Innervation of the Levator Ani and Coccygeus Muscles of the Female Rat

RONALD E. BREMER,[1] MATTHEW D. BARBER,[2] KIMBERLY W. COATES,[3]
PAUL C. DOLBER,[1,4] AND KARL B. THOR[1,4,5]*
[1]Research Services, Veterans Affairs Medical Center, Durham, North Carolina
[2]Department of Obstetrics and Gynecology, Cleveland Clinic Foundation, Cleveland, Ohio
[3]Department of Obstetrics and Gynecology, Scott and White Clinic, Temple, Texas
[4]Department of Surgery, Duke University Medical Center, Durham, North Carolina
[5]Dynogen Pharmaceuticals, Inc., Durham, North Carolina

ABSTRACT

In humans, the pelvic floor skeletal muscles support the viscera. Damage to innervation of these muscles during parturition may contribute to pelvic organ prolapse and urinary incontinence. Unfortunately, animal models that are suitable for studying parturition-induced pelvic floor neuropathy and its treatment are rare. The present study describes the intrapelvic skeletal muscles (i.e., the iliocaudalis, pubocaudalis, and coccygeus) and their innervation in the rat to assess its usefulness as a model for studies of pelvic floor nerve damage and repair. Dissection of rat intrapelvic skeletal muscles demonstrated a general similarity with human pelvic floor muscles. Innervation of the iliocaudalis and pubocaudalis muscles (which together constitute the levator ani muscles) was provided by a nerve (the "levator ani nerve") that entered the pelvic cavity alongside the pelvic nerve, and then branched and penetrated the ventromedial (i.e., intrapelvic) surface of these muscles. Innervation of the rat coccygeus muscle (the "coccygeal nerve") was derived from two adjacent branches of the L6-S1 trunk that penetrated the muscle on its rostral edge. Acetylcholinesterase staining revealed a single motor endplate zone in each muscle, closely adjacent to the point of nerve penetration. Transection of the levator ani or coccygeal nerves (with a 2-week survival time) reduced muscle mass and myocyte diameter in the iliocaudalis and pubocaudalis or coccygeus muscles, respectively. The pudendal nerve did not innervate the intrapelvic skeletal muscles. We conclude that the intrapelvic skeletal muscles in the rat are similar to those described in our previous studies of humans and that they have a distinct innervation with no contribution from the pudendal nerve. Anat Rec Part A 275A:1031–1041, 2003. © 2003 Wiley-Liss, Inc.

Key words: pelvic floor; levator ani; pelvic organ prolapse; pudendal nerve

In human females, the pelvic floor muscles include the levator ani and coccygeus muscles (Hollinshead and Rosse, 1985). The levator ani is composed of the iliococcygeus, the pubococcygeus, and the puborectalis muscles. Dysfunction of these muscles may contribute to pelvic organ prolapse and/or incontinence, the prevalence of which is correlated with vaginal parity (DeLancey, 1988). In addition, a number of studies have demonstrated evidence of nerve damage in pelvic floor muscles of patients with pelvic organ prolapse (Hjartardottir et al., 1997; Bump and Norton, 1998; Wester and Brubaker, 1998). These two findings led to the proposal that parturition-induced nerve damage may be an etiologic factor in pelvic organ prolapse (Smith et al., 1989; Allen et al., 1990; Snooks et al., 1990; Barnick and Cardozo, 1993).

Anatomical texts and review articles suggest that the levator ani muscles are dually innervated by the pudendal nerve, which travels outside the pelvic cavity (i.e., extrapelvically) to reach its target muscles, and by branches of the S_3–S_5 spinal nerves, which travel within the pelvic

cavity (i.e., intrapelvic) to reach their target muscles (Hollinshead and Rosse, 1985; Wall, 1993; Strohbehn, 1998; Wester and Brubaker, 1998). However, this has never been verified experimentally, and the relative contributions (if any) from each source are unknown. For instance, postmortem dissection studies suggest that both the puborectalis muscle and the external anal sphincter are innervated via the pudendal nerve from the caudal (i.e., extrapelvic) side of the muscle (Shafik et al., 1995). However, electrophysiological studies in humans (Percy et al., 1981; Snooks and Swash, 1986), and neuroanatomic tracer studies in animals suggest that the levator ani is innervated via "sacral nerve roots" from the cephalic (i.e., intrapelvic) side of the muscle, without any contribution from the pudendal nerve (Schroder, 1980; Thuroff et al., 1982; Vanderhorst and Holstege, 1997). Furthermore, in our previous study of human cadavers, we were unable to demonstrate penetration of the levator ani or coccygeal muscles by the pudendal nerve (Barber et al., 2002).

In order to fully elucidate the impact that parturition and denervation injury have on the development, treatment, and prevention of pelvic floor dysfunction, a clearer understanding of the pattern of innervation of the levator ani in animal models is necessary. The primary objectives of this study were to determine the peripheral innervation of the levator ani and coccygeus muscles in the rat, and to specifically determine the contribution of the pudendal nerve to their innervation.

The present study refers to the levator ani in the rat as the muscle group composed of the iliocaudalis and pubocaudalis muscles, consistent with nomenclature in all other species. Unfortunately, numerous articles by various well-respected scientists have referred to the dorsal bulbospongiosis muscle in the rat as the "levator ani" (Forger et al., 1992; Jordan et al., 1997). This misnomer can be traced to a figure from a 1935 rat anatomy text (Greene, 1935) in which a line from the "levator ani" text label mistakenly indicated the dorsal bulbospongiosis muscle. Various investigators have called attention to this misnomer (Hayes, 1965; Holmes and Sachs, 1994; Poortmans and Wyndaele, 1998). In an effort to align the nomenclature in the rat with that in all other species, in the present work we will refer to the pubocaudalis and iliocaudalis muscles of the rat as the levator ani muscles.

METHODS

Pelvic Dissections

Gross dissection was performed on nine virgin female Charles River (Wilmington, MA) CD/SD rats (175–200 g). The rats were anesthetized with 65 mg/kg sodium pentobarbital (i.p.) and then perfusion-fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS). After fixation the rats were opened with a midline abdominal incision, and the thoracic and abdominal viscera were removed. The pelvic viscera were initially left in place. The innervation of the levator ani muscles was identified and traced from the point where the nerve penetrated the muscle to the L6-S1 trunk.

A dorsal incision was made through the skin over the lumbosacral vertebrae, and the superficial glutealis, external caudae medialis, transverse spinal muscles, and semitendinosus muscles were sectioned to allow access to the ischiorectal fossa and L6-S1 trunk. The L6-S1 trunk was traced from the vertebral foramina to the branch point of the levator ani nerve, pelvic nerve, pudendal

nerve, and coccygeus nerve, continuing on until the nerves penetrated the muscle.

Pieces of each nerve (1 cm in length) were fixed and embedded in Epon for sectioning (1 μm) and staining with toluidine blue to histologically verify the tissue as nerve, and allow cross-sectional measurements. After the innervation of the levator ani and coccygeal muscles was identified, the pelvic muscles were dissected to reveal their origins and insertions. (Nomenclature is based on that used by Brink and Pfaff (1980).)

Acetylcholinesterase Staining of the Motor Endplate Zone

To investigate the distribution of motor endplates, a variant of the Koelle stain for acetylcholinesterase (Karnovsky and Roots, 1964; Boriek et al., 1998) was used on unfixed muscles from six virgin female rats. Briefly, the rats were deeply anesthetized with isoflurane and killed by exsanguination. The abdomen was opened and the viscera were carefully removed to expose the surfaces of the levator ani and coccygeus muscles. For the iliocaudalis, this further required removal of the flexor caudae muscles that partially overlaid it. The pelves were then left overnight in a solution made by combining, in sequence, 130 ml 0.1 M phosphate buffer containing 0.05% acetyl thiocholine iodide (Sigma, St. Louis, MO), 10 ml of 0.1 M sodium citrate (Sigma), 20 ml of 30 mM cupric sulfate, 20 ml of distilled water, and 20 ml of 5 mM potassium ferricyanide (Sigma). After the pelves were rinsed three times with tap water, they were immersed overnight in ammonium sulfide (Sigma) until a brown reaction product could be clearly observed under a dissecting microscope. After the motor endplates were adequately delineated, which took approximately 5 min, the muscles were rinsed with tap water again, immersed in 4% paraformaldehyde in PBS, and photographed.

Neurectomy Effects on Myocyte Size and Muscle Mass

Nine female rats (175–200 g, three per group) were subjected to unilateral transection of the levator ani nerve (levator ani neurectomy), pudendal nerve (pudendal neurectomy), and coccygeal nerve (coccygeus neurectomy). Two weeks posttransection, the rats were perfusion-fixed with 4% paraformaldehyde in PBS, and the three intrapelvic skeletal muscles (the coccygeus, pubocaudalis, and iliocaudalis) were removed bilaterally. The muscles were weighed. Four transverse sections from the middle of each muscle were cut at 20 μm thickness on a cryostat and mounted on a series of five slides (i.e., 100 μm between each section). One slide from each series was subsequently stained with wheat germ agglutinin conjugated to tetramethylrhodamine isothiocyanate to label the myocyte surfaces and thereby enable the size of the myocytes to be determined (Dolber et al., 1994). The micrographs were viewed at a final magnification of 700× on the monitor screen.

Preliminary measurements indicated that a sample size of 100 myocytes/muscle provided a standard error of the mean that was within 10% of the mean. To obtain measurements on 100 myocytes, five myocyte diameters were measured in each of 20 equally-distributed videomicrographs taken of a single section. The five myocytes selected for measurement were those that had cellular

boundaries closest to the top of the micrograph and were completely contained within the micrograph.

Cross-sectioned myocyte profiles were outlined using the Scion Image 1.62 (Scion Corporation, Frederick, MD) software package, and the length of the minor axis of the best-fit ellipse was automatically determined for each profile. We used this length measurement as an estimator of myocyte "least diameter" (i.e., the shortest distance across the myocyte through its center). The least diameter is a preferred indicator of myocyte size because it compensates for oblique section angles (Dubowitz, 1985).

Differences in mean muscle mass and myocyte diameters were tested for statistical significance using Student's paired two-tailed *t-test*.

RESULTS
Intrapelvic Skeletal Muscles (Figs. 1–4)

Dissection of the rat pelvic cavity revealed the iliocaudalis muscle, with origin on the ilium and insertion on the caudal (Ca) Ca_5–Ca_6 vertebrae; the pubocaudalis muscle, with origin on the pubic bone and insertion on the Ca_3–Ca_4 vertebrae; and the coccygeus muscle, with origin on the pubic bone and insertion on the Ca_1–Ca_2 vertebrae. The coccygeus and pubocaudalis muscle fibers were interdigitated near their origin on the pubic bone, but were readily separated along most of their length. From their origin on the pubic bone to their insertion on the Ca_1–Ca_2 and Ca_3–Ca_4 vertebrae, respectively, the coccygeus and pubocaudalis muscles had dorsoventral and dorsocaudal fiber orientations, respectively (Figs. 2 and 3). This difference in fiber orientation allowed us to discriminate their individual origins on the pubic bone and determine that the origin of the coccygeus muscle was slightly lateral and caudal to that of the pubocaudalis. The orientation of the iliocaudalis muscle was primarily rostrocaudal, running from the ilium to the Ca_5–Ca_6 vertebrae (Figs. 1 and 2). At the point at which the muscles lay adjacent to each other, the iliocaudalis muscle was medial to the pubocaudalis muscle (Fig. 1), which in turn was medial to the coccygeus muscle (Figs. 2 and 3). A distinct puborectalis muscle could not be identified grossly. The pubic bone, intrapelvic skeletal muscles, and pelvic viscera were invested with areolar connective tissue, which joined them (Fig. 4).

Intrapelvic Skeletal Muscle Innervation

Dissection of the pelvis revealed a nerve that entered the pelvic cavity alongside the pelvic nerve (Fig. 4), projected caudally, and branched to penetrate the iliocaudalis and pubocaudalis muscles. This nerve will be referred to as the "levator ani nerve." Innervation of the coccygeus muscle separated from the L6-S1 trunk as two branches and penetrated the coccygeus muscle (Figs. 3 and 5). This innervation will be referred to as the "coccygeal nerve."

Levator ani nerve. The levator ani nerve and pelvic nerve separated from the L6-S1 trunk approximately 5 mm distal to the junction of the L6 and S1 spinal roots, and subsequently entered the pelvic cavity between the flexor caudalis brevis and iliocaudalis muscles (Figs. 1, 4, and 5). This entry point was just dorsal and medial to the internal iliac artery and vein. We identified the entry point by first distinguishing the rostral border of the pubic bone, near the junction of the ilium and pubic bone, to initiate dissection.

The much larger obturator nerve also served as a convenient landmark (Fig. 4). The levator ani nerve was always found in the pelvis 1–2 mm medial to the obturator nerve and about 1–2 mm rostral to the point where the obturator nerve entered the obturator foramen. In three rats, the pelvic and levator ani nerves were distinct nerve fascicles from their origin along the L6-S1 trunk to their termination. In the other six animals, the nerves separated from the lumbosacral trunk as a single entity and traveled together until they entered the pelvic cavity, at which point they were consistently distinct fascicles traveling alongside each other. The levator ani and pelvic nerves were similar in size (about 0.2–0.5 mm in diameter).

Within 1–3 mm of entering the pelvic cavity, the levator ani nerve and pelvic nerve diverged, with the pelvic nerve continuing ventromedially toward the major pelvic ganglion and the levator ani nerve turning caudally to run along the ventromedial surface of the iliocaudalis muscle (Fig. 4). Within 1–3 mm after separating from the pelvic nerve, the levator ani nerve bifurcated to innervate the iliocaudalis and pubocaudalis muscles. We refer to these separate branches of the levator ani nerve as the iliocaudalis and pubocaudalis nerves, respectively. The iliocaudalis nerve immediately penetrated the "intrapelvic" (i.e., ventromedial) surface of the iliocaudalis muscle as one to three fascicles. The pubocaudalis nerve traveled ventrocaudally along the fascial plane between the iliocaudalis and pubocaudalis muscles, and penetrated the intrapelvic surface of the pubocaudalis muscle as one to three fascicles about midway between the origin and myotendinous junction. Light microscopic examination of plastic-embedded sections showed that the pubocaudalis nerve contained both large and small myelinated axons (Fig. 6A).

Coccygeal nerve. The coccygeal nerve was comprised of two branches (each about 100 μm diameter (Fig. 6B)) that emerged from the sacral plexus about 1–2 mm distal to the point where the levator ani and pelvic nerves had emerged (Fig. 5). These nerves traveled caudally about 2–4 mm and penetrated the rostral edge of the coccygeus muscle midway between its origin and tendons.

Pudendal nerve. The pudendal nerve emerged from the sacral plexus about 2–4 mm distal to the branch point of the coccygeus nerve (Fig. 5). Dissection of the pudendal nerve in the current study corroborates previous studies (McKenna and Nadelhaft, 1986) describing its course from the L6-S1 trunk to the striated urethral sphincter, striated anal sphincter, clitoris, and perineum. We specifically searched for branches of the pudendal nerve that innervated intrapelvic skeletal muscles, but found none. Although the myelinated axons in the motor branch of the pudendal nerve (Fig. 6C) are not yet quantified, they did not appear to be as large as those in the pubocaudalis (Fig. 6A) or coccygeal nerve (Fig. 6B).

Motor Endplate Zone

The Koelle technique consistently revealed a single band of intense acetylcholinesterase staining (by definition, the motor endplate zone) across the width of each of the intrapelvic skeletal muscles (Fig. 7). In the iliocaudalis muscle, the motor endplate zone was U-shaped, with the apex toward the origin. In the pubocaudalis and coccygeus muscles, the motor endplate was a straight band that was
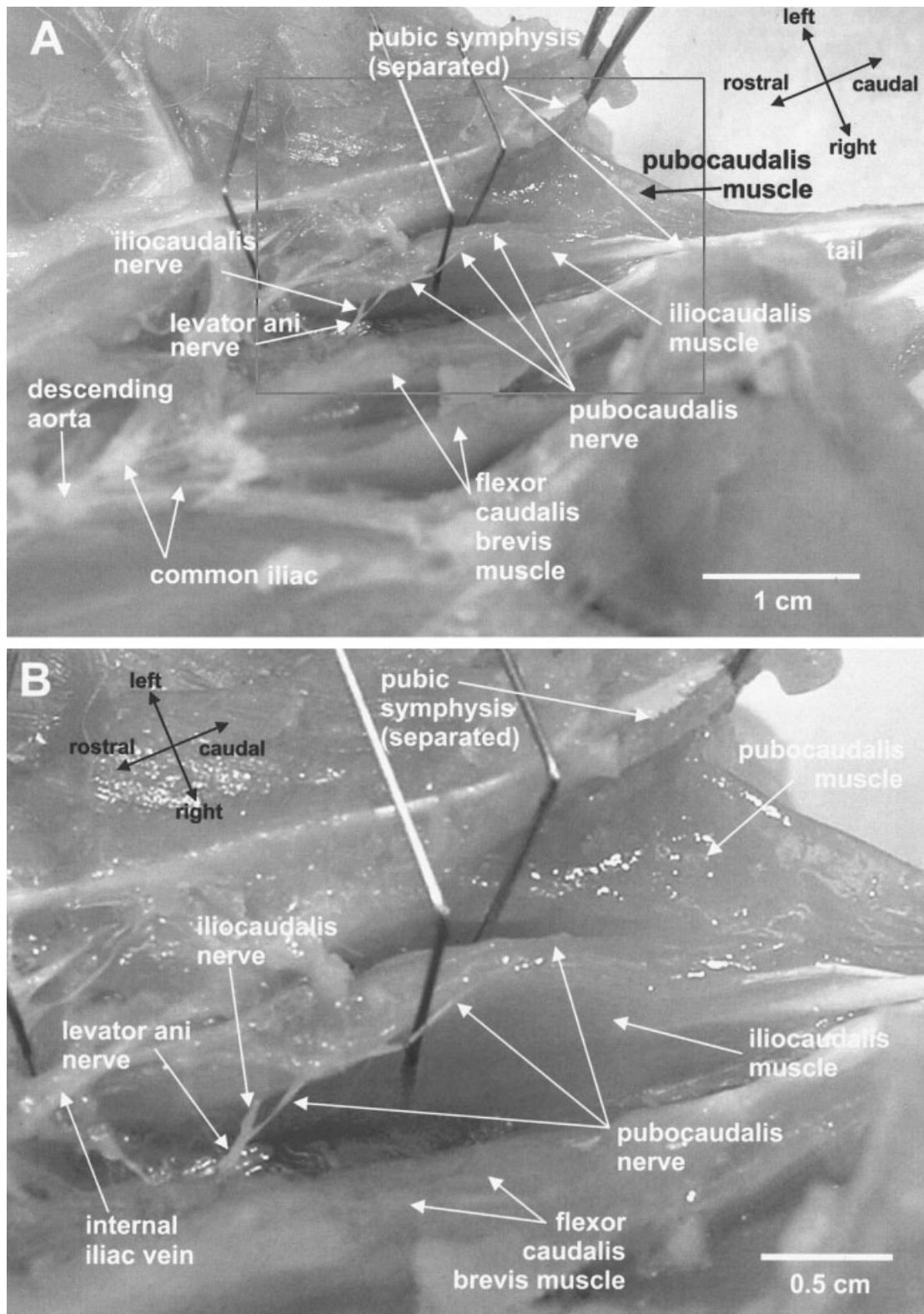
Fig. 1. Ventral view of the intrapelvic musculature after division of the pubic symphysis, removal of pelvic viscera, and separation of the iliocaudalis and pubocaudalis muscles. The dashed box in panel **A** indicates the area shown at higher magnification in panel **B**. Note the levator ani nerve entering the pelvic cavity between th flexor caudalis brevis and iliocaudalis muscles. Shortly after the nerve enters the cavity, it divides into the iliocaudalis nerve, which immediately penetrates the iliocaudalis muscle and the pubocaudalis nerve, which runs caudally along the fascial plane between iliocaudalis and pubocaudalis before it penetrates the pubocaudalis muscle. The pubocaudalis nerve is hidden behind the iliocaudalis muscle shortly after it passes the dissecting pin, but can be seen again in Figure 2.
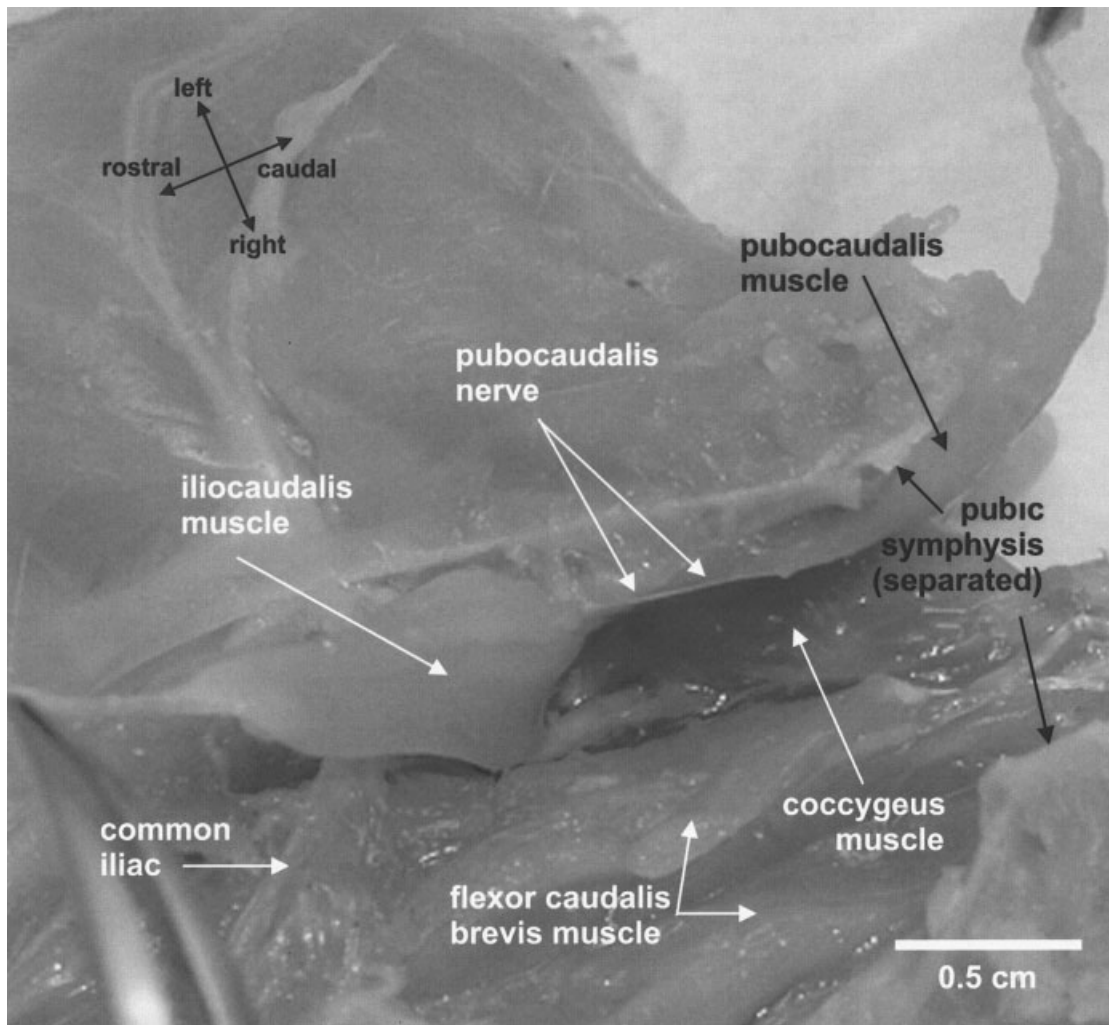
Fig. 2. Same specimen shown in Figure 1 after insertions of iliocaudalis and pubocaudalis muscles were cut and reflected to allow visualization of the underlying coccygeus muscle. Note the continuation of the pubocaudalis nerve across the iliocaudalis muscle to the pubocaudalis muscle.

consistently located close to the middle of the muscle. The location of the motor endplate zone correlated with the point where the nerve penetrated the epimysium of the muscle.

Effects of Selective Neurectomies

In a live rat, the levator ani nerve prior to branching, and the most central branch of the iliocaudalis nerve were extremely difficult to reach using an abdominal surgical approach (Figs. 1 and 4), due to interference from the internal iliac artery and vein, and the levator ani nerve trunk was impossible to distinguish from the pelvic nerve using an ischiorectal fossa approach (Fig. 5). Therefore, we were unable to obtain a reliable denervation of the iliocaudalis muscle. Postmortem analyses of our levator ani neurectomies indicated that the transections occurred distal to the point at which the most central branch of the iliocaudalis nerve separated from the levator ani nerve trunk. Thus, our "levator ani" neurectomies often yielded only a partial denervation of the iliocaudalis muscle, but complete denervation of the pubocaudalis muscle. The

coccygeal nerve and the pudendal nerve were easily accessible in the live animal.

Tables 1 and 2 summarize the effects of the levator ani, coccygeal, and pudendal neurectomies on the mass and myocyte diameters of the iliocaudalis, pubocaudalis, and coccygeus muscles. Statistically significant muscle atrophy and myocyte shrinkage occurred in the pubocaudalis muscle ipsilateral to a levator ani nerve transection, while the coccygeus muscle showed statistically significant muscle atrophy and myocyte shrinkage ipsilateral to a coccygeal nerve transection. Although the mean muscle mass and myocyte diameter were reduced in the iliocaudalis muscle ipsilateral to the levator ani nerve transection, this did not reach statistical significance. A pudendal neurectomy produced no statistically significant changes in the muscle mass or myocyte diameter of any pelvic floor muscles.

DISCUSSION

The major findings from this study can be summarized as follows: The levator ani nerve originates from the L6-S1
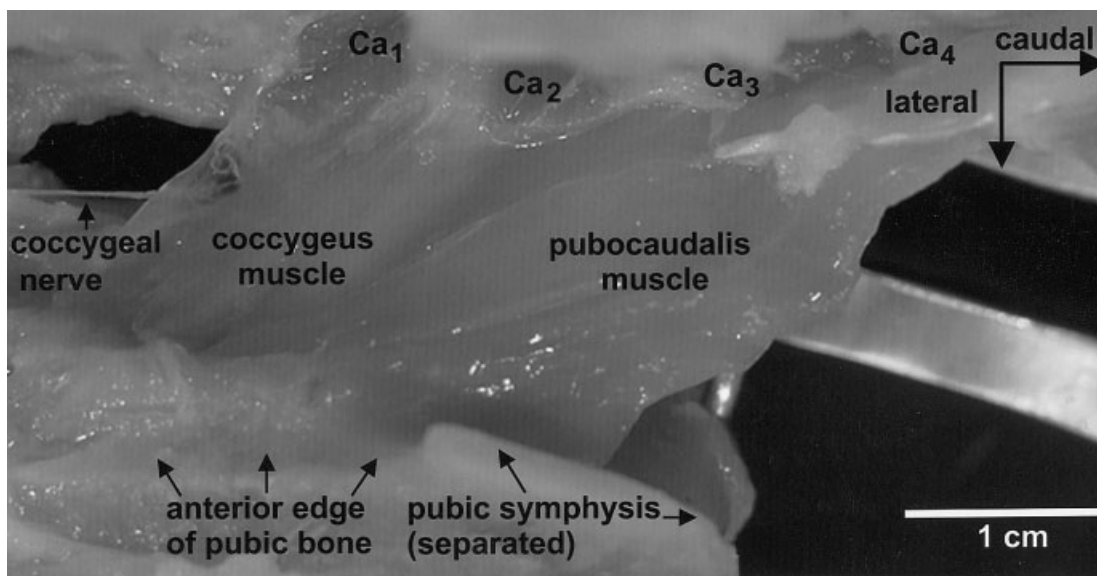
Fig. 3. Medial view of the right pubocaudalis and coccygeus muscles after division of the pubic symphysis and removal of viscera, showing overlapping origins from pubic bone and distinct insertions onto the Ca_{3-4} and Ca_{1-2} vertebrae, respectively.

spinal roots and enters the pelvis alongside the pelvic nerve. It then branches into the pubocaudalis and iliocaudalis nerves, which penetrate their respective muscles on their intrapelvic side. The coccygeal nerve also originates from the L6-S1 spinal roots, but does not enter the pelvic cavity along with the levator ani nerve. No evidence of pudendal nerve innervation of the intrapelvic skeletal muscles was observed. The intrapelvic skeletal muscles each contain only a single motor endplate zone, which is associated with the point at which the respective nerves penetrate the epimysium of each muscle. Selective neurectomies demonstrated atrophy of the respective muscles, while a pudendal neurectomy produced no evidence of atrophy. It is reasonable to conclude that the pudendal nerve does not innervate the levator ani or coccygeus muscles in the rat.

In humans, the pelvic floor muscles are comprised of the levator ani and coccygeus muscles (Hollinshead and Rosse, 1985). The major subdivisions of the levator ani muscles are the iliococcygeus and pubococcygeus muscles. A similar overall structural organization of the intrapelvic skeletal muscles was found in the rat. The primary interspecies differences in levator ani muscles are that 1) the rat iliocaudalis muscle originates from the ilium, while the human iliococcygeus muscle primarily originates from the arcus tendineus (which does not exist in rats); and 2) the rat pubocaudalis and iliocaudalis muscles have tendons that attach directly to the caudal vertebrae, while the human pubococcygeus and iliococcygeus muscles primarily attach to connective tissue (i.e., the anococcygeal ligament) that subsequently attaches to the coccyx. The primary interspecies difference in the coccygeus muscle is that the rat coccygeus muscle has its origin from the pubic bone and the human coccygeus muscle has its origin from the ischial spine. A further difference is that each muscle is more distinct (i.e., easily separated) in rats compared to humans, where the muscles are more integrated. It is a general feature that pelvic muscle groups are more integrated or "blended" as the phylogenetic scale is ascended (Wilson, 1973).

Anatomical textbooks and publications routinely indicate that human levator ani muscles receive innervation not only from the S3-S5 spinal roots, which penetrate the muscle's intrapelvic (or superior) surface, but also from the pudendal nerve, which penetrates the extrapelvic (or inferior) surface (Hollinshead and Rosse, 1985; Wall, 1993; Strohbehn, 1998; Wester and Brubaker, 1998). Our previous studies of human cadavers (Barber et al., 2002) support the notion that sacral root innervation courses within the pelvic cavity to penetrate the intrapelvic surface of the iliococcygeus and pubococcygeus muscles. However, pudendal nerve innervation of the levator ani muscles was not found in those studies. In general, the present findings in rats were similar to our observations in humans in that only a single innervation, derived from the lumbosacral plexus, entered the pelvic cavity to penetrate the levator ani muscles from the intrapelvic surface. The rat coccygeus muscle was innervated along its rostral edge, and thus the point of penetration could not be called either intra- or extrapelvic. The human coccygeus nerve penetrates the muscle from the intrapelvic side, as well as along its anterior (or rostral) portion (Barber et al., 2002).

A minor difference between the innervation of the levator ani and coccygeus muscles of rats and humans is that the innervation originates from the L6-S1 spinal roots in rats and the S3-S5 spinal roots in humans. This difference is consistent with the differences in origin of other nerves of the pelvis—for example, the pelvic and pudendal nerves, which also originate from the L6-S1 spinal segments in the rat, but from the more caudal sacral segments in humans (Nadelhaft and Booth, 1984; McKenna and Nadelhaft, 1986).

In the present study, as in our previous study in humans, we refer to the innervation of the levator ani mus-
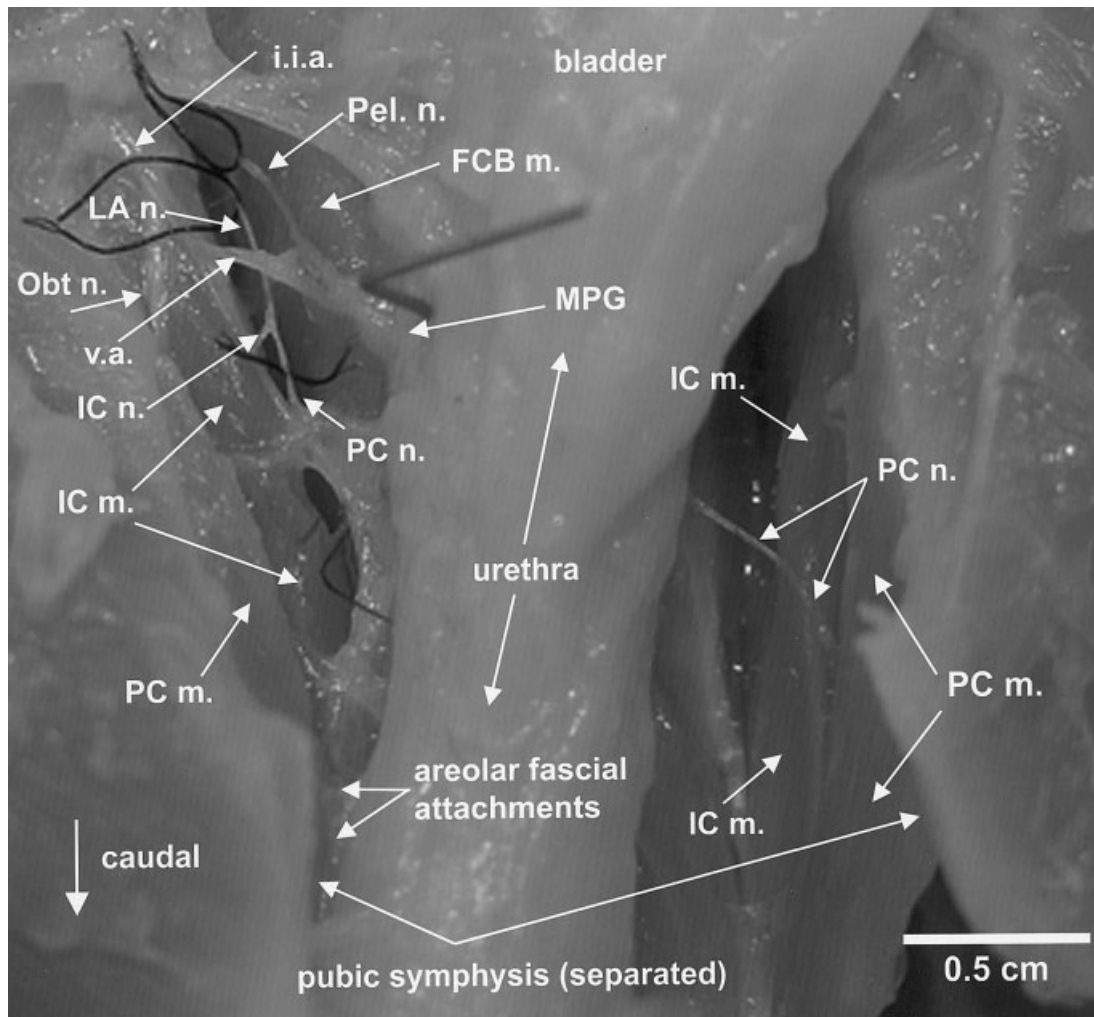
Fig. 4. Ventral view of the abdomen after division of the pubic symphysis, showing intrapelvic skeletal muscles and their nerves in relationship to pelvic viscera. The bladder has been slightly retracted to show the areolar fascial attachments between urethra, iliocaudalis muscle, pubocaudalis muscle, and pubic bone. FCB m. = flexor caudalis brevis muscle; i.i.a. = internal iliac artery; IC m. = iliocaudalis muscle; IC n. = iliocaudalis nerve; LA n. = levator ani nerve; MPG = major pelvic ganglion; Obt. n. = obturator nerve; PC m. = pubocaudalis muscle; PC n. = pubocaudalis nerve; Pel n. = pelvic nerve; v.a. = vesical artery.

cles as the "levator ani nerve." To indicate the subsequent branching of the levator ani nerve into distinct fascicles that penetrate the iliocaudalis and pubocaudalis muscles, the terms "iliocaudalis nerve" and "pubocaudalis nerve," respectively, are proposed. Similarly, we propose that the innervation of the coccygeus muscle be called the "coccygeal nerve." This nomenclature was adopted over the older nomenclature of "S3-S5 spinal roots" (human) or "L6-S1 spinal roots" (rat) for the following reasons. First, the S3-S5 (human) or L6-S1 (rat) spinal roots can provide innervation to structures other than the levator ani muscles (Hollinshead and Rosse, 1985), and thus the terms are ambiguous. Second, the innervations of all other muscles (except possibly the axial muscles) have been named (Hollinshead and Rosse, 1985). Third, names (as opposed to spinal root numbers) are convenient in that they allow one to easily and unambiguously discuss these nerves. Fourth, providing specific names may increase awareness among physicians and scientists that these muscles have a dis-

tinct innervation, and facilitate better understanding of the pathophysiology of the pelvic floor. Finally, the proposed nomenclature is also favored for its brevity. For example, "coccygeus nerve" is simpler to say or write than "the innervation of the coccygeus muscle."

Previous studies of pelvic floor muscle innervation used various names to describe nerves that may innervate the levator ani muscles. These various names include "the intrapelvic somatic nerve" (Borirakchanyavat et al., 1997), "the somato-motor branch of the pelvic nerve" (Pacheco et al., 1989, 1997), "the intrapelvic branch of the pudendal nerve" (Hollabaugh et al., 1997), and the "extra-pudendal nerve" (Zvara et al., 1994). These terms may be ambiguous and confusing. For example, the pelvic nerve is commonly and classically accepted as a parasympathetic nerve that innervates the pelvic viscera (Langley and Anderson, 1895). Thus, referring to the levator ani innervation as the "somatic branch of the pelvic nerve" could generate confusion with the classical literature, and it is
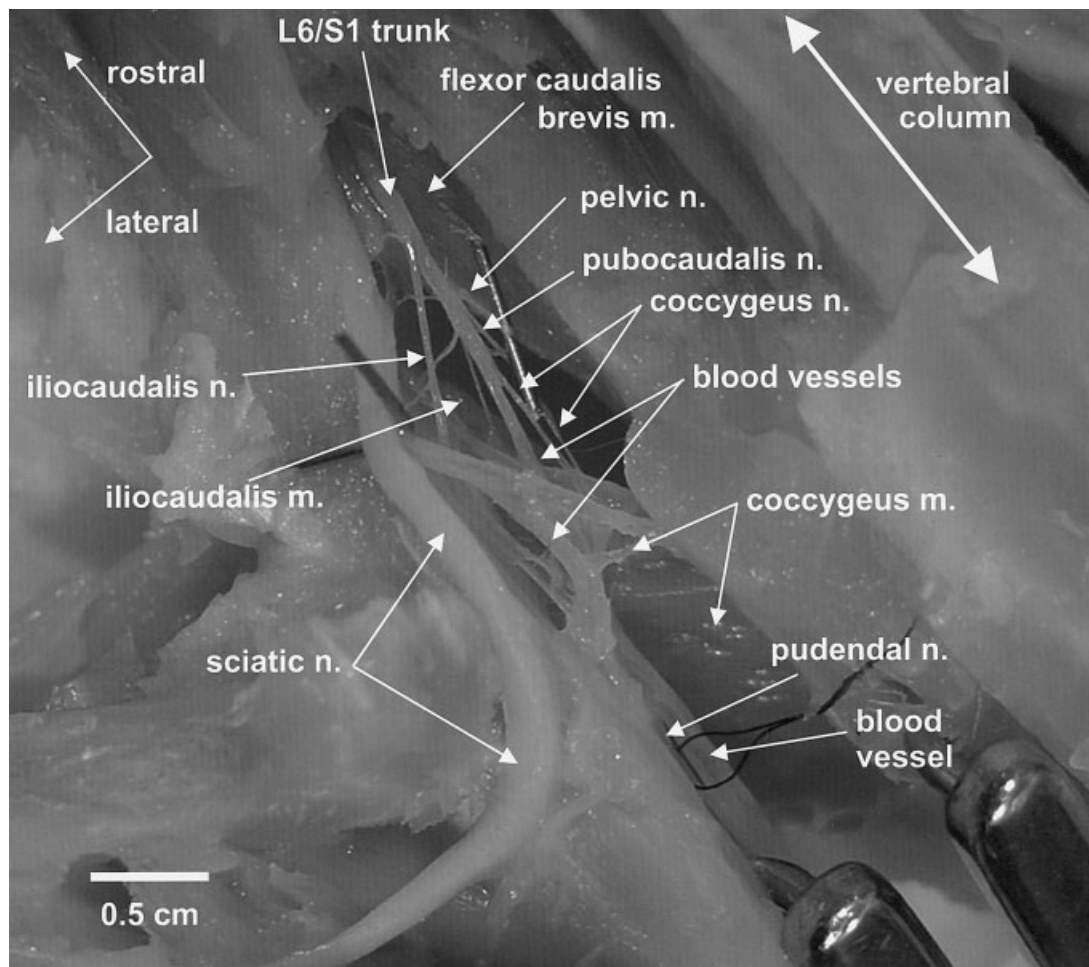
Fig. 5. Dorsal view of the ischiorectal fossa and surrounding area after removal of external hip muscles, separation of the iliosacral junction, and separation of the flexor caudalis brevis and iliocaudlis muscles, showing the lumbosacral trunk and nerves derived from it. The levator ani nerve is hidden behind the L6/S1 trunk, and the branch point of the iliocaudalis and pubocaudalis nerves can not be seen. Note the penetration of two coccygeus nerve branches penetrating the rostral edge of the coccygeus muscle.

ambiguous because it does not tell which somatic structures in the pelvic cavity (e.g., tail flexors, vertebral muscles, rhabdosphincters, levator ani, etc.) are innervated. Similarly, a combination with the pudendal nerve name will only generate confusion. As indicated by the sheer number of names, and the fact that none of these previous names have been accepted into usage by anyone other than the originators, it is obvious that a commonly accepted term for the innervation of the levator ani muscles is needed. We recommend the simple and explicit "levator ani nerve." This term can be used to describe the nerve from the lumbosacral plexus to where it terminally branches into the iliocaudalis nerve and pubocaudalis nerve.

In this study, selective neurectomies were performed to verify that a specific nerve provided innervation of a specific muscle by demonstrating that the target muscle exhibited a reduced mass and myocyte fiber diameter (i.e., atrophy). This was readily achieved in the pubocaudalis and coccygeus muscles following levator ani and coccygeus neurectomies. However, less consistent results were ob-

tained in the iliocaudalis muscle following levator ani neurectomy. This inconsistency was due to difficulties encountered when we tried to surgically isolate and identify all branches of the iliocaudalis nerve. Some branches separated from the main trunk of the levator ani nerve within 1 mm of penetrating the pelvic cavity, which made them inaccessible in a living animal in which the internal iliac vein could not be reflected. In other words, it is extremely difficult to access all iliocaudalis nerve branches in vivo.

As mentioned above, evidence for pudendal nerve innervation of the levator ani muscles was not found in either of our previous human studies (Barber et al., 2002) or the present rat studies (or ongoing studies in squirrel monkeys). In the present study, we stained for acetylcholinesterase to reveal motor endplate zones (Karnovsky and Roots, 1964; Boriek et al., 1998) in the pubocaudalis and iliocaudalis muscles, and the results indicated an absence of pudendal innervation. This technique revealed that only a single motor endplate zone could be identified in these muscles. Furthermore, the location of the motor endplate zone in each muscle showed an obvious correla-
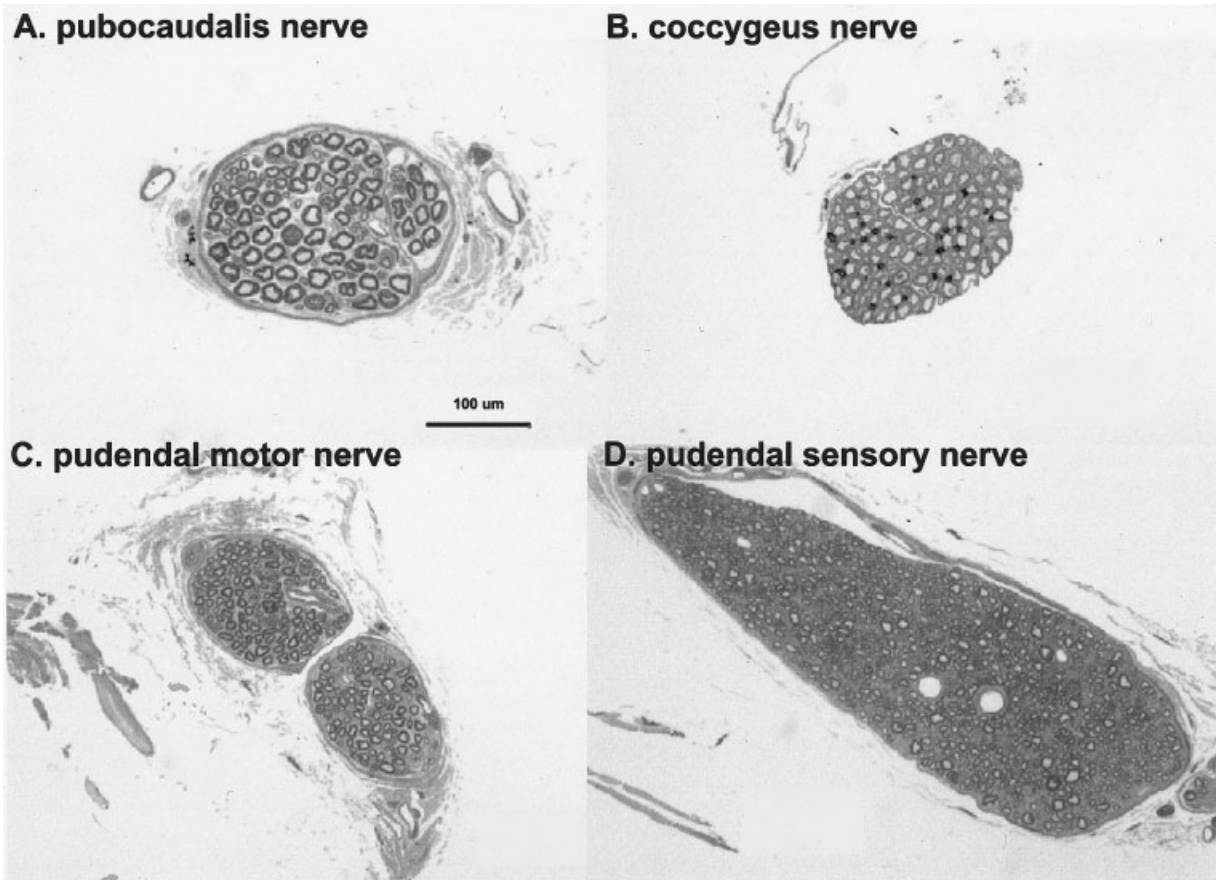
Fig. 6. Cross sections of the pubocaudalis (**A**), coccygeus (**B**), pudendal motor (**C**), and pudendal sensory (**D**) nerves stained with toluidine. Note that the largest fibers in the pubocaudalis and coccygeus nerves are greater than the largest fibers in the pudendal motor nerve. Magnification is the same for all four panels, and is referenced in panel A.
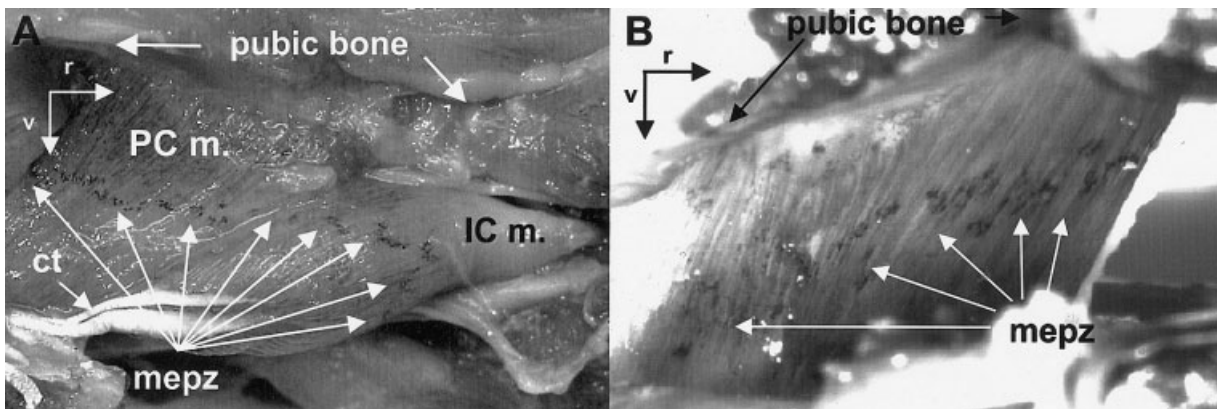

Fig. 7. Motor endplate zone of iliocaudalis and pubocaudalis (**A**), and coccygeus (**B**) muscles stained for acetylcholinesterase. Medial view of iliocaudalis and pubocaudalis (A) and coccygeus (B) muscles after hemisection of the pelvis and removal of viscera. In part A, the flexor caudalis brevis muscle has been removed. In B, the iliocaudalis and pubocaudalis muscles have been removed. ct = central tendon of iliocaudalis muscle; ic = iliocaudalis muscle; mepz = motor endplate zone; pc = pubocaudalis muscle; r = rostral; v = ventral.

tion with the point at which the respective branches of the levator ani nerve or coccygeus nerve penetrated the muscle. If the pudendal nerve penetrated the pubocaudalis or iliocaudalis muscles from the perineal side of the pelvis (as classically described in anatomy texts), closer to their insertions, and provided secondary innervation of the

TABLE 1. Effect of selective neurectomy on muscle mass (g)

Neurectomy	Side	Pubocaudalis	Iliocaudalis	Coccygeus
Levator ani	Ipsi	0.07 ± 0.01	0.12 ± 0.02	0.11 ± 0.01
	Contra	0.16 ± 0.02	0.19 ± 0.02	0.10 ± 0.01
	P	**0.05**	0.21	0.48
Coccygeal	Ipsi	0.19 ± 0.03	0.20 ± 0.01	0.09 ± 0.01
	Contra	0.20 ± 0.03	0.18 ± 0.01	0.13 ± 0.01
	P	0.29	0.19	**0.04**
Pudendal	Ipsi	0.12 ± 0.01	0.19 ± 0.01	0.11 ± 0.02
	Contra	0.14 ± 0.01	0.17 ± 0.02	0.12 ± 0.02
	P	0.16	0.20	0.34

Mean muscle mass (g ± SE) in the ipsilateral (ipsi) and contralateral (contra) pelvic muscles following selective unilateral nerve transections. *P* values are based on statistical comparisons between the ipsi and contra muscles. (See Methods for details.) Bold numbers indicate statistical significance.

TABLE 2. Effect of selective neurectomy on myocyte diameter (μm)

Neurectomy	Side	Pubocaudalis	Iliocaudalis	Coccygeus
Levator ani	Ipsi	22.2 ± 1.2	31.8 ± 5.0	29.8 ± 1.7
	Contra	34.9 ± 0.6	42.0 ± 1.9	30.2 ± 3.1
	P	**0.012**	0.100	0.893
Coccygeal	Ipsi	37.1 ± 2.5	37.7 ± 5.2	20.0 ± 3.8
	Contra	34.8 ± 1.6	38.4 ± 2.2	30.8 ± 3.2
	P	0.215	0.788	**0.002**
Pudendal	Ipsi	34.5 ± 2.4	38.0 ± 0.8	29.1 ± 0.5
	Contra	33.0 ± 0.7	36.0 ± 2.3	29.8 ± 2.3
	P	0.608	0.516	0.652

Mean myocyte diameter (μm ± SE) in the ipsilateral (ipsi) and contralateral (contra) pelvic muscles following selective unilateral nerve transections. *P* values are based on statistical comparisons between the ipsi and contra muscles. (See Methods for details). Bold numbers indicate statistical significance.

muscles, it is logical to assume that an additional motor endplate zone would have been seen in those regions of muscle. Finally, our findings that pudendal neurectomy did not reduce muscle mass or myocyte diameter in any of the intrapelvic skeletal muscles also supports our conclusions that the pudendal nerve does not innervate the levator ani or coccygeus muscles.

Axonal tracing studies in the rat (Schroder, 1980; McKenna and Nadelhaft, 1986; Manzo et al., 1999), cat (Thor et al., 1989b; Vanderhorst and Holstege, 1997), dog (Thuroff et al., 1982), and monkey (Roppolo et al., 1985) also support our conclusion that the levator ani is not innervated by the pudendal nerve. Various studies (Roppolo et al., 1985; Thor et al., 1989b; McKenna and Nadelhaft, 1989) have shown that virtually all motor neurons that are labeled following application of retrograde tracers to the pudendal nerve are located in distinct, well-circumscribed nuclei (e.g., the dorsolateral and dorsomedial nuclei in rats, and cell group Y of Romanes in cat) that are homologues of Onuf's nucleus in humans. In contrast, motor neurons labeled following injection of tracers into the levator ani muscles are dispersed throughout the ventral horn of the sacral spinal cord (or lumbosacral spinal cord in rats), and not in Onuf's nucleus homologues. In addition, these studies showed that the levator ani motor neurons are morphologically distinct from pudendal motor neurons. Finally, electrical stimulation of the pudendal nerve does not produce contraction of the levator ani muscles (Pacheco et al., 1997).

Based on both anatomical and physiological studies of the pudendal nerve in the various species described above, researchers have formed a general consensus that this nerve contains somatic motor axons only from neurons in Onuf's nucleus (and species-specific homologues) that innervate the urethral or anal rhabdosphincters, or the bulbospongiosus or ischiocavernosus muscles. Therefore, we decided that the pudendal nerve should be distinguished from the innervation of the levator ani and coccygeus muscles. This distinction is equivocal because the pudendal nerve and the levator ani and coccygeal nerves eventually form a common trunk in the sacral plexus (Fig. 5). In other words, one could consider the levator ani and coccygeal nerves as branches of the pudendal nerve, depending on where one decides that the L6-S1 trunk ends and the pudendal nerve begins. In fact, in the electrophysiological studies of Pacheco et al. (1997), they considered the coccygeal innervation to be branches of the pudendal nerve. However, since most other electrophysiological (McKenna and Nadelhaft, 1989; Thor et al., 1989a) and all tracing studies (Ueyama et al., 1984; Kawatani et al., 1986; McKenna and Nadelhaft, 1986; Thor et al., 1989b) isolated the pudendal nerve in the ischiorectal fossa distal to the branch point of the levator ani and coccygeal nerves, these studies excluded the levator ani and coccygeal nerve fibers from analyses. Thus, distinguishing the levator ani and coccygeal nerves from the pudendal nerve by the use of separate names will prevent confusion in interpreting the conclusions of previous studies.

In summary, in this study we described the intrapelvic skeletal muscles in the rat and compared them with the pelvic floor muscles in humans. The innervation of the intrapelvic muscles was described by dissection, and corroborated with nerve lesion experiments and motor endplate identification. The term "levator ani nerve" is pro-

posed to indicate the innervation of the levator ani muscles as it enters the pelvis, and the terms "iliocaudalis nerve" and "pubocaudalis nerve" are proposed to designate the two terminal branches of the levator ani nerve that innervate the iliocaudalis and pubocaudalis muscles, respectively. Finally, the "coccygeal nerve" is proposed as the name for the innervation of the coccygeus muscle.

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