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The Innervation of the External Urethral Sphincter; An Ultrastructural Study in Male Human Subjects

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Summary. Innervation of the external urethral sphincter (EUS) was studied in male human subjects. In the region of EUS at the distal end of prostatic urethra, a large axon bundle surrounded by perineurium was evident in the intramural connective tissue gap. Because of the presence of dense core vesicles, the small nonmyelinated axon profiles in the bundle were considered to be adrenergic. After ramifications to smooth musculature, the axons were traced to the EUS. In the EUS, axon bundles containing many nonmyelinated axons were recognized as a sole autonomic nerve among the striated muscle cells. A single or at most two or three axons were surrounded by a Schwann cell, and some possessed dense core vesicles which suggested an adrenergic function. These autonomic adrenergic nerve ends formed surface junctions with the striated muscle of EUS. The clinical relevance of these data are discussed.

Key words: External urethral sphincter, Adrenergic innervation, Surface junction.

The part of the EUS located distally in the urethra is crucial for the maintenance of urinary continence after prostatectomy [12, 28]. In neurogenic vesical dysfunction this area is responsible for dyssynergia of somatic [16] or sympathetic nature [1, 17]. There is agreement as to the innervation of the EUS region by autonomic sympathetic nerves. This has been confirmed by many different investigators, both histochemically [6, 26, 29] and ultrastructurally [6, 30]. Considering the innervation of the EUS, some debate exists although there is general acceptance of the EUS as an intrinsic urethral rhabdosphincter [3, 7, 21]. Tanagho insists that the somatic pudendal nerve is the principal innervation system with its motor neuron nucleus of Onuf juxtaposed to the sacral pelvic nerve [27]. Gosling and Dixon dispute the adrenergic component of the innervation of the EUS, although they claim that the autonomic pelvic nerve contains efferent motor neurons to the EUS [10]. In 1974 Elbadawi and Schenk proposed the triple innervation theory of EUS based on a histochemical study [5]. This theory has subsequently been confirmed by an electron microscopic study of male rats by Watanabe and Yamamoto [30] and most recently in male cats by Elbadawi [6] and Elbadawi and Atta [8]. Because of the paucity of morphological data on the innervation of the EUS in human subjects, coupled with our own experience [14, 18] on neurogenic vesical dysfunction which functionally correlates to the aforementioned animal studies, the innervation of the EUS and its region was studied electron microscopically in male humans.

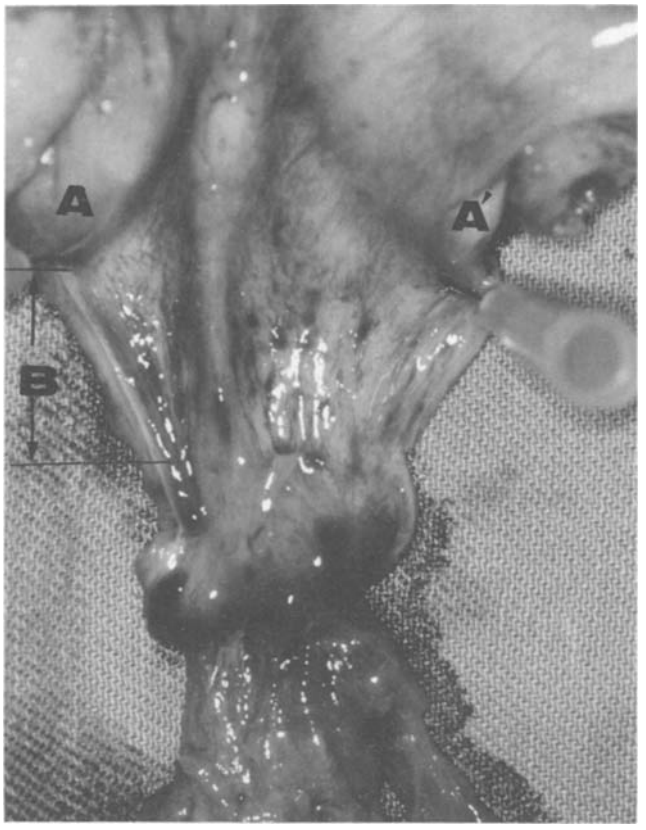
Materials and Methods

Adult human male urethra were used. Urethra was obtained at the time of total cystectomy for bladder tumor. For the purpose of the study the urethra was separated from the bladder neck and was further divided into two parts. The first corresponded to the distal end of prostatic urethra (McNeal's peripheral zone) and to the region of EUS described in this study, and the second was the EUS itself (Fig. 1). For electronmicroscopy the tissues were fixed for 2 h in 0.1 M phosphate buffer containing 2.5% glutaraldehyde, and then post-fixed for 2 h in 2% osmium tetroxide in the same buffer. After dehydration in ethanol the tissues were embedded in epoxy resin. Thin sections were cut and stained with 3% aqueous uranyl acetate followed by lead solution. Several sets 5–10 μm of thin sections were cut and mounted on Cu grids. 5-hydroxydopamine was not injected preoperatively. For the light microscopic study, several sections were cut from epoxy resin embedded tissue and stained in heated 1% toluidine blue.

Results

1. The Distal End of Prostatic Urethra – The Region of the External Urethral Sphincter

In the light microscopic (L.M.) study, a large axon bundle surrounded by perineurium was recognized. The fibers were mostly unmyelinated, though a few were myelinated in the

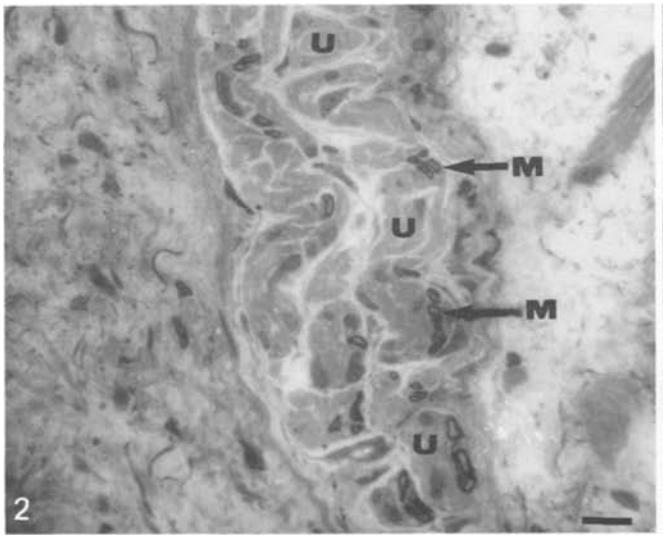


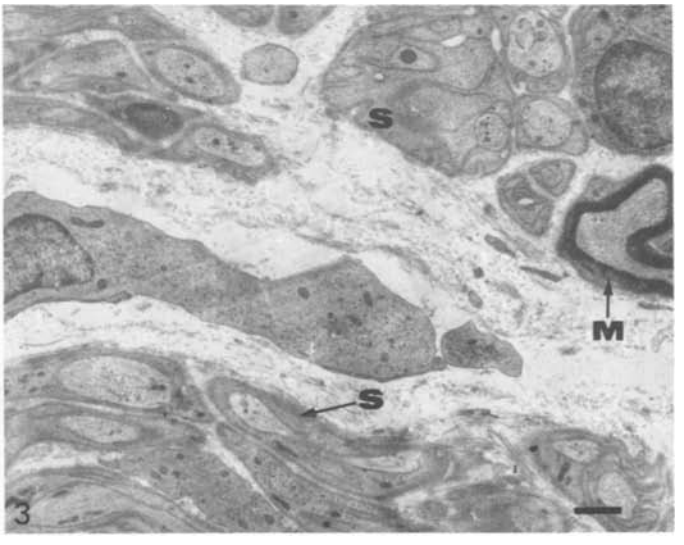
wide connective tissue space between the smooth muscle layer (Fig. 2). In the electron microscopic (E.M.) study, each axon bundle consisted of five or more unmyelinated axons and was surrounded by a Schwann cell (Fig. 3). Such bundles divided into finer branches as they proceeded into the narrow spaces between the smooth muscle cells. The finest branches contained a single, or at most two or three axons. These preterminal axons contained many small granular vesicles (about 50 nm in diameter) and large granular vesicles (about 100 nm) (Fig. 4). These were considered to be adrenergic nerves. There were no axons containing small agranular vesicles, considering to be cholinergic, in the connective tissue between the smooth muscle cells. These autonomic nerves, mostly adrenergic, were recognized to run distally towards the EUS. Some myelinated axons contained in the large fiber became unrecognizable, probably terminating in intramural ganglia in the prostatic urethra.

2. External Urethral Sphincter

Among the striated muscle cells, a large axon bundle surrounded by perineurium was recognized (more than 7 μm

◀ **Fig. 1.** The urethral specimen was bivalved at 12 o'clock position to expose the distal sphincteric zone. The region of EUS (*A* and *A'*) and EUS per se (*B*)





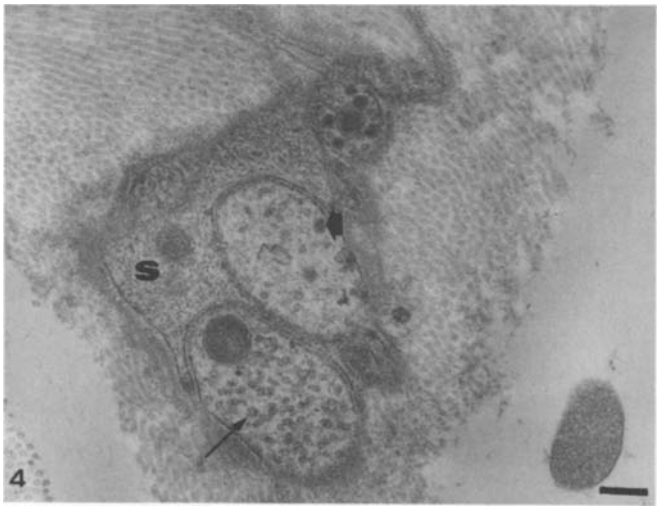
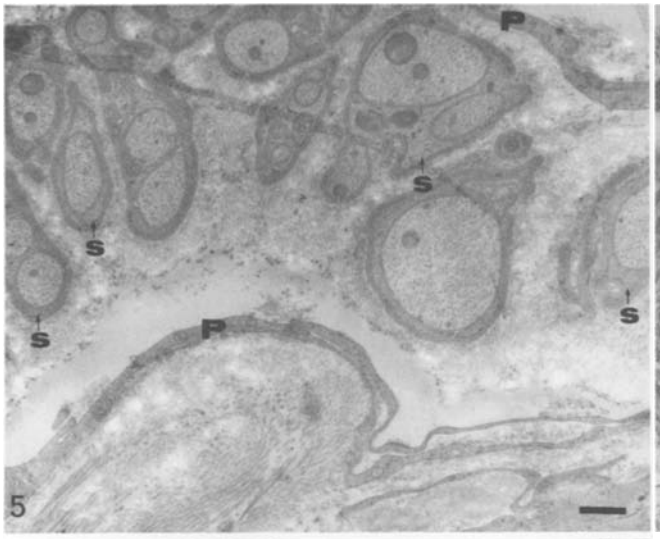
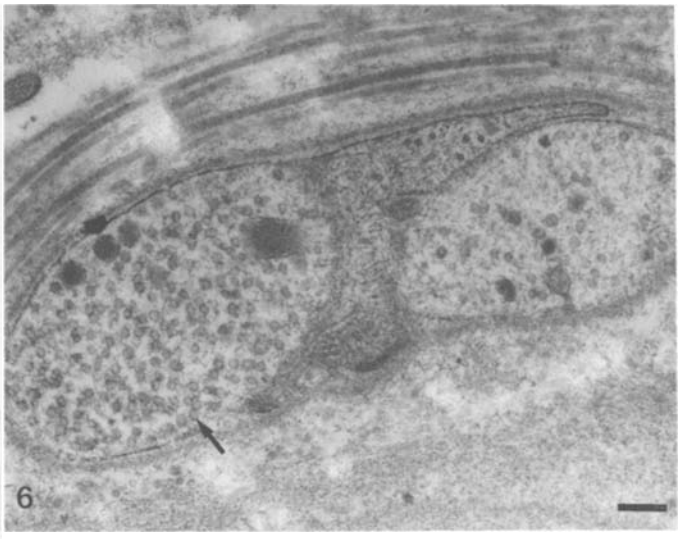


Fig. 2. A large axon bundle in the wide space of connective tissue between the smooth muscle layer at the region of EUS. This nerve consists largely of unmyelinated nerves fibers (*U*) and a small quantity of myelinated nerve fibers (*M*). (L.M. scale bar = 5.6 μm)

Fig. 3. In the interstitium of the region of EUS, a large number of unmyelinated axon bundles exists among the smooth musculatures. Each axon bundle consists of five or more unmyelinated axons and is surrounded by a Schwann cell (*S*). *M*, myelinated nerve. (E.M. scale bar = 1.5 μm)

Fig. 4. Preterminal axon bundle in the region of EUS. The bundle which in a terminal ramification of the aforementioned nerve (Fig. 2 and 3), contains a single, or at most two or three axons surrounded by a Schwann cell (*S*), and each axon contains many small granular vesicles (*thin arrow*) and large granular vesicles (*thick arrow*) considered to be adrenergic. (E.M. scale bar = 300 nm)





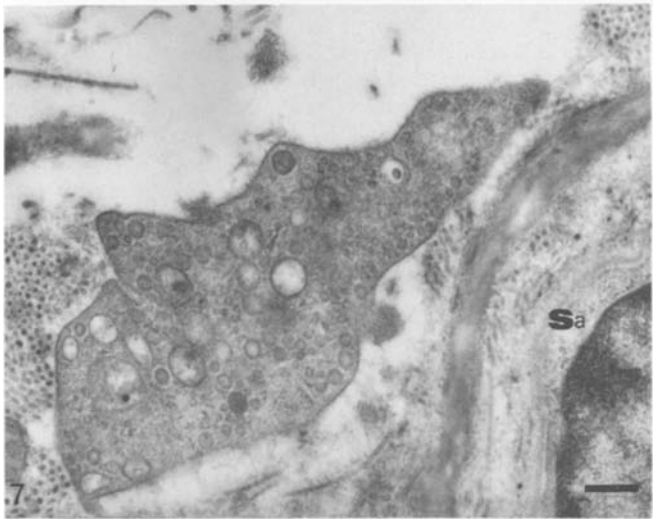


Fig. 5. A large axon bundle among the striated muscle cells of EUS. This axon bundle is surrounded by perineurium (*P*) and contains many unmyelinated axon bundles. Each bundle was surrounded by a Schwann cell (*S*) and contains a single or at most two or three axons (E.M. scale bar = 700 nm)

Fig. 6. Preterminal axon bundle among the striated muscle cells of EUS. Each axon in the bundle contains many small granular vesicles (*thin arrow*) and large granular vesicles (*thick arrow*), considered to be adrenergic. (E.M. scale bar = 200 nm)

Fig. 7. Neuromuscular contact at EUS. These bare nerve endings do not form any terminal swellings but instead form the so-called surface junction (1.1 μm gap). *Sa*, sarcolemma of striated muscle cell (E.M. scale bar = 500 nm)

in diameter) (Fig. 5). All axons were nonmyelinated and each axon bundle contained a single or at most two or three axons. Moreover, each preterminal axon contained many small granular vesicles and larger granular vesicles which were considered to be adrenergic (Fig. 6). The adrenergic nerve endings between striated muscle cells were denuded of their Schwann cell sheeth. These nerve endings, unlike those innervating smooth muscle cells, did not form any terminal swellings, but instead formed so-called surface junctions. The cleft between the nerve ending and the striated muscle fiber was about 1.1 μm wide (Fig. 7), close enough to suggest its direct involvement in the function of the EUS.

Discussion

The axon profiles in the interstitium of the distal prostatic urethra were mostly adrenergic, agreeing with similar work in animals [6, 29, 30] and in humans. This correlates well with functional data which ascribe a predominant role to alpha-adrenergic system in the region of the EUS [1, 17]. Unmyelinated cholinergic axons were not recognized in the present study. Nonetheless this is in concordance with the concept of the cholinergic system in the prostatic urethra which has a secretomotor rather than a motor function [11, 29]. Even in the latter role it has been suggested that the cholinergic system acts through the adrenergic neurons in the vesicourethral short neuron system [9, 13].

The present study failed to demonstrate the intramural ganglia suggested by others [4, 24, 29], although similarly negative data was reported by Watanabe et al. [30]. This may have been because the present study was restricted to the most distal end of prostatic urethra, rather than to more proximal area of the bladder neck. Nonetheless myelinated nerves recognized along the adrenergic axon profiles must represent autonomic preganglionic or afferent neurons. These myelinated nerves were not thought to be the somatic pudendal nerve because of their size and location.

The adrenergic axon profile extended into the EUS itself. Here it was noted to form a surface junction with the striated muscle of EUS. This type of autonomic termination has been noted in the male feline rhabdosphincter [8], in the striated muscle of mouse oesophagus [23],

and in feline skeletal muscle [2]. There were no cholinergic unmyelinated or myelinated fibers in the EUS. Certainly more serial sections along the both longitudinal and transverse axis of EUS would be needed to exclude or confirm the presence of cholinergic myelinated or unmyelinated axon profiles [8].

Nevertheless the fact that it was the sole autonomic representation in the EUS and that neuro-muscular surface junctions, ultrastructurally identical to other neuroeffector junctions of the vesicourethral smooth muscle [8], were noted in the present study, indicated that the human EUS received direct autonomic adrenergic innervation.

What is the clinical relevance of these ultrastructural findings? Pathophysiologically, these findings support the observation alpha-adrenergic activity in the distal urethral sphincter of the neurogenic bladder [13, 14, 17]. The sympathetic dyssynergia of Awad [1] and Koyanagi [17] suits the present data which show clear adrenergic involvement in the region of the EUS. It would be interesting to correlate ultrastructural changes in these adrenergic axon profiles to an established phenomenon of urethral supersensitivity [23] in neurogenic bladder dysfunction. Currently such a study is ongoing in our institution using transurethrally resected prostatic tissue from patients with bladder neuropathy.

Elaborate functional studies [14, 18] have suggested that the EUS itself receives an alpha-adrenergic input. Although our findings as to electron microscopic features of muscle subtypes of the EUS [19] do not correlate with the observations of Gosling and Dixon [10], the fact that the EUS receives direct adrenergic innervation does suggest its functional role as a sole mechanism of passive continence after the proximal sphincter system was incapacitated [12]. Therapeutically it offers a sound basis for adrenergic manipulation of sphincter disorders in neurogenic vesical dysfunction. The use of alpha-adrenolytic drugs for dyssynergia in the region of the EUS has already been discussed [1, 14, 17]. In view of adrenergic axon profiles in the region of the EUS and their subsequent passage to the distal sphincter, the role of our modified loop sphincterotomy for neurogenic dysfunction of the bladder [15] needs to be reevaluated. Radical TURP specifically aims to resect all the prostatic tissue (peripheral zone of McNeal) [20] down to the anatomical capsule for the maximal reduction of outlet resistance, and yet not to touch the EUS for the purpose of preserving urinary continence.

Conjoining our data and Vaalasti which demonstrated adrenergic ganglion cells beneath the anatomical capsule [29] this radical TURP must be accomplishing what classical sympathectomy cannot [9, 13, 26] to the sympathetic system of neurogenic bladder by resecting activated intramural adrenergic axon profiles. Moreover electromyographic study of EUS after surgery, where an analysis at a single motor unit level was employed, suggested that suppression of somatic sphincter dyssynergia was caused by an efferent interruption to the EUS [25]. This may be possible in view of the intramural efferent adrenergic pathway to the EUS. In this regard our proposal of radical TURP as surgical "sympathectomy" [15] would not be as hypothetical as was commented [22].

Admittedly more work is needed to clarify the innervation of the distal sphincteric zone with its known variations by species, age and sexual differences [6]. We believe this is the first ultrastructural study in the human urethra documenting the adrenergic involvement in the region of EUS and of EUS itself, and we offer a morphological explanation of our functional studies [14, 17, 18].

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