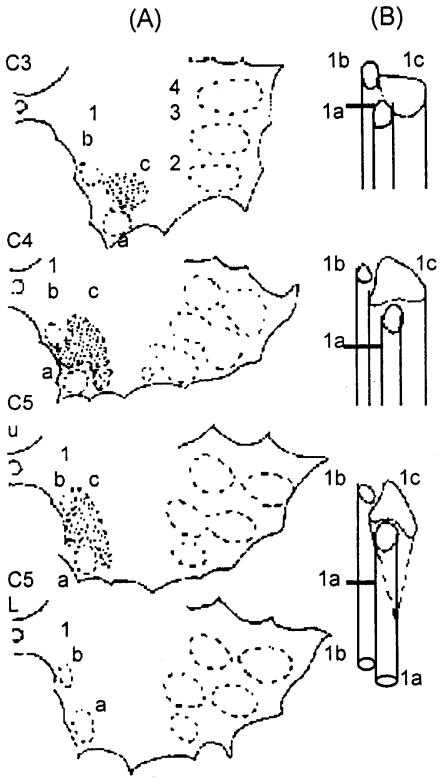

Fig. 2. (*a*) Cross sections at the level of 3rd, 4th and 5th cervical segments depicting subdivisions of group 1. Group 2, 3 and 4 are members of the lateral division. L, lower; U, upper. (*b*) Segmental extent of column 1c and its relation with columns 1a and 1b at above mentioned levels.

presented a considerable enlargement at the level of C3 to C5 and the major bulk of this enlargement was due to subdivision 1c (Figs 1, 2).

DISCUSSION

The structure of the phrenic nucleus has been reported by Hirako (1928) and Greene (1963) in the rat; by Sano (1898), Keswani et al. (1954) and Wilson (1969) in the cat; by Kohnstamm (1898), Marinesco (1898) and Ullah (1978) in the rabbit; and by Bijlani & Kewsani (1961) and Warwick & Mitchell (1956) in the rhesus monkey as a distinct and separate nucleus located between the ventromedial and ventrolateral columns. Table 1 depicts the segmental levels of the

Table 1. *Segmental extent of the phrenic nucleus in different mammals**

Animal	Segmental level	Reference
Albino rat	C 4, 5	Greene (1963)
Cat	C 5, 6	Wilson (1969)
Rabbit	C 4, 5, 6	Ullah (1978)
Rhesus monkey	C 3, 4, 5, 6	Mitchell et al. (1954)

* Adapted from Ullah (1978)

Table 2. *Localisation of the phrenic nucleus in man as reported by different investigators*

	Phrenic nucleus	
Reference	Segmental level	Position in the ventral horn
Kaiser (1891)	C 3, 4, 5	DM and VL
Collins (1894)	C 3, 4, 5	Between VM and N. XI
Sano (1898)	C 3, 4, 5, 6	Between VM and VL
Bruce (1901)	C 3, 4, 5, 6	Middle of the ventral horn
Urechia & Mihalescu (1927)	C 3, 4, 5	Middle of the ventral horn
Kristenson (1934)	C 4, 5	Complete ventral horn
Elliott (1942)	C 4, 5	VM
Keswani & Hollinshead (1956)	C 3, 4, 5	VM

C, cervical; D, dorsal; L, lateral; M, medial; V, ventral; N.XI, spinal nucleus of accessory nerve.

phrenic nucleus in different mammals. Despite variations in its segmental extent the position of the phrenic nucleus within the ventral horn was found to be constant. In all the above mentioned mammals the phrenic nucleus was situated centrally in the ventral horn between the 'back muscle' and 'shoulder muscle' columns, (Sano, 1898; Ullah, 1978).

Bruce (1901) and Urechia & Mihalescu (1927) were of the opinion that in man, the central column of neurons at C3 to C5 is the phrenic nucleus. The account by Williams et al. (1995) of the human phrenic nucleus mentioned that 'The central group, the least extensive, is found only in cervical and lumbosacral segments. In the cervical cord through the 3rd to 7th segments, is a central columnar phrenic nucleus; abundant experimental and clinical evidence shows that its neurons innervate the diaphragm, being probably the least controversial motor pool in the entire cord'. This statement may be true for other mammals but not for the human phrenic nucleus (see below).

Review of literature reveals that there are many controversies regarding the position and segmental extent of the human phrenic nucleus (Table 2).

Sano (1898) described the phrenic nucleus lying between the 'back muscle' group (ventromedial) and the 'shoulder muscle' group (ventrolateral). The nucleus becomes more and more applied to the 'back muscle' group in C4 and becomes more and more dorsal the ventral border of the ventral horn in C5 and C6. According to Sano, while tracing the phrenic nucleus in a craniocaudal direction it shifts from lateral to medial close to the ventromedial group and is also shifted dorsally. Kristenson (1934) found the phrenic nucleus to be a diffuse mass occupying almost the entire grey horn, including the ventral grey commissure. Elliott (1942) assumed that in C3 and C4 the ventromedial group of cells gives rise to the phrenic nucleus.

The findings of Keswani & Hollinshead (1956) seem more reliable as compared with previous reports as most of these were based on a single spinal cord. Keswani & Hollinshead located the phrenic nucleus by retrograde chromatolysis, after unilateral phrenicotomy in 10 patients. The phrenic nucleus was located between C3 and C5, as a part of the ventromedial column. They also observed that the position of the phrenic nucleus was not constant in all segments. At mid C3, the nucleus was found to be in close proximity to the medial margin of the ventral horn where it formed a major part of the ventromedial cell column. At C4 it retained its close relationship to the medial border but extended more dorsally. At mid C5 it was much reduced in size and occupied an area in the middle of the ventromedial column.

In the present study columns 1a and 1b were observed throughout the cervical cord. According to Smith (1983) and Williams et al. (1995) dorsally placed motoneurons of the medial column innervate hypaxial (prevertebral) muscles by ventral rami, while those situated ventrally innervate epaxial (back) muscles through dorsal rami. Hence we assumed that columns 1a and 1b innervate prevertebral and back muscles respectively. The difference in the sizes of 1a and 1b corresponded to the muscle mass they innervate.

Column 1c was the shortest but a prominent subdivision of column 1, located between C3 and C5. Such a large neuronal bulk with a close proximity to neurons innervating axial muscles has tempted us to think about its target area. On the basis of following evidence we assume that 1c could be considered as the phrenic nucleus.

Clinical evidence

With regard to the segmental extent and is position in the ventral horn (as a part of medial column) the description of the phrenic nucleus by Keswani & Hollinshead (1956) is similar to that of column 1c of the present study. However, the position of the phrenic nucleus within medial column, as described by these authors differed from that of column 1c.

Keswani & Hollinshead described the phrenic nucleus as a part of the ventromedial column (1a of

present study) without mentioning its relation to the other subdivisions of the medial column (1b, 1c). This might be due to the fact that the retrograde tracing methods provide an accurate position of labelled cells in the ventral horn but hardly provide any clue for surrounding cells. Keswani & Hollinshead did not study the subdivisions of the medial column in the normal spinal cord for reference. In the absence of the normal pattern of motor columns at that level, it becomes very difficult to determine whether the labelled cells are part of a main column or part of a subdivision of the main column. This may be the reason why Keswani & Hollinshead were unable to trace the lateral subdivision (1c) of the medial column.

Another important factor in the findings of Keswani & Hollinshead was that they were based on longitudinal (frontal) sections. It is quite surprising that they preferred only longitudinal sections. Probably their main aim was to trace the longitudinal extent of the phrenic nucleus. Conventionally, cross sections are preferred for the reconstruction of a nucleus or neuronal column. According to Elliott (1942) 'Cross sections are most suitable to accurate compiling of images. Longitudinal sections are more difficult, since outside a short range the width of the section is bound to vary due to curvature'. It is quite possible that the reconstruction of cross section from longitudinal sections may not give a correct morphology of the medial column and in that case it is difficult to identify the closely situated subdivisions of the medial column. Hence the position of the phrenic nucleus within the medial column may not be correct in the study of Keswani & Hollinshead. As mentioned earlier, they may have failed to notice the 3 subdivisions of the medial column. However, on developmental grounds, their findings seem more accurate as compared with other previous reports where the phrenic nucleus has been described as a separate column situated between the 'back' and 'shoulder muscle' columns (Sano, 1898) or in the central area of the ventral horn (Bruce, 1901; Urechia, 1927).

Developmental evidence

According to Keith (1948), the human diaphragm develops from 3 elements, ventrolateral, central and dorsal. The central tendinous part is derived from the septum transversum. The remaining 2 elements are muscular. The ventrolateral part (sternocostal) is larger and develops from the right and left pleuroperitoneal folds. The dorsal element develops from the subvertebral (hypaxial) musculature (C3, C4, C5 somites). Thus it is very clear that in man the diaphragm is an axial muscle. Hence, its motoneurons will tend to remain close to those innervating other axial muscles and together they form the medial column (column 1). Logically it thus seems that the phrenic nucleus is a part of the medial column (column 1) and this supports the findings of the Keswani & Hollinshead (1956). Hence, in man the phrenic nucleus cannot be a central column (cf. Bruce, 1901; Urechia, 1927; Williams et al. 1995) or a separate nucleus situated between back and shoulder muscle columns (Sano, 1898). Moreover in the present study we did not observe a central column in the cervical region (Fig. 1).

According to the development of the diaphragm, motoneurons innervating its sternocostal and dorsal elements should have specific positions within the phrenic nucleus. The dorsal element develops from the hypaxial muscle mass, hence logically its neurons will tend to lie close to neurons innervating other hypaxial (prevertebral) muscles, i.e. in line with 1b, whereas motoneurons innervating the sternocostal fibres will be lateral to those innervating the dorsal element as the sternocostal part of the diaphragm develops from the body wall. Thus the diaphragm is a composite muscle (derived from different elements) and its motoneurons may group together within the medial column as a separate subdivision, but it cannot be a part of ventromedial column (1a) as mentioned by Keswani & Hollinshead as column 1a projects to back muscles (Smith, 1983).

The developmental aspect of the diaphragm suggests that the phrenic nucleus is part of a column that innervates axial musculature (column 1). Within this column it may have a specific position, i.e. dorsal to motoneurons innervating epaxial or back muscles (1a) and close to those innervating hypaxial or prevertabral muscles (1b). It is quite possible that its medial and lateral motoneurons project to the dorsal and sternocostal fibres respectively.

Column 1c of the present study provides all the above features of the phrenic nucleus. In particular, its segmental extent, being a part of column 1, its relation with columns 1a and 1b and its smaller medial and larger lateral parts strongly support the possibility of column 1c being the phrenic nucleus.

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