Morphologie 91 (2007) 38–43

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MORPHOLOGIE
Bulletin de l'Association des Morphologistes

ORIGINAL ARTICLE

Anatomy of the deep fascia of the upper limb. Second part: study of innervation

Anatomie du fascia profond du membre supérieur. Deuxième partie : étude de l'innervation

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KEYWORDS
Fascia;
Innervation;
Proprioception;
Motor coordination;
Ruffini corpuscles;
Pacini corpuscles

Abstract Analysis of specimens taken from different areas of the deep fascia in 20 upper limbs was made in order to establish which kind of nerve fibres and endings are present in the deep muscular fascia. The flexor retinaculum and the lacertus fibrosus were also evaluated because they are anatomically hardly separable from the deep muscular fascia, although they have different functions. In particular, specimens were taken at the level of: (a) the expansion of pectoralis major onto the bicipital fascia, (b) the middle third of the brachial fascia, (c) the lacertus fibrosus, (d) the middle third of the antebrachial fascia, (e) the flexor retinaculum. This study demonstrated an abundant innervation of the fascia consisting in both free nerve endings and encapsulated receptors, in particular, Ruffini and Pacini corpuscles. However, differences in innervation were verified: the flexor retinaculum was resulted the more innervated element whilst lacertus fibrosus and the pectoralis major expansion the less innervated. These results suggest that the retinaculum has more a perceptive function whereas the tendinous expansions onto the fascia have mostly a mechanical role in the transmission of tension. The hypothesis that the fascia plays an important role in proprioception, especially dynamic proprioception, is therefore advanced. In fact, the fascia is a membrane that extends throughout the whole body and numerous muscular expansions maintain it in a basal tension. During a muscular contraction these expansions could also transmit the effect of the stretch to a specific area of the fascia, stimulating the proprioceptors in that area.

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doi:10.1016/j.morpho.2007.05.002

MOTS CLÉS
Fascia ;
Innervation ;
Proprioception ;
Coordination motrice ;
Corpuscules de Ruffini ;
Corpuscules de Pacini

Résumé Des prélèvements différents du fascia profond du membre supérieur ont été analysés pour établir si le fascia musculaire est innervé et surtout la nature des fibres présentes. On a évalué aussi le retinaculum du fléchisseur et le *lacertus fibrosus* car ils sont anatomiquement inséparables du fascia musculaire profond, même s'ils ont des fonctions différentes. Les spécimens ont été prélevés dans 20 membres supérieurs : a) au niveau de l'expansion de grand pectoral sur le fascia brachial ; b) au tiers médian du fascia brachial ; c) au niveau du *lacertus fibrosus*, d) au tiers médian du fascia antébrachial ; e) au niveau du retinaculum du fléchisseur. Cette étude a démontré une riche innervation du fascia. Cette innervation est formée par des terminaisons nerveuses libres et par des récepteurs encapsulés, en particulier des corpuscules de Ruffini et Pacini. On a trouvé des différences dans la quantité et le type d'innervation dans les structures analysées. En particulier le retinaculum du fléchisseur est la structure la plus innervée, tandis que le *lacertus fibrosus* est la structure la moins innervée. Ces résultats nous suggèrent ces conclusions : a) le retinaculum a une fonction perceptive ; b) les expansions tendineuses sur le fascia (*lacertus fibrosus*) ont principalement un rôle mécanique de transmission de tension ; c) le fascia joue un rôle important dans la proprioception, particulièrement dynamique. Le fascia est une membrane qui s'étend dans tout le corps et les expansions des muscles sur elle la maintiennent dans une tension basale. Quand une contraction musculaire se réalise, le fascia est tiré par les expansions tendineuses musculaires, en stimulant de cette façon les propriocepteurs d'une région spécifique.

Introduction

Recent studies [3,11,18–20] have proposed that the fascia is an important element in muscular biomechanics, peripheral motor coordination, proprioception and regulation of posture. Wheater [23] describes it like a flexible skeleton onto which muscle fibres are anchored and with the function of distributing and directing the force of muscular action to bone, skin etc. However, there is poor anatomical and physiological explanation about this role of the fascia. In the previous paper, we have demonstrated that the fascia is maintained at a basal tension by many muscular insertions and that, when the muscles contract, these insertions transmitted part of the traction to the fascia. It had been therefore hypothesized that these stretching could activate nerve terminations implanted within the fascia itself. In effect, some authors have described a specific innervation of deep fascia. In particular, Stilwell [21], Hirsch et al. [10] and Yahia et al. [24,25] established that there are numerous free nerve endings and mechanoreceptors in the thoracolumbar fascia, Palmieri et al. [12] in the lacertus fibrosus and Staubersand and Li [17] in the crural fascia. Von Düring and Andres [22] demonstrated how all proprioceptors in lamprey are to be found in the fascia corporis or in the intermuscular septa and that the single receptors connect to the fascia by the capsular collagen fibres. Furthermore, Bednar et al. [3] found an alteration in both the histological structure (inflammation and microcalcifications) and the degree of innervation of the thoracolumbar fascia in patients with chronic lombalgia, indicating a possible role of the fascia in lumbar pain. Nonetheless, the available data in the literature are poor and the fascia is still considered, more or less, an amorphous layer of collagenic tissue whose function is inert structural support [10] or containment. It therefore seemed important to study this tissue and, in particular, we have concentrated on the innervation of the deep fascia of the upper limb.

Materials and methods

The approval of our research protocol by the Anatomy Institute of René Descartes University, Paris, allowed us to analyse 20 upper limbs without pathological lesions from 13 cadavers (10 men, 3 women and mean age 79.9 years), neither embalmed nor frozen prior to examination. In order to evaluate any differences in the innervations of the fascia of the upper limb, five different samples were taken from the deep fascia in each specimen. The areas sampling were:

- the expansion of the pectoralis fascia onto the anterior region of the brachial fascia;
- the middle third of the anterior region of the brachial fascia;
- the lacertus fibrosus;
- the middle third of the anterior region of the antebrachial fascia;
- the central region of the flexor retinaculum.

All samples had the size of 1 × 1.5 cm. All the specimens were immediately preserved in formaldehyde 4% in phosphate buffer saline (PBS) 0.1 M, pH 7.0 and subsequently included in paraffin. Five-micron slices were treated with a solution of H_2O_2 0.15% for 15 minutes in order to inhibit endogenous peroxidase activity and, after washing in PBS, incubated with normal goat serum 1:100 for 30 minutes and then with primary antibody S100, which bonds specifically the S100 protein present in the Schwann cells, diluted 1:500 in PBS at 37 °C in a humid chamber for 60 minutes. Repeated washings were performed and then the sections were incubated with the secondary antibody (Goat anti-rabbit IgG peroxidase-coniugated antibodies DAKO) 1:50 for 30 minutes and, lastly, the reaction was highlighted with 3,3'-diaminobenzidine (DAB substrate tablets, Sigma, 0.1%

Table 1 Mean number of nerve elements found in every sample. Horizontal line: types of receptors; vertical lines: analysed areas

Tableau 1 Nombre moyen de nerfs observés dans chaque zone. Ligne horizontale : types de récepteurs ; lignes verticales : régions analysées

	Pectoralis major expansion	Bicipital fascia	Lacertus fibrosus	Ante-brachial fascia	Flexor retinaculum
Free nerve endings	25.59	48.57	27.36	44.37	53.55
Pacini corpuscles	0.47	0.43	0.26	0.26	0.66
Ruffini corpuscles	0.12	0.29	0.1	0.26	0.55

v/v H_2O_2). The negative controls were obtained by omitting the primary antibody or substituting the same previously absorbed primary antibody with the antigen in excess. The preparations, contrasted with hematoxylin, were dehydrated and mounted with Canadian balsam (BDH, Italy) and observed with a Leica DMR microscope. The S100 positive nerve elements were counted in each sections at 100× magnification and the values obtained are reported in Table 1.

Other sections were stained with hematoxylin-eosin in order to highlight tissue morphology.

Results

Nerve elements were present in all of the specimens analysed although specific differences existed according to the area, or the subjects. Small unmyelinated nerves were revealed in all specimens, whereas Ruffini corpuscles, Pacini corpuscles, Golgi-Mazzoni and rare spherical clubs were present only in some. Table 1 reports the mean number of the nerves and the types of mechanoreceptors found in each area.

The Pacini corpuscles appeared in anti-S100 immunohistochemistry staining with a distinctly positive central core and a series of surrounding lamina (Fig. 1) stained only with

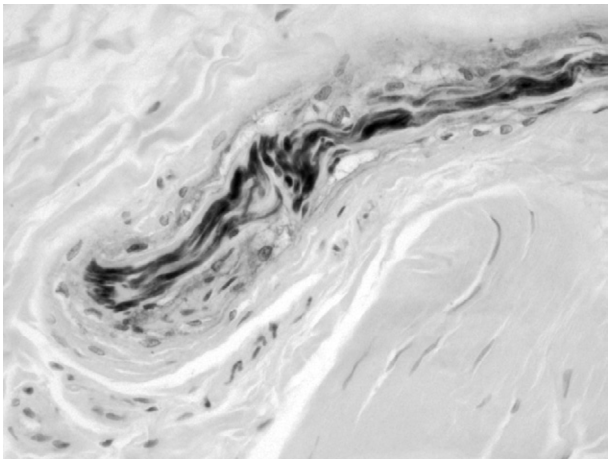

Figure 2 Golgi-Mazzoni corpuscles or Golgi clubs, 200× enlargement. Note the elongated form of these corpuscles, the presence of only 2–3 connective tissue laminae and the nerve fibre that divides into different filaments at the centre.

Figure 2 Corpuscules de Golgi-Mazzoni, 200× agrandissement. Notez la forme étirée de ces corpuscules, la présence de seulement 2–3 lames de tissu conjonctif et la fibre nerveuse ramifiée au centre.

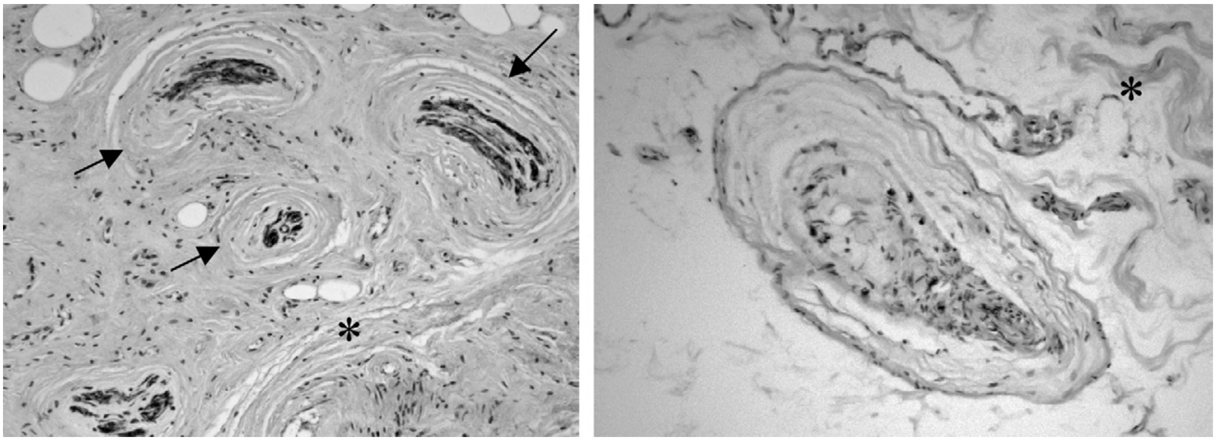

Figure 1 Pacini corpuscles (↑): on the left enlargement 100×, on the right a typical Pacini corpuscle, enlargement 400×. A large nerve fibre at the centre of numerous concentric laminae forms the corpuscle, which is embedded in loose connective tissue. Bundles of collagen fibres (*) can be noted next to these Pacini corpuscles.

Figure 1 Corpuscules de Pacini (↑) : à gauche grandissement 100×, à droite un corpuscule de Pacini typique, grandissement 400×. Le corpuscule est formé par une grande fibre nerveuse au centre de nombreuses lames concentriques. Des fibres de collagène (*) peuvent être observées à côté de ces corpuscules de Pacini.

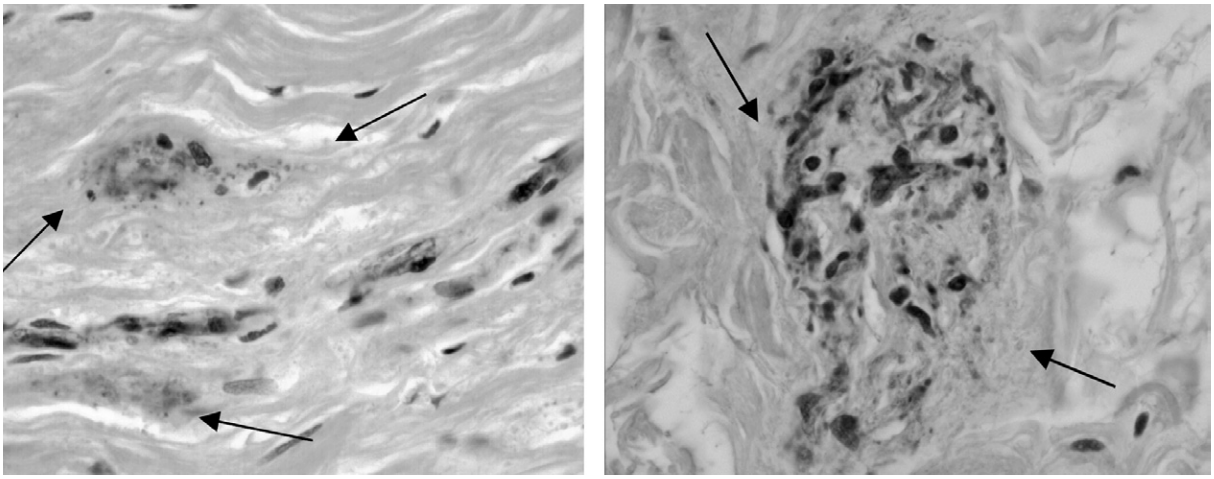

Figure 3 Ruffini corpuscles, on the left enlargement 100×, on the right a typical Ruffini corpuscle, 400×. Note how they are formed by a fine connective tissue capsule that has numerous points of contact (↑) with the surrounding collagen fibres. The nerve divides into numerous fine and twisted branches.

Figure 3 Corpuscules de Ruffini, à gauche grandissement 100×, à droite un corpuscule de Ruffini typique, 400×. Noter comme ils sont formés par une capsule de tissu conjonctif très fine qui a de nombreux points de contact (↑) avec les fibres de collagène environnantes. Le nerf se divise en de nombreuses branches à l'intérieur du corpuscule.

hematoxylin. The corpuscles were oval in shape and presented different dimensions (length from 1 to 4 mm, width from 1 to 2 mm) being apparently larger and more numerous at the flexor retinaculum level. In addition, corpuscles of a similar morphology, but smaller (length 0.1–0.3 mm, width 0.05 mm), more elongated and with just a few lamina are also present. The nerve fibres appeared more irregular, presenting numerous varicosities and often subdivided into various, rather twisted, secondary branches. These corpuscles corresponded morphologically to the Golgi-Mazzoni type or Golgi clubs (Fig. 2).

Yet other corpuscles, identified as Ruffini corpuscles (Fig. 3), appeared as a ball of fine twisted nerve fibres that connected in different directions, surrounded by a delicate capsule. These corpuscles were often associated together in groups of two-three.

Sections of nerves and free nerve endings were numerous in the fascia (Fig. 4) both around vessels and regularly distributed among the fibrous components. In particular, the intra-fascial nerves were often oriented perpendicularly to the collagen fibres, and in this manner stimulated by any stretching of the collagen fibres.

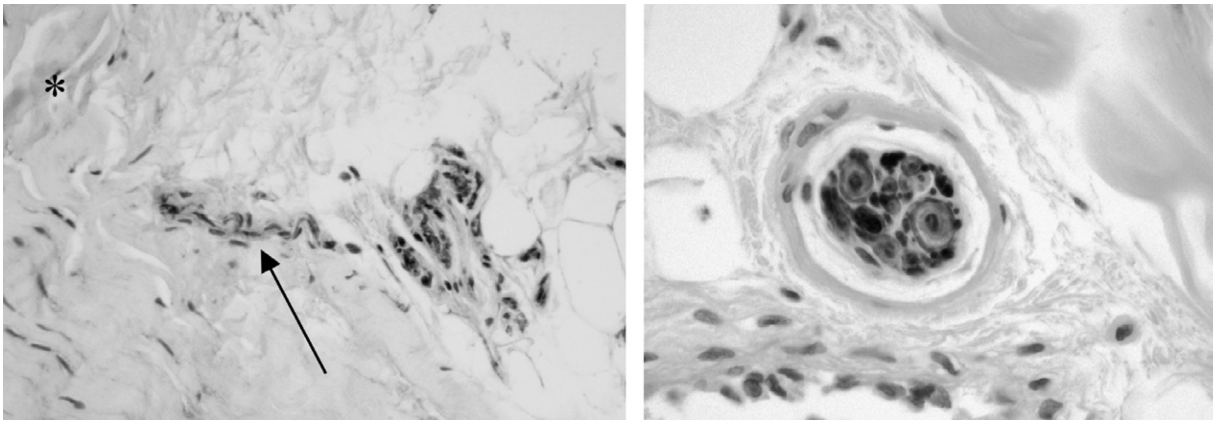

Figure 4 On the left, free nerve ending, enlargement 200×. Note how the fibre (↑) separates from the main trunk at a right angle (90°) crossing the bundles of collagen fibres of the fascia perpendicularly (*). On the right, transverse section of a small myelinated nerve, enlargement 400×. The nerve proceeds within a loose connective tissue matrix that separates it from the bundles of collagen fibres of the fascia, which are nevertheless nearby. Note the continuity of the perineurium with the surrounding connective tissue.

Figure 4 Sur la gauche, terminaison nerveuse libre, grandissement 200×. Noter comme le nerf (↑) se sépare du tronc principal selon un angle droit (90°) et croise les fibres de collagène du fascia perpendiculairement (*). À droite, section transversale d'un petit nerf, grandissement 400×. Notez la continuité du périnèvre avec le tissu conjonctif environnant.

Discussion

Our research established that the deep fascia of the upper limb is richly innervated by free nerve endings and encapsulated receptors. In our previous article, it was demonstrated how the fascia receives many expansions from the underlying muscles to maintain the fascia at a basal tension and to stretch it along different lines of force according to muscular traction to the movement performed [7]. This stretching of the fascia could activate the free nerve endings and the other proprioceptors, which, as we have demonstrated in this new study, are embedded within the fascia itself. The mechanoreceptors, which are immersed in a fibrous stroma, are in this way sensible to the traction of the muscles, thanks to the tendinous expansions into the fascia. This hypothesis is also substantiated by embryogenetic studies that have established how the fibrous capsule of all mechanoreceptors is derived from the surrounding connective tissue [15].

Our preliminary data underline a difference in the density of innervation of the fascia in relation both to the area from which the sample was taken and to the subject. In particular, from these results it can be deduced that both the tendinous expansion of the pectoralis major onto the bicipital fascia and the lacertus fibrosus are less innervated than the brachial and the antebrachial fascia, probably because they have a prevalent function of mechanical transmission. Furthermore, the nerve endings found in these tendinous expansions was above all perivascular, and so relating to the innervation of the vessel wall rather than the fascial tissue. On the contrary, samples taken from the flexor retinaculum were resulted the better innervated. Effectively, it has many functions: it is a pulley for the flexor tendons; it is an extrinsic ligament of the carpus, but also a thickening of the fascia at the periarticular level, probably having a fundamental role in motor perception as well as signalling variations in joint volume [9]. Instead, because the role of the deep fascia situated between the two joints is above all to guarantee a directional-perceptive continuity along a particular myokinetic chain [19,20] it therefore requires minor innervation [11].

Analysis of Table 1 reveals that the Pacini corpuscles, as compared to the Ruffini corpuscles, are relatively few. Nonetheless, comparing the few studies that report a quantitative analysis of the encapsulated receptors of the upper limb (17–29 Pacini corpuscles in the shoulder region) [2,4], we can understand how the fascia is relatively rich even with these elements. The mechanoreceptors within the fascia carry out different functions and they are activated in different situations. Pacini corpuscles only perceive rapid variations in their state of compression/relaxation, whereas Ruffini corpuscles respond to chronic stimuli [13,16]. It is probable that when the fascia is stretched, the compression exerted by the surrounding collagenic fascicles activates the Pacini corpuscles. Quite differently, the Ruffini corpuscles are activated by traction on the capsule by the surrounding collagenic fibres. In addition, if mechanoreceptors are stretched beyond their physiological limit, they have the ability of becoming nocìceptors. Thus, the fascia could be implicated in the etiopathogenesis of many extra-articular pain syndromes that result as a consequence of an excessive stimulation to these receptors.

It is, however, emphasised that these results require further confirmation and, since the mean age of the subjects was elevated, it would be interesting to examine young subjects to ascertain if their innervation is the same.

Conclusions

In section 1 the intuitions of some authors about the role of the fascia in peripheral motor coordination and proprioception were reported, our results confirm its decisive involvement. In particular, the fascia could have a role not only in static proprioception but also, and above all, in dynamic proprioception (perception of movement and its direction) because it perceives the stretching of the tendinous expansions of the underlying muscles. The works of Grigg and Greenspan [8], Clark et al. [5,6] and Rossi and Grigg [14] demonstrate how joint receptors are activated only at maximum flexion and extension, placing doubts on the exclusive role of articular receptors in Kynesthesia. This fact further implicates intramuscular and fascial receptors as indicators of the intermediate grades of movement. These authors made no distinction between muscular and fascial receptors but, seeing the type of fascial innervation and the structure of the fascia, we can advance the hypothesis that it is above all the fascia and/or the myofascial unit that has a role in proprioception, particularly dynamic proprioception [18–20].

We conclude by quoting a sentence from Baldissera [1]: “only if the receptors are mapped out they can have a directional significance”; in our opinion the fascia could be the framework upon which these receptors are mapped. Specific physiological experiments will be required in order to better clarify the functional aspect of the fascia.

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