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Motoneuron organisation of the muscles of the spinal accessory complex of the sheep investigated with the fluorescent retrograde tracer technique

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ABSTRACT

Retrograde transport of the fluorescent tracers Diamidino Yellow dihydrochloride and Fast Blue was used to determine the location of the spinal nucleus of the accessory nerve in the sheep. We also considered whether in this species the sternocephalic, brachiocephalic, omotransversarius and trapezius muscles, i.e. the muscles of the spinal accessory complex, are supplied by more than one population of motoneurons. The spinal accessory nucleus extends as a single column of neurons from C1 to C7 spinal cord segments and occupies a lateral position within the ventral horn. The most rostral portion of this column is located dorsolaterally, whereas the remaining portion from C2 to C7 occupies a ventrolateral position. At C1 and C4 levels the nucleus also possesses some cells with a medial location. All the muscles of the spinal accessory complex receive their motor innervation both from the spinal accessory nucleus and from motoneurons forming the cervical spinal nerves. A double motor innervation of these muscles is thus present in the sheep.

INTRODUCTION

The innervation of the muscles of the spinal accessory nuclear complex, i.e. the sternocleidomastoid and trapezius muscles, has been studied in detail in the monkey (Karim & Nah, 1981; Augustine & White, 1986; Jenny et al. 1988), cat (Rapoport, 1978), rabbit (Karim & Nah, 1981) and rat (Gottschall et al. 1980; Karim & Nah, 1981; Kitamura & Sakai, 1982; Brichta et al. 1987; for review, see Krammer et al. 1987). In addition, in the rat (Kitamura & Sakai, 1982) attention has been paid to verify the existence of a double efferent innervation of sternocleidomastoid and trapezius. These muscles have been described as receiving their motor innervation both from the spinal accessory nerve and from cervical spinal nerves.

In the present study, the motor innervation of the spinal accessory muscle group was investigated in the sheep. We consider that a comparison of the organisation in this species with the findings observed in other mammals to be of interest, since the sheep, as is true for ungulates in general, lacks a clavicle. As a consequence, instead of a true sternocleidomastoid muscle, 2 other muscles, the sternocephalic and brachiocephalic, are present in the cervical region and shoulder girdle.

The retrograde transport of fluorescent dyes (Bentivoglio et al. 1980; Keizer et al. 1983) was used in the present experiments to investigate the localisation of the motoneurons of the spinal accessory nerve in the sheep, and to establish whether in this species the sternocephalic, brachiocephalic, omotransversarius and the cervical part of trapezius are innervated only by the spinal accessory nerve or both by this nerve and cervical spinal nerves.

MATERIALS AND METHODS

A total of 12 sheep weighing between 15 and 25 kg were used in the study. Each animal was anaesthetised with ketamine (4 mg kg^{-1}, i.v.) and diazepam (2 mg kg^{-1}, i.m.). The fluorescent tracers Diamidino

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Yellow dihydrochloride (DY) and Fast Blue (FB) were used as 2% aqueous suspensions and injected by means of Hamilton microsyringes.

In 4 animals a 4 cm incision was made on the right lateral aspect of the neck, rostral and parallel to the transverse process of the atlas, and the external ramus of the right spinal accessory nerve was exposed. 5–10 µl of DY suspension were slowly injected into the nerve proximal to its division into the ventral and dorsal branches. In 4 other animals the sternocephalic and omotransversarius muscles were identified and dissected free of the surrounding muscles and connective tissue. FB and DY were then injected at multiple sites (6 sites for each muscle, 10 µl/site) into the left sternocephalic and omotransversarius muscles. The same muscles on the right were similarly injected after cutting of the right spinal accessory nerve just before it branches. In the remaining 4 animals FB and DY were injected into the left brachiocephalic and trapezius muscles. The same muscles were then injected contralaterally after cutting of the right spinal accessory nerve. Each injection was performed very carefully so as to avoid any leakage of the tracer from the injected muscle.

After a survival period of 5–7 d, each animal was perfused, under deep anaesthesia, through the common carotid arteries with heparinised saline solution followed by 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The brainstem and cervical spinal cord were removed, postfixed in the paraformaldehyde perfusate for 3 h and subsequently rinsed and stored in 10% sucrose solution in phosphate buffer (pH 7.4) at 4 °C for 1–2 d. Serial transverse sections (60 µm) through the caudal brainstem and cervical spinal cord were cut on a freezing microtome, collected in chilled phosphate-buffered saline (pH 7.3) onto uncoated slides and allowed to air dry. Slides were then coverslipped and the sections examined with a Leitz Ploemopack fluorescence microscope at 360 nm excitation wavelength (filter system A) which elicits the blue FB fluorescent labelling of the neuronal cytoplasm and the yellow DY labelling of the nucleus. The most cranial level of the ventral roots of the 1st cervical (C1) nerve was defined as the most cranial level of spinal cord and specific spinal cord segments were identified by the entrance of the C1–C8 dorsal roots.

RESULTS

Spinal nucleus of the accessory nerve

After injection of DY into the external ramus of the spinal accessory nerve, labelled cells could be observed forming a column in lamina IX of the ipsilateral ventral horn of the spinal cord from C1 to C7 segments (Fig. 1). This population of cells was located laterally. In the rostral half of C1 the cells occupied a dorsolateral position, whereas at a level caudal to C1 the column shifted to a ventrolateral position without any discontinuity. After this ventral shift, the spinal nucleus of the spinal accessory nerve was located in the ventrolateral part of the ventral horn from C2 to C7. At C5, C6 and C7, in particular, the column appeared to be formed by 2 groups of cells (Fig. 1), only slightly separated, one placed along the most lateral margin of the ventral horn and the other in a more medial region of the ventrolateral portion of the anterior horn. In addition, at C1 and C4 levels some labelled cells could also be observed in a medial location. No labelled cells were detected in the medulla oblongata.

Location of sternocephalic, brachiocephalic, omotransversarius and trapezius motoneurons

Sternocephalic motoneurons (Table, Fig. 2).

The injection of the fluorescent tracer into the sternocephalic muscle labelled cells ipsilaterally from C1 to C3 segments (Fig. 2*a*). The cells were located at both the dorsolateral and dorsomedial borders of the ventral horn at the level of C1, whereas at C2 they showed a ventrolateral and a ventromedial position. At C3 segment labelled cells were only located ventrolaterally.

After cutting the spinal accessory nerve, the tracer injected into the sternocephalic muscle labelled cells from C1 to C2 only (Fig. 2*b*). At C1, cells were located both laterally and medially, whereas at C2 level they were only medially placed.

Brachiocephalic motoneurons (Table, Fig. 2)

Following i.m. injection of the tracer, labelled cells were observed ipsilaterally between C1 and C6 segments (Figs. 2*a*, 3). The motoneurons were distributed along 2 cell columns, 1 located medially and 1 laterally. The medial column extended from rostral C1 to C4, whereas the lateral column extended from caudal C1 to C6. With the exception of C1 segment in which the cells were located dorsomedially and dorsolaterally, in all other segments the cells had a ventromedial and ventrolateral position.

After cutting the spinal accessory nerve, the tracer injected into the brachiocephalic muscle labelled cells from rostral C1 to C4 in the medial portion of the

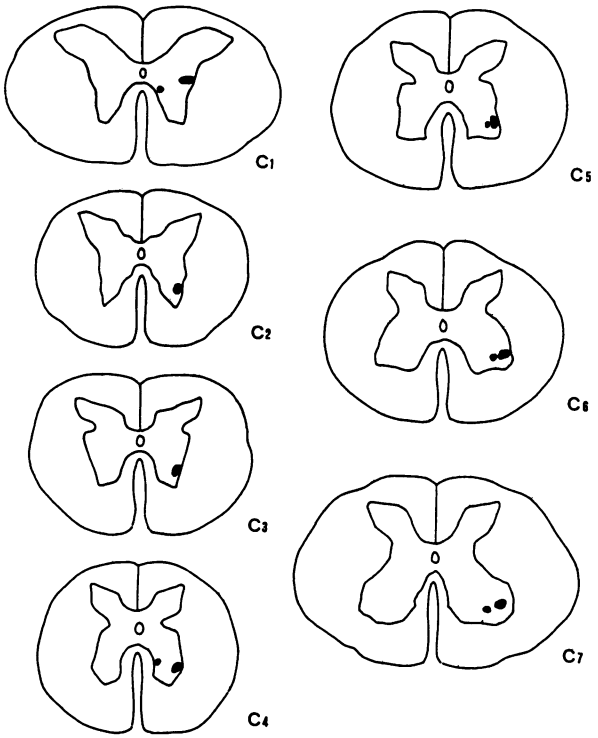

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Fig. 1. Drawings of representative transverse sections through cervical spinal cord segments C1–C7 of the sheep. Black areas indicate the localisation of labelled accessory nucleus neurons after injection of the tracer into the ipsilateral external ramus of the spinal accessory nerve. The composite results of injections in 4 different animals are depicted.

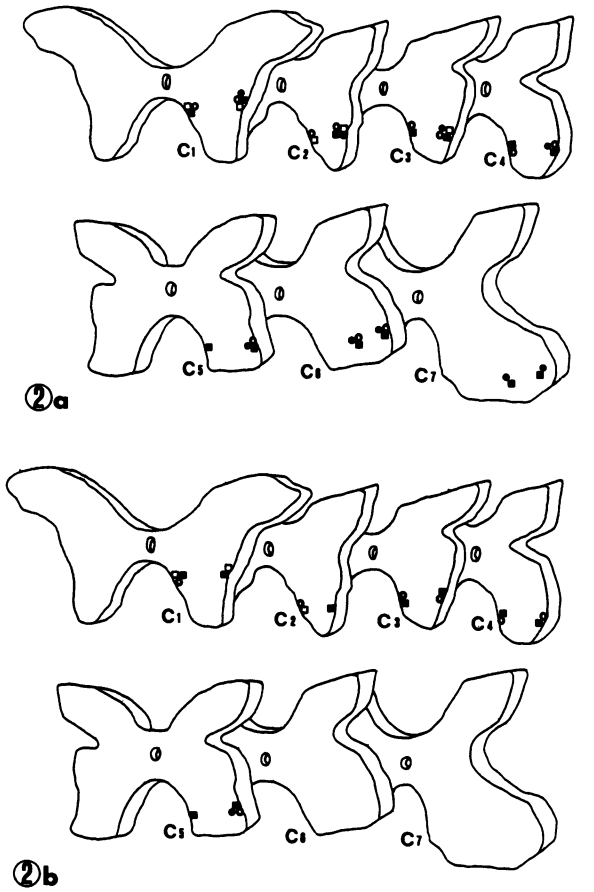

Fig. 2. Drawings of representative transverse sections through cervical spinal cord segments C1–C7 of the sheep. The different symbols represent the location of labelled neurons following injection of the tracers into sternocephalic (□), brachiocephalic (○), omotransversarius (●) and cervical part of trapezius (■) muscles. (*a*) shows the result of the injections into the muscles with an intact nerve supply. (*b*) shows the result of injections into the muscles after cutting the ipsilateral spinal accessory nerve.

ventral horn, but from C3 to C5 in the lateral position (Fig. 2*b*).

Omotransversarius motoneurons (Table 1, Fig. 2)

Omotransversarius neurons labelled with the tracer formed a cell column extending from caudal C1 to C7. With the exception of C1, the column was in the ventrolateral portion of the ventral horn, ipsilateral to the injected omotransversarius muscle (Fig. 2*a*).

Table. *Presence of labelled cells in cervical spinal cord segments following injection of fluorescent tracer into the sternocephalic (SC), brachiocephalic (BC), omotransversarius (OT), and trapezius (TRAP) muscles either with an intact nerve supply* (*) *or after cutting the ipsilateral spinal accessory nerve* (▲).

	SC				BC				OT				TRAP			
	Med.		Lat.		Med.		Lat.		Med.		Lat.		Med.		Lat.	
C1 rostral	*	▲	*	▲	*	▲	–	–	–	–	–	–	*	▲	*	▲
C1 caudal	*	▲	*	▲	*	▲	*	–	–	–	*	–	–	–	*	▲
C2	*	▲	*	–	*	▲	*	–	–	–	*	–	–	–	*	▲
C3	–	–	*	–	*	▲	*	▲	–	–	*	–	*	▲	*	▲
C4	–	–	–	–	*	▲	*	▲	–	–	*	–	*	▲	*	▲
C5	–	–	–	–	–	–	*	▲	–	–	*	▲	*	▲	*	▲
C6	–	–	–	–	–	–	*	–	–	–	*	–	–	–	*	–
C7	–	–	–	–	–	–	–	–	–	–	*	–	–	–	*	–

Med., medial cell column and Lat., lateral cell column in the ventral horn.

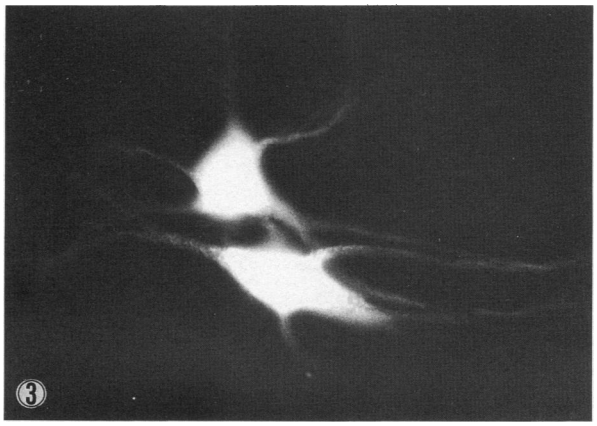

Fig. 3. Labelled accessory nucleus neurons at the level of C3 spinal cord segment after injection of the fluorescent tracer Fast Blue into the ipsilateral brachiocephalic muscle with its nerve supply intact. × 200.

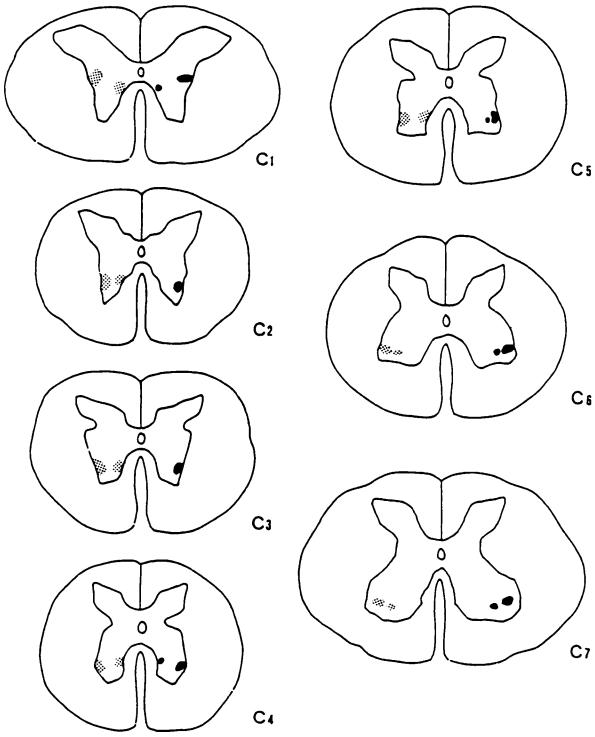

Fig. 4. Drawings of representative transverse sections through spinal cord segments C1–C7 of the sheep. Comparison of the spinal accessory nucleus (black areas) and the areas occupied by motoneurons labelled after tracer injection into the 4 muscles of the spinal accessory complex with their nerve supply intact (dotted areas).

After cutting the spinal accessory nerve, the tracer injected into the muscle labelled cells only at the C5 level (Fig. 2*b*).

Trapezius motoneurons (Table, Fig. 2)

Trapezius neurons labelled with the tracer formed a cell column extending from rostral C1 to C7 in the lateral portion of the ventral horn. In addition, labelled cells were also found in the medial portion of the ventral horn at the C1 (rostral), C3, C4 and C5 levels (Fig. 2*a*).

After cutting the spinal accessory nerve, the tracer injected into the trapezius muscle labelled cells with a distribution comparable to that described above, between rostral C1 and C5 segments. No labelled cells were found below the C5 level (Fig. 2*b*).

The diagrams of Figure 4 summarise the topographic position of the sternocephalic, brachiocephalic, omotransversarius and trapezius motoneurons obtained with their nerve supply intact, and compare their distribution with that of the spinal nucleus of the accessory nerve.

DISCUSSION

The general location of the motoneurons of the spinal nucleus of the spinal accessory nerve in the sheep is similar to that described for other mammals. Some lack of correspondence may exist, however, if either the rostral or the caudal limits of the nucleus are considered. According to our present labelling experiments, in the sheep the nucleus extends from C1 to the C7 level. In many other species, including man (see Augustine & White, 1986; Ullah & Salman, 1986), and also in the sheep on the basis of findings obtained by a retrograde degeneration method (Flieger, 1964), the rostral limit of the nucleus has been described as being situated in the caudal part of the medulla oblongata. Our results, however, never demonstrated labelled cells above the C1 level. Concerning the caudal limit of the nucleus, the observations of the present study are in agreement with those of Flieger (1964) and Krammer et al. (1987) which showed, in the sheep and in the rat respectively, that the caudal extent of the accessory neurons was at the level of C7 segment. In all the other species examined, the nucleus has been described as extending only as low as the C5 or C6 segments (Rapoport, 1978; Augustine & White, 1986; Ullah & Salman, 1986). This could reasonably be explained, according to Krammer et al. (1987), by the fact that there is a gradual decrease in the nucleus below this level.

There is also some disagreement in the literature concerning the distribution of the cells of this nucleus in the ventral horn. Some authors, in many species including man, have described only a single cell column representing the spinal nucleus of the accessory nerve, whereas other authors have described the nucleus as being composed of 2 separate cell columns, designated dorsal and ventral (see Ullah and

Salman, 1986). Our results show that in the sheep the nucleus extends as a single cell column and occupies a lateral position within the ventral horn. In its most rostral portion, this column is located dorsolaterally, whereas starting from caudal levels of C1 it displays a ventral shift, becoming ventrolaterally placed. The few cells described in the medial portion of the ventral horn are observable only at C1 and C4 levels and do not form a cell column.

Concerning the innervation of the muscles of the spinal accessory complex, our present findings clearly show that these muscles in the sheep receive their motor innervation both from the spinal accessory nucleus and motoneurons forming the cervical spinal nerves. This observation is supported by 2 considerations. (1) Following injection of the tracer into muscles with their nerve supply intact, the labelled motoneurons occupy not only the area corresponding to that of the nucleus of the spinal accessory nerve (Fig. 4), but also, as far as C5, an area wider than that of the nucleus and even a portion of the ventral horn in which spinal accessory motoneurons have never been detected. (2) For muscles injected with the tracer after cutting the spinal accessory nerve, labelled motoneurons have consistently been observed. The labelling was confined to a reduced rostrocaudal length of the ventral horn when compared with the experiments performed with their nerve supply intact. This means that the motoneurons of the cervical spinal nerves contribute to the muscle innervation to a lesser extent than those of the spinal accessory nucleus. In fact, the motoneurons of the cervical spinal nerves involved in the muscle innervation were limited to the C1–C5 tract. In addition, it is of interest that the sternocephalic, brachiocephalic and trapezius muscles receive a large contribution from cervical spinal nerves, whereas omotransversarius is primarily innervated by the spinal accessory nerve.

In conclusion, although morphological and functional differences exist between the accessory musculature of the sheep and that of the rat, our present findings on the sheep confirm the results obtained by Kitamura & Sakai (1982) in the rat concerning the motor innervation of the accessory complex muscles, i.e. that they are innervated both by motoneurons of the spinal accessory nerve and motoneurons giving origin to the fibres of cervical spinal nerves.

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