GYNECOLOGY

Detailed muscular structure and neural control anatomy of the levator ani muscle: a study based on female human fetuses

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BACKGROUND: Injury to the levator ani muscle or pelvic nerves during pregnancy and vaginal delivery is responsible for pelvic floor dysfunction.
OBJECTIVE: We sought to demonstrate the presence of smooth muscular cell areas within the levator ani muscle and describe their localization and innervation.
STUDY DESIGN: Five female human fetuses were studied after approval from the French Biomedicine Agency. Specimens were serially sectioned and stained by Masson trichrome and immunostained for striated and smooth muscle, as well as for somatic, adrenergic, cholinergic, and nitriergic nerve fibers. Slides were digitized for 3-dimensional reconstruction. One fetus was reserved for electron microscopy. We explored the structure and innervation of the levator ani muscle.
RESULTS: Smooth muscular cell beams were connected externally to the anococcygeal raphe and the levator ani muscle and with the longitudinal anal muscle sphincter. The caudalmost part of the pubovaginal muscle was found to bulge between the rectum and the vagina. This bulging was a smooth muscular interface between the levator ani muscle and the longitudinal anal muscle sphincter. The medial (visceral) part of the levator ani muscle contained smooth muscle cells, in relation to the autonomic nerve fibers of the inferior hypogastric plexus. The lateral (parietal) part of the levator ani muscle contained striated muscle cells only and was innervated by the somatic nerve fibers of levator ani and pudendal nerves. The presence of smooth muscle cells within the medial part of the levator ani muscle was confirmed under electron microscopy in 1 fetus.
CONCLUSION: We characterized the muscular structure and neural control of the levator ani muscle. The muscle consists of a medial part containing smooth muscle cells under autonomic nerve influence and a lateral part containing striated muscle cells under somatic nerve control. These findings could result in new postpartum rehabilitation techniques.

Key words: anatomy, innervation, levator ani muscle, pelvic floor, smooth muscle cells

Introduction

Pelvic floor dysfunction affects nearly 25% of all women and is responsible for altered quality of life.[1] It might be the consequence of injury to the levator ani muscle or pelvic nerves during pregnancy and vaginal delivery.[2-4] Improved knowledge of the structure and innervation of the pelvic floor muscles may have clinical and therapeutic consequences.

In 2004, Kearney et al[5] published a new anatomical description of the levator ani muscle following a literature review. The resulting nomenclature is based on origin-insertion pairs and describes 3 components: (1) the pubovisceral muscle composed of 3 bundles called the puboperineal, puboanal, and puboanal muscles; (2) the puborectal muscle; and (3) the iliococcygeal muscle.[5] This description has the advantage of clarifying the nomenclature of the levator ani muscle, but is not based on its structure. Furthermore, the review does not provide a full description of the levator ani muscle.

We have previously described dual autonomic (inferior hypogastric plexus) and somatic (pudendal nerve) control of pelvic viscera with a communicating loop within the levator ani muscle.[6] Subsequently, we introduced a new concept of levator ani muscle innervation based on female human fetuses and using 3-dimensional (3D) reconstruction in a recent study. In this study, autonomic nerve fibers were identified as arising from the inferior hypogastric plexus and reaching the medial part of levator ani muscle,[7] supporting the possible existence of smooth muscle cells within the levator ani muscle. However, to date, classic anatomical literature textbooks describe the levator ani muscle as an entire striated muscle.[8] Even though some authors have reported the existence of smooth muscle cells in the pelvic floor,[9-11] data about the exact relation between the levator ani muscle and these smooth muscle cells remain scarce. Moreover, few studies have focused on the organization and innervation of smooth muscle cell areas within the pelvic floor.

Hence, the aim of this study was to demonstrate the presence of smooth muscle cell areas within the levator ani muscle, and to clarify their localization and innervation.

Materials and Methods

Human fetuses

The fetal specimens were obtained from late miscarriages. All parents gave written consent and authorization for the scientific use of the cadaver. Only specimens without maceration and without morphological or neurological macroscopic abnormalities on pathology examination were used. No infectious

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conditions were reported and no abnormalities of the central nervous system were observed during the autopsy of the fetuses.

The French Biomedicine Agency approved the study (PFS15-011). The work was compliant with the provisions of the 2013 revised version of the Declaration of Helsinki.

Five female fetuses were studied with a crown-rump length (CRL) of 150-185 mm. Fetuses had a gestational age of between 18-22 weeks, as determined from the CRL and fetal heel-to-toe length, corrected for the first-trimester ultrasound CRL measurement and confirmed by postmortem examination estimating organ maturation.[12] Fetuses A, B, C, D, and E were at 19, 19, 21, 21, and 22 weeks of gestation, respectively (Supplemental Figures 1 and 2). The external body aspect of each fetus was carefully restored after the autopsy out of respect and in case parents expressed a wish to see their fetus once more.

Macroscopic dissection

The entire pelvis was removed en-bloc with the pelvic organ and pelvic bone, and was fixed in formalin (10% formaldehyde) for 48 hours. Tissues were then cut into transverse or sagittal slices at 4-mm intervals. The tissue slices were placed in baskets, processed, and embedded in cardboard molds filled with paraffin. To avoid alteration of the visceral topographical relationship, the slices were kept warm in water at 37°C and then mounted whole on Superfrost glass slides. They were then dried at 37°C overnight. Series of 5 μm-thick sections were then created without intervals. We obtained a total of 150-320 sections from each fetus, depending on the age and size of the specimen.

Staining and immunolabeling

The first section of each level was taken as the reference and stained with Masson trichrome or hematoxylin-eosin to provide information about the topography and localization of anatomical structures (collagen tissue in dark blue, cytoplasm in red, nucleuses in black). Smooth muscles were detected with polyclonal antibodies against alpha smooth muscle actin (SMA)[13] and striated muscle with antibodies against myogenin (anti-myogenin)[14] (Supplemental Table).

Neuronal markers were detected with polyclonal antibodies against protein S100 for labeling all nerves, peripheral myelin protein (PMP22) for the somatic peripheral nervous system,[15] tyrosine hydroxylase (TH) for adrenergic nerves,[16] vesicular acetylcholine transferase (VAChT) for cholinergic neurons,[17] and the neural isoform of nitric oxide synthase (nNOS) for the proerectile nerve bundles[18] (Supplemental Table).

The avidin-biotin-peroxidase detection procedure was carried out with a Vectastain ABC kit (reference PK6100; Vector Laboratories, Burlingame, CA). Chromogenic detection was performed with a DAB detection kit (DAB, reference SK-4100; Vector Laboratories). Nonimmune serum or IgG at an equivalent dilution was used as a control for all the immunohistochemical analyses.

Histological examination

Serial stained and immunolabeled 2-dimensional sections were used for 3D reconstruction. Using high-magnification (×4-40) analyses of the Masson trichrome– and hematoxylin-eosin–stained sections, it was possible to identify the various anatomical structures (organs, bones, and fascia). Subsequent sections, treated with an antibody against S100, were used to identify pelvic-perineal nerves and communicating branches. By comparing the Masson trichrome–stained sections with sections stained with specific antibodies against SMA, myogenin, S100, TH, VAChT, PMP22, and nNOS, it was possible to determine the structure of muscle (smooth [SMA] or striated [myogenin]) and the nature of the nerve fibers identified: ie, somatic (PMP22), autonomic (TH, VAChT), or nitriergic (nNOS).

The sections were taken at almost the same level with a negligible interval between sections (5 μm). The computer system comprised a personal laptop computer (Windows XP, Microsoft, Redmond, WA) equipped with a digitization system (Perfection V750, Epson), digitization software (Silverfast AI, reference B11B178071), image-processing software (Photoshop, Adobe), and Surfdriver software for Windows (Winsurf image reconstruction software, Version 4.3). All sections were digitized at a resolution of 4800 dpi, and the images were then stacked and aligned. The brightness and contrast of the histological tissue images were adjusted using Photoshop (Adobe). The pelvic anatomical structures and nerve fibers were outlined manually on all histological sections. A 3D analysis of the location, course, and distribution of the nerve fibers and muscle structure was then carried out.

Electron microscopy

An additional fifth fetus was reserved for electron microscopy. The entire pelvis was removed en-bloc including the pelvic organ and pelvic bone. The bony pelvis was then separated from other muscular and fatty tissue using a binocular magnifying glass. The sample was sectioned in 4 parts: right anterior, left anterior, right posterior, and left posterior. Pelvic viscera (bladder, rectum, vagina) were removed.

The fragments were postfixed in 1% osmium tetroxide in buffer for 3 hours at 4°C and embedded in Epon. We used 1 μm-thick cross sections stained with thionin. Then ultrathin sections were stained with uranyl acetate and lead citrate. Electron microscopy was then performed to identify smooth actin. The positive control was human colon.

Results

Global morphology

The levator ani muscle inserted onto the pubis with an anteroposterior course and ended on the vaginal wall (for the pubovaginal muscle component of the pubovisceral muscle) and behind the rectum (for the puborectal muscle) describing muscular bundles. The iliococcygeal muscle joined via the anococcygeal raphe posterior to the rectum (Figure 1, A). [F1]

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