Sensory nerve formations in the myodural bridge complex of adult humans

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SUMMARY

The myodural bridge complex (MDBC) consists of connective tissue bands connecting the spinal dura mater to the suboccipital muscles passing throughout the posterior atlanto-occipital and atlanto-axial spaces. It is a universal evolutionarily conserved anatomical structure present in most vertebrate species including humans. Its physiology is unknown, but it is thought that it may be related to cerebrospinal fluid circulation. On the other hand, there is no information about the possible innervation of MDBC in humans. In the present study, immunohistochemistry (S100 protein and neurofilament proteins) was used to study the innervation of MDBC in five specimens of adult human cadavers. In all cases, nerve profiles were observed forming isolated nerve fibers or small nerve bundles, sometimes associated with blood vessels. In one case (1/5), at the level of the junction of the MDBC with the *posterior rectus capitis minor* muscle, complex capsulated formations containing a variable number of sensory nerve formations were found. We consider that such formations may be related to the proprioceptor system of the suboccipital muscles, but further studies are needed to determine their frequency, density and possible function.

Key words: Myodural bridge complex – Innervation – Sensory nerve formations – Human

INTRODUCTION

In 1995, Hack et al. described in human a dense bilateral band of connective tissue that connects the spinal dura mater to the suboccipital musculature (primarily the *rectus capitis posterior minor* muscle, but also the *rectus capitis posterior major* and *obliquus capitis inferior* muscles) and the *ligamentum nuchae* (LN), passing throughout the posterior atlanto-occipital and atlanto-axial membranes (see Humphreys et al., 2003). All together these structures are currently known as myodural bridge complex (MDBC) (Kahkeshani and Ward, 2012; Scali et al., 2011, 2015, 2022; Pontell et al., 2013; Zheng et al., 2020), regarded as an universal evolutionarily conserved anatomical structure present in different vertebrate species (Zheng et

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al., 2017) including horses (McElroy et al., 2019), rats (Lai et al., 2021; Song et al., 2023), marine mammals (Liu et al., 2017, 2018; Zhang et al., 2021), reptilians (Zhang et al., 2016; Huangfu et al., 2019; Grondel et al., 2022), and birds (Okoye et al., 2018; Dou et al., 2019; Chen et al., 2021).

After the description of Hack et al. (1995), numerous studies have been conducted in humans to establish in detail the anatomy of MDBC (see Scali et al., 2015). Using imaging techniques, Humphreys et al. (2003) compared magnetic resonance with anatomical dissection images and reported that MDBC appeared as a small low signal intensity band on T_1-weighted images. Later, Scali et al. (2013b) examined the anatomy of the posterior atlanto- axial space using T_2-weighted MR imaging and identified MDBC as oblique hypointense fibers (see for a review Sun et al., 2020). Structurally, MDBC consists of connective tissue containing vessels and very scarce nerves (Pontell et al., 2013; Scali et al., 2013a).

Functionally, MDBC is theorized to stabilize the dura mater during the extension of the head and neck, thus serving as dural tension monitors, preventing compression of the dura mater during motion of the spinal column and infolding of the dura mater and disruption of the flow of cerebrospinal fluid (Hallgren et al., 1997; Pontell et al., 2013; Enix et al., 2014; Chen et al., 2015; Sillevis and Hogg, 2020).

In this study, it is hypothesized that, in addition, MDBC are sources of proprioceptive inputs that regulate the contraction of the suboccipital muscles. These muscles are involved in head movements, but they also play important roles as joint stabilizers of the upper cervical spine or postural controllers (Hallgren et al., 2014a, b, 2017). Here, we have studied the presence of sensory nerve formations in MDBC using immunohistochemistry techniques. The purpose of the study is to contribute to the knowledge of the possible functions of MDBC in humans, and to demonstrate that they are sensory-innervated structures able to transmit stimuli, via afferent pathways, to the central nervous system related to the control of head stability.

MATERIAL AND METHODS

Tissues

Five formalin-fixed hemi-heads, corresponding to 5 different subjects, 3 males and 2 females, were obtained at the Área de Anatomía y Embriología Humana de la Universidad de Oviedo, Spain. These materials were obtained in accordance with Spanish legislation (RD 1301/2006; Law 14/2007; RD 1716/2011; Order ECC/1404/2013). Thereafter, MDBC were identified (mobilizing the cephalo-cervical junctions and observing the tension of the connective bands arranged between the dura mater, the atlanto-occipital membrane, or the suboccipital muscles) and dissected (Fig. 1). The pieces were washed in tap water for 24 hours, then processed for routinely paraffin embedding. The pieces were cut into10 µm sections, mounted on gelatin-coated microscope slides and processed for indirect immunohistochemistry using the peroxidase-antiperixidase method. To ascertain structural details, some sections were stained with hematoxylin & eosine.

Immunohistochemistry

Deparaffinized and rehydrated sections were processed for immunohistochemical detection of S100 protein (S100P) to label Schwann cells and terminal glial cells in sensory nerve formations and neurofilament proteins (NFP) which selectively label the axons (see Vega et al., 2009; Cobo et al., 2021). The EnVision antibody complex detection kit (Dako, Glostrup, Denmark) was used following the manufacturer's instructions. Briefly, the endogenous peroxidase activity was inhibited (3% H_2O_2 for 15 minutes) and non-specific binding was blocked (10% bovine serum albumin for 20 minutes). The sections were then incubated overnight at 4°C with primary antibodies against S100P (Dako; polyclonal raised in rabbit used diluted 1:5000) and against NFP (Dako; clone 2F11, monoclonal antibody raised in mouse, used diluted 1:100). After incubation with the primary antibodies, sections were washed in tris-phosphate buffered saline (PBS-T) for 15 minutes and incubated at room temperature with the corresponding peroxidase-conjugated secondary antibody for 90 minutes (Dako EnVision labeled

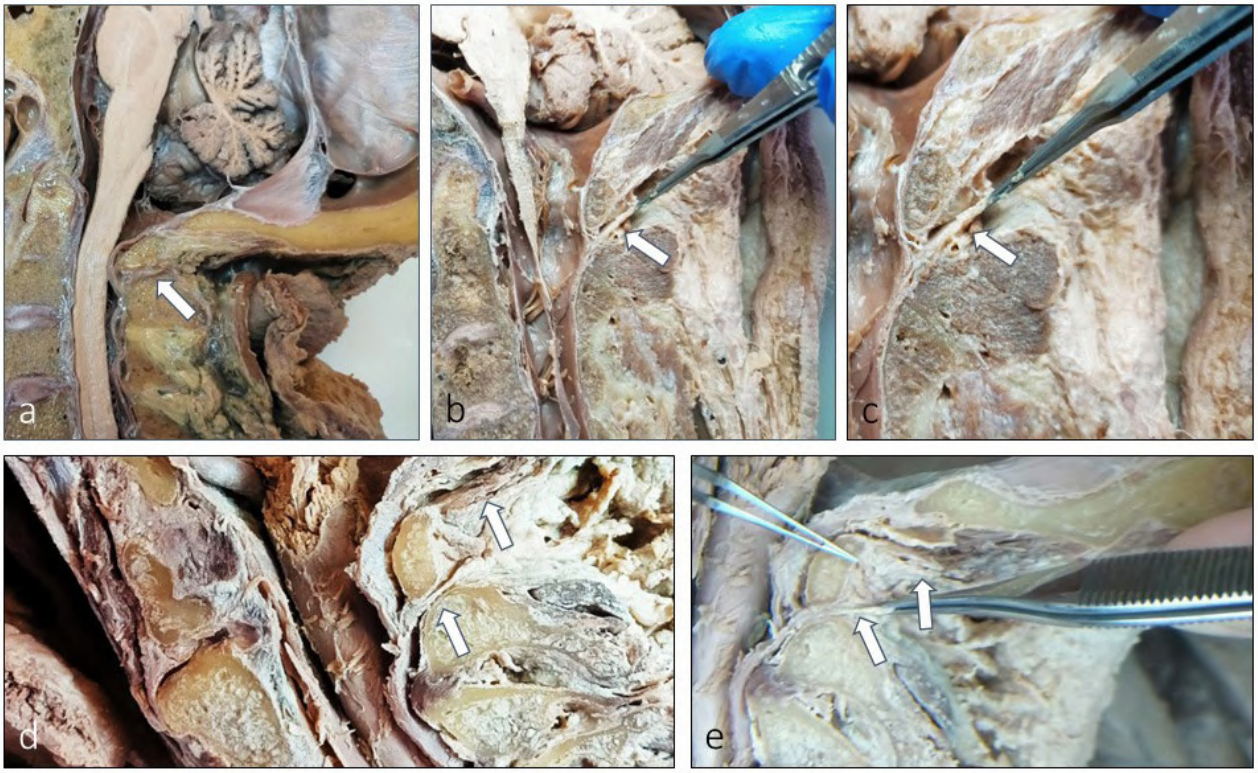

Fig. 1.- Myodural bridge complexes (arrows) in all five specimens examined (**a-e**).

polymer-HRP anti-rabbit IgG or anti-mouse IgG). After washing in PBS-T, the immunoreaction was revealed with a solution of 3-3' diaminobenzidine (Leica BondTM Polymer Refine Detection Kit, Leica BiosystemsTM). The sections were contrasted with hematoxylin, washed in water, dehydrated with alcohols of increasing concentration, diaphanized in xylol and mounted with Entellan®. Processed slides were reviewed and photographed on a Nikon Eclipse® 80i light microscope coupled to a Nokia® DS-5M camera. For control purposes, some sections were processed in the same way described above but using mouse or rabbit serum instead of the primary antibody, or omitting incubation with secondary antibodies, to obtain a negative immunoreaction (not shown). On the other hand, nerve trunks and fibers from the same sections served as positive internal controls for the antigens investigated (Vega et al., 2009).

RESULTS

MDBC in adult humans consist of bands of connective tissue, between 1 and 3, extending from the upper segment of the spinal dura mater and the suboccipital muscles. The fibrous bands follow an oblique direction, and between the fibrillary fascicles there are blood vessels and nerves of different calibers. Nerve profiles are isolated nerve fibers or small nerve bundles, and the innervation density of MDBC can be regarded as low; on the other hand, within the thickness of the MDBC no differentiated structures identifiable as sensory nerve formations have been found.

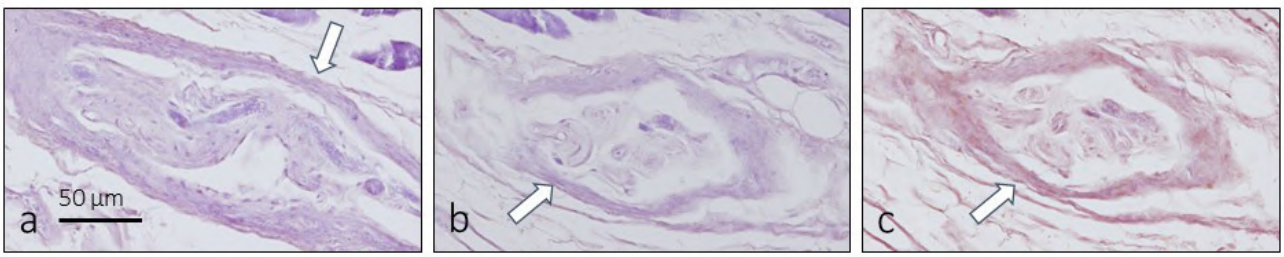

Fig. 2.- Ovoidal structures (arrows) localized in the junction of one myodural bridge complexes with the *rectus capitis posterior minor* muscle. Scale bar = 50 µm.

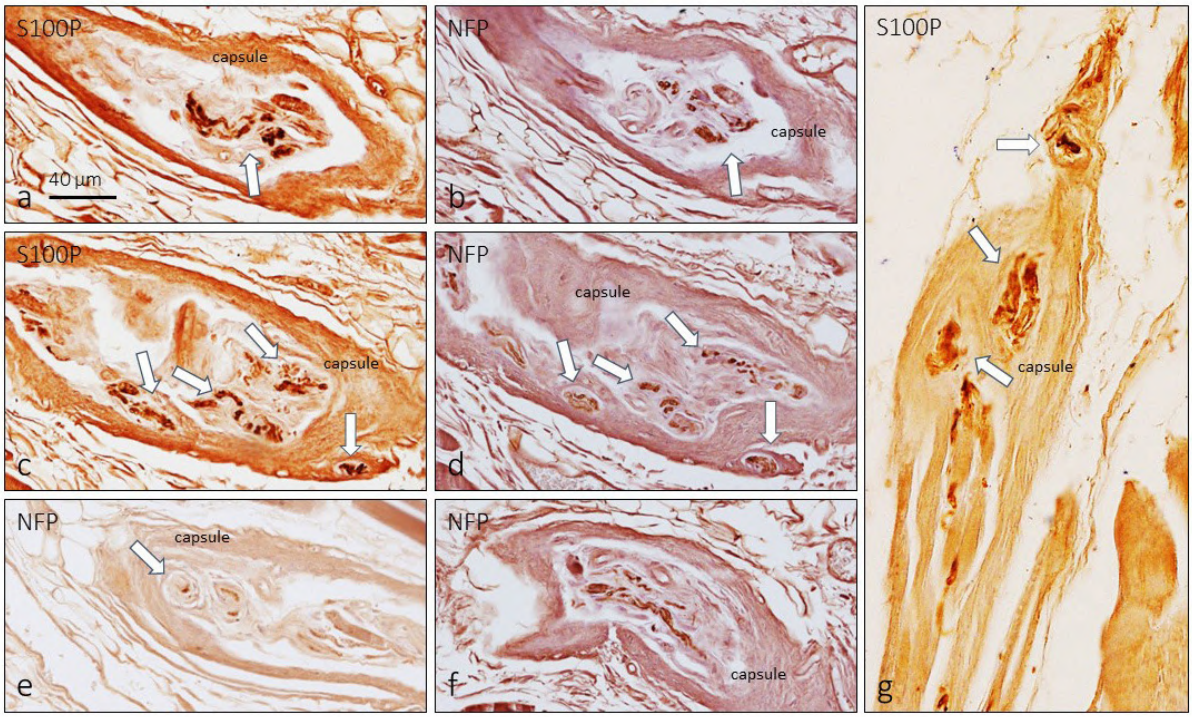

Fig. 3.- Serial sections (**a** and **b**; **c** and **d**) of the ovoidal structures localized in the junction of one myodural bridge complexes with the *rectus capitis posterior minor* muscle immunostained for the detection of S100P (**g**) and NFP (**e, f**). These formations contain sensory nerve formations and isolated nerve fibers. Scale bar = 40 µm.

However, in one case (1/5), at the junction level of one band of connective tissue of the upper MDBC with the *rectus capitis posterior minor* muscle three ellipsoid formations with a well- developed capsule were observed (Fig. 2). The immunohistochemical study revealed that these structures contain clusters of nerve structures, variably arranged and grouped in a common capsule, which were identified as sensory nerve formations. They consist of NFP positive axons and Schwann-related or terminal glial cells (Fig. 3). These sensory nerve formations are immersed in an amorphous connective tissue, and in no case muscle fibers were found inside; therefore, it must be ruled out that they are muscle spindles.

DISCUSSION

Over the past years, the suboccipital region has received an increasing attention, since research has revealed multiple soft-tissue connections extending from the suboccipital structures to the cervical dura mater through the posterior intervertebral spaces; these formations were called MDBC (Kahkeshani and Ward, 2011; Scali et al., 2011, 2015; Pontell et al., 2013; Zheng et al., 2020). Different studies have concluded that MDBC serve as monitors of dural tension (Hallgren et al., 1997; Pontell et al., 2013; Enix et al., 2014; Scali et al., 2013a, b; Chen et al., 2015; Sillevis and Hogg, 2020). Here, we hypothesized that MDBC are sources of proprioceptive inputs, and thus are involved in the regulation of cervical spine positioning and movements, which in turn inform the central nervous system about the tension of the spinal dura mater. To perform this function, MDBC must have a nervous system that, as far as we know, has never been investigated. Only Scali et al. (2013a) have reported the occurrence of nerves in 1/11 human specimen examined. Our findings about the presence of isolated nerve fibers and small nerves bundles in MDBC are in good agreement with those authors.

The most striking finding of the present research was the discovery of sensory nerve formations in one specimen of our small series (1/5). These structures are formed by a well- developed capsule containing an amorphous tissue and, inside, groups of independent sensory nerve formations, of variable morphology. These nerve formations cannot be classified within the typical nerve formations (see Cobo et al., 2021; Martín-Aguacil et al., 2022) but, presumably, they represent propi-

oceptor organs, different from muscle spindles or tendon organs. Given the unexpectedness of the findings and the small sample analyzed, further studies are needed to deepen the study of the innervation of MDBC and to draw definitive conclusions regarding its function.

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