Review

Urethral pharmacological mechanisms incontinence and bladder emptying: An updated review

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Abstract

Background: The urethral wall consists of layers of striated muscle, circular and longitudinal smooth muscles, collagen fibers, and a vascular plexus. However, the relative contributions of these components to urethral pressure in humans remain poorly understood. The circular and longitudinal smooth muscle components can develop a spontaneous contractile activity, generating a basal tone. They can further contract or relax in response to excitatory or inhibitory stimuli. Animal studies suggest that smooth muscle activity in the mid-urethra plays a crucial role in determining maximal urethral closing pressure. Notably, the highest sympathetic activity occurs in the middle segment of the female urethra during increasing smooth muscle tone. This finding is supported by human studies that did not detect any electromyographic activity from striated muscle in this region. **Objectives:** This study was conducted to review the contributions of the primary structural components and control mechanisms of urethral. **Conclusion:** In females, the external urethral striated sphincter is located at the distal urethra, which is not the segment associated with the highest closing pressure. Rather, the sphincter has been shown to modulate urethral pressure during exercise and physical stress. Basic science research does not support the notion that mid-urethral pressure is caused by the external striated sphincter tone in females. Instead, findings suggest that, at rest and during bladder filling, maximal urethral pressure is primarily determined by the activity of the urethral smooth muscles.

Keywords: Urethra, Striated muscle, Smooth muscle, Vascular plexus

1. INTRODUCTION

Structures within the urethra that may contribute to maintaining continence and initiating micturition have been the subject of several recent studies and reviews [1-4]. The prevailing understanding is that the urethral sphincter complex comprises the striated external urethral sphincter (EUS) surrounding the smooth muscle of the internal urethral sphincter (IUS) [4]. It is widely accepted that the urethral closing pressure (UCP) is the most essential factor in maintaining continence, yet the mechanisms generating this pressure remain unclear. In a seminal study, Rud *et al.* [5] employed simultaneous urethrocystometry in five continent women undergoing cancer treatment to determine the roles of various urethral structural components to intraurethral pressure (IUP) at rest. Their urethral pressure profile measurements, taken before, during, and after curarization and after clamping the arterial blood supply to the urethra, led to the conclusion that the striated muscle of the urethra and pelvic floor accounted for one-third of the total IUP. Meanwhile, the urethral vascular bed, as well as the smooth muscles and connective tissues in the urethra and the periurethral tissues, contributed to the remaining thirds. However, this study could not differentiate the specific contributions of the longitudinal and circular smooth muscle layers. Pipitone *et al.* [2] divided the female urethra into five

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segments, with the first segment comprising the IUS. This segment is characterized by a trigonal ring of smooth muscle contiguous with the trigonal muscle, along with a detrusor loop that passes lateral to and in front of (ventral to) the lumen. The subsequent two segments contain varying wall layers: an outer circular striated muscle layer surrounding a thin circular smooth muscle layer, a thicker longitudinal smooth muscle layer, and a vascular plexus. In the fourth segment, a striated muscle arch passes over the urethra, forming the compressor urethra and urethrovaginal sphincter. The distal segment is a fibrous "nozzle" devoid of muscles [2]. Defining the role of each component in generating IUP is difficult. Previous assertions that (i) the entire urethra acts as a sphincter [6] with all structural components contributing to IUP, and (ii) urethral pressure results from both urethral muscle activity and external pressure forces around the urethra [7], have been recently re-evaluated. Venema *et al.* [3] posited that the maximal urethral pressure at rest and during normal bladder filling is solely determined by the activity of the urethral smooth musculature in females, challenging earlier perspectives on urethral pressure dynamics.

This article briefly reviews the primary structural components and control mechanisms of the urethra. It also questions whether recent efforts to define the contributions of various components to urethral function can lead to improved pharmacological interventions for urological dysfunctions, such as stress urinary incontinence (SUI), or whether they are mainly of theoretical interest. The focus will be on articles published in the past 5 years (Table 1).

2. STRIATED MUSCLES

Gosling [8,p.1] stated that "urodynamic studies consistently demonstrate that maximum closure pressure typically occurs in the mid-portion of the urethra, corresponding to the location of maximum thickness of the intrinsic urethral striated muscle sphincter (rhabdosphincter)." He further suggested that the rhabdosphincter is structurally adapted to maintain a relatively constant tone, which is essential for urethral occlusion and urinary continence at rest. The endopelvic connective tissue separates the rhabdosphincter from the extrinsic periurethral striated muscles [9,10]. Gosling *et al.* [9] showed that human EUS striated muscle fibers have a typical sarcomeric structure rich in mitochondria, suggestive of an oxidative metabolic phenotype. Continuous activity of the striated muscle is essential to prevent urine leakage. Two major fiber types of striated muscle have been demonstrated: type 1 and type 2. Type 2 fibers are subdivided into type 2A, 2X, and 2B fibers [11]. McCloskey *et al.* [4] reported that type 1 muscle fibers are the dominating type in human EUS, which remains unexplained. Compared to type 2 fibers, type 1 fibers are more fatigue-resistant under continuous tetanic stimulation, have a smaller fiber diameter, and possess a more extensive capillary network. They have an oxidative rather than glycolytic metabolism. EUS muscle from laboratory animals has a preponderance of type 2 fibers. For instance, the rat urethra has a significant proportion of 2A fibers, demonstrating considerable resistance to fatigue. The disparity between human and animal EUS fiber types is striking and should be considered when extrapolating data from animal studies to human conditions [4]. This difference could be due to the quadrupedal posture of animals (for example, sheep, dog, rabbit, guinea pig, and rat). In contrast, adult humans stand on their hind limbs (legs), which add hydrostatic pressure to bladder pressure due to gravity.

At rest, the urethral striated muscle contracts, keeping the urethra closed and preventing involuntary leakage of

Table 1. Important recent articles giving new aspects on urethral function/dysfunction

Authors	Title	Publication	Type	Comments
Attari *et al*. [1]	On structure-function relationships in the female human urethra: A finite element model approach	Ann Biomed Eng. 2021 Aug;49(8):1848-1860	Research article	Important aspects of urethral function based on a new model
Pipitone *et al*. [2]	Urethral function and failure: A review of current knowledge of urethral closure mechanisms, how they vary, and how they are affected by life events	Neurourol Urodyn. 2021 Nov;40(8):1869-1879	Review	An extensive overview of the known factors related to urethral structures and their relations to both function and dysfunction
Venema *et al*. [3]	The maximal urethral pressure at rest and during normal bladder filling is only determined by the activity of the urethral smooth musculature in the female	J Clin Med. 2023 Mar 29;12(7):2575	Review	An opinion paper arguing that mid-urethral pressure is not generated from forces around the urethra, including the external striated sphincter, but rather by urethral smooth muscle
McCloskey *et al*. [4]	What do we really know about the external urethral sphincter?	Continence Volume 10, June 2024, 101223	Review	The authors' contributions to a Roundtable Discussion at the 2023 International Continence Society Conference in Toronto
van Geelen *et al*. [6]	The female urethra: Urethral function throughout a woman's lifetime	Int Urogynecol J. 2023 Jun;34(6):1175-1186.	Review	A constitutional or genetic predisposition, along with aging and senescence, are the most prominent etiological factors in the development of urinary incontinence and other pelvic floor disorders

urine. Attari *et al.* [1] used a 3-D multiphysics finite-element urethra model to reject the hypothesis that the striated and smooth muscles contribute equally to the urethral closure pressure. Their simulations indicated that contraction of the outer circular striated muscle increased closure pressure. Meanwhile, contraction of the inner longitudinal smooth muscle reduced closure pressure and shortened urethral length. These observations suggest a role in micturition initiation. Their model demonstrated that arteriovenous pressure is essential in maintaining luminal closure in the proximal urethra and functional urethral length. They rejected Rud *et al.*'s [5] hypothesis that the striated and smooth muscles (including the vascular plexus) contribute equally to UCP, although they acknowledged that their model of a healthy urethra did not include the vascular plexus along the entire urethra and suggested that integrating vasculature and muscle layers could yield more realistic results.

Upon initiating voiding, the central nervous system signals the relaxation of the urethral striated muscle, reducing the resistance to urine flow. Onuf's nucleus, a distinct group of motor neurons located in the ventral horn of the sacral spinal cord (specifically in segments S2 to S4) [12], provides somatic motor innervation to the EUS through the pudendal nerve. Relaxation of the urethral striated muscle is coordinated with detrusor muscle contraction, which ensures efficient urine expulsion. Onuf's nucleus is densely innervated by serotonergic (5-HT) and noradrenergic nerve terminals, with 5-HT released from the brainstem raphe nuclei, binding to various receptors (5-HT1A, 5-HT2A, 5-HT2C, 5-HT3, and 5-HT7) [13]. In rats, Xu *et al.* [14] identified several groups of motor neurons in Onuf's nucleus that innervate different pelvic striated muscles, with differential expression of 5-HT2A receptors and 5-HT5A receptors. Notably, 5-HT5A receptors are specifically expressed in neurons innervating the EUS and bulbospongiosus muscles [14]. Animal studies have shown that during the storage phase, serotonin and noradrenaline potentiate glutamate effects in stimulating Onuf's nucleus, enhancing acetylcholine release from the pudendal nerve and consequently activating nicotinic acetylcholine receptor in the rhabdosphincter, resulting in striated muscle contraction [13,15]. This understanding underpinned the introduction of duloxetine, a dual 5-HT and noradrenaline re-uptake inhibitor, to increase IUP and improve SUI. Studies of the selective 5-HT2C receptor agonist (TAK-233) in female rats and humans demonstrated enhanced striated urethra-closing functions during both evoked and momentary events [16-18]. Therefore, 5-HT2C receptor agonists may offer treatment options for SUI. Kamo *et al.* [19] showed that TAK-233 lowered the threshold for urethral sphincter contraction induced by transcranial magnetic stimulation in healthy women, suggesting that 5-HT2C receptor agonists might enhance the active urethral-closing reflex rather than merely affecting resting urethral pressure. Meanwhile, Klarskov *et al.* [20] evaluated the effect of ASP2205, a selective serotonin agonist, on urethral pressure in healthy females, comparing it with duloxetine and placebo. Contrary to expectations, the 5-HT2C receptor agonist resulted in a dose-dependent decrease in urethral pressure compared to placebo, while duloxetine significantly increased the opening urethral pressure (OUP) during both resting and squeezing conditions. Further clinical studies are needed to establish whether selective 5-HT2C receptor stimulation positively impacts SUI frequency in humans.

The IUP-increasing action of duloxetine is well-documented, prompting interest in the selective inhibition of its components. Reboxetine, a potent and selective noradrenaline re-uptake inhibitor, and citalopram, a highly selective 5-HT reuptake inhibitor (SSRI), have been shown to influence OUP in women as demonstrated by urethral pressure reflectometry [21]. Citalopram resulted in a slight increase in OUP compared to placebo, which was attributed to the complex action of serotonin on the lower urinary tract. Increasing the amount of serotonin in the synapse by citalopram might increase the activity of both agonistic and antagonistic 5-HT receptors in Onuf's nucleus, leading to a minor increase in urethral pressure. Observations in female rats [17] suggest that activation of 5-HT receptors may both inhibit and enhance urethral closure, supporting this interpretation. Collectively, these results imply that SSRIs are unlikely to decrease urethral pressure and, thus, will not induce or aggravate SUI in women.

3. SMOOTH MUSCLES

The spontaneous mechanical activity of the urethral longitudinal and circular smooth muscle layers provides the basic tone of the urethra [22,23]. These muscle layers have different physiological properties, notably a lower shortening velocity in circular compared to longitudinal smooth muscles [24]. The circular smooth muscle plays a significant role in developing and maintaining urethral tone and continence [22,25]. Conversely, during the emptying phase, the relatively faster longitudinal smooth muscle becomes more active. This shortens the urethra and facilitates urine flow [22,26]. The hypogastric nerve modulates the circular smooth muscle, producing tension that exceeds the spontaneous baseline [26].

A contraction of the thick inner longitudinal smooth muscle has been shown to reduce the closure pressure and shorten the urethral length, indicating its role in initiating micturition [1]. Venema *et al.* [3] concluded, from a comprehensive literature review, that maximal urethral pressure at rest and during normal bladder filling is primarily dictated by the activity of the urethral smooth musculature in females. The smooth

muscle activity in the mid-urethra, where the most significant sympathetic responses are found, determines the maximal pressure. Importantly, human studies have demonstrated an absence of striated electromyographic activity in this region, with EUS contributing additional urethral pressure only during exercise and physical activity [3]. Thus, basic science research does not support the idea that mid-urethral pressure results from external forces around the urethra, including contributions from the striated sphincter.

The primary factor driving contractions in the urethral smooth muscle is sympathetic activity, particularly through the release of noradrenaline and stimulation of α1-adrenergic receptors (α1-ARs). Among the three high-affinity α1-AR subtypes identified in molecular cloning and functional studies, the α1A subtype predominates in both male and female urethras [27]. The physiological role of β-adrenergic receptors (β-ARs) within the urethra has not been established [27,28]. However, stimulation of β-ARs by exogenous agonists can induce urethral relaxation, particularly through β3-ARs [29,30]. Immunohistochemical analyses have revealed the presence of β3-ARs in the human female urethra, particularly in the epithelial layer of the mid-urethra [31], as well as in the striated muscle layer at the EUS level. These findings provide a mechanistic basis for the clinical use of β3-AR agonists, such as mirabegron, which can induce relaxation of the urethral smooth muscle [28,32]. Interestingly, endogenous catecholamines appear to have limited effects on urethral β-ARs. While β-AR antagonists have been proposed as a treatment for SUI [33], their efficacy has not been established. The role of muscarinic receptors (and their subtypes) in urethral function is also unclear [27]. Nitric oxide, synthesized by nitric oxide synthase in cholinergic nerves, is considered the predominant inhibitory neurotransmitter [34]. However, there is compelling evidence for other unidentified non-adrenergic, non-cholinergic inhibitory mediators, such as vasoactive intestinal polypeptide, adenosine 5'-triphosphate, and carbon monoxide, which can relax urethral smooth muscles [33]. However, their precise roles in urethral function remain to be elucidated. Upon stimulation, multiple receptors within the urethral musculature can induce either contraction (such as vasopressin receptors) or relaxation (for instance, β3-ARs), yet their specific contributions to urethral function are not well defined [32].

Hashitani *et al.* [35] investigated urethral smooth muscle vascularity in female rats, focusing on the intramural arteriolar network. They dissected the mucosal layer and found that nitric oxide released from parasympathetic post-ganglionic nerves counteracted sympathetic vasoconstrictions, both pre- and post-synaptically, thereby restricting arteriolar contractility [35]. The researchers observed that sympathetic vasoconstriction was predominantly suppressed by α, β-methylene ATP but not by prazosin. The phosphodiesterase type 5 inhibitor, tadalafil, was found to diminish these vasoconstrictions.

4. VASCULAR PLEXUS

Augsburger and Muller [36] investigated the urethral vascular plexus using serial histological sections, vascular corrosion casts, and scanning electron microscopy. They found that the plexus, located in the lamina propria connective tissue between the epithelium and the longitudinal smooth muscle layer, consists of blood-filled sinusoids. The proximal segment of the plexus is composed of interconnected longitudinal tubes, whereas the distal portion adopts a net-like configuration, continuous with the vestibular plexus. Arterial pressure inflates the plexus, with the pressure in the intraurethral veins regulated by the arteriovenous anastomoses [37]. The vascular plexus is highly developed not only to provide simple urethral vascularization [38] but also to maintain a watertight seal. The arterial blood inflow causes the plexus to expand toward the urethral lumen, constrained by a rigid muscular sheath surrounding it. Given that the inner diameter of the muscular tube forming the urethra is approximately 5 mm, the absence of a sealing mechanism would lead to urine leakage [2]. Rud *et al.* [5] found that the (venous) vascular bed was responsible for 30% of the IUP. However, as noted by Venema *et al.* [3], this assertion warrants further examination due to the artificial occlusion of vessels in Rud *et al.*'s study [5], which may not only decrease the vascular bed's influence on urethral pressure but could also induce hypoxia, thereby impairing the ability of the urethral musculature to maintain a tonic response to α-adrenergic stimulation and facilitating smooth muscle relaxation.

Research into the pharmacological regulation of vessels involved in sinusoidal filling has been proven challenging. However, isolated lamina propria preparations from the female rabbit urethra, which contained the vascular plexus, exhibited both contractile and relaxant properties [39]. Contraction responses to noradrenaline were inhibited by α-AR blockers, vasoactive intestinal polypeptide, and electrical stimulation (mediated by nitric oxide mechanism). Folasire *et al.* [40] explored porcine urethral tissues, both intact and denuded of urothelium/lamina propria, concluding that the urothelium/lamina propria of the urethra has an inhibitory effect on receptor-mediated urethral contraction. This inhibition is due to the release of a diffusible factor that is not mediated by nitric oxide or prostaglandins, nor is it affected by age.

Studies in both animals and humans have shown that estrogen increases vascularity in the periurethral plexus. Vascularity can be measured through vascular pulsations using methods such as urethral pressure profilometry or Doppler velocimetry, and these measurements have been validated by immunohistochemical staining [41,42].

5. DISCUSSION

Surgical interventions such as artificial sphincters and slings for SUI, subtotal cystectomies, and the creation of a neobladder for severe urge incontinence have demonstrated favorable clinical outcomes. Notably, these procedures do not require a detailed understanding of the cellular mechanisms underlying urethral function, sphincter innervations, or ion channels in the sphincter cells. This benefit suggests that surgical solutions can effectively address the problem and potentially cure the patient. However, a large number of patients are either unwilling or unfit for surgery and thus would benefit from effective pharmacological treatments. Current pharmacological options for lower urinary tract disorders, such as duloxetine for SUI, yield only modest effects, underscoring the need for more effective interventions. The question arises: can efforts to better define the contributions of various structural components of the urethra lead to improved treatments, or are such efforts mainly of theoretical interest?

Gosling [8] posited that the rhabdosphincter is the most essential factor in achieving urethral occlusion. In contrast, many animal and human studies indicate that the smooth muscle activity in the mid-urethra determines the maximal UCP. This mid-urethral region is where the highest levels of smooth muscle activity and sympathetic innervation occur during tone development. Notably, human studies have found no evidence of striated electromyographic activity in this segment. The EUS, mainly located in the distal urethra, does not correspond with the area exhibiting the highest closing pressure. However, during EUS contraction, such as during exercise or physical stress, the highest urethral pressures are indeed recorded in the distal part of the urethra.

Thus, both basic and clinical science research does not support the notion that mid-urethral pressure is caused by EUS tone. Instead, findings suggest that in females, maximal urethral pressure at rest and during bladder filling is predominantly dictated by the activity of the urethral smooth muscles [3]. A prerequisite for effectively targeting specific structural components is that they must be able to respond to pharmacological stimulation. If each structural component contributes to urethral pressure [5], a question presents itself: Could increase stimulation of one component compensate for the dysfunction of others? Is it possible to achieve a "normalized" function in such patients? Among the various structural components, targeting the smooth muscle appears to be the most promising approach for increasing IUP.

The pharmacological treatment of SUI aims to enhance the tone of both smooth and striated muscles, either directly or indirectly. The studied drugs for female SUI treatment have included α-AR agonists, β-AR antagonists, β-AR agonists, and serotonin-noradrenaline uptake inhibitors. However, the limited efficacy and/or adverse effects associated with these drugs have constrained their clinical application [28,41]. The main factor responsible for contraction in urethral smooth muscle is sympathetic activity mediated by noradrenaline release and α1-AR stimulation. Efforts to stimulate α1-ARs or counteract the relaxant effects of β-ARs have not yielded successful outcomes [28,41]. So far, approaches targeting the striated muscle (for example, using duloxetine) or the vascular plexus (using estrogen) have shown modest success [28,41].

6. FUTURE PERSPECTIVES

It has been well demonstrated that the clinical effects of stimulating any single urethral structural component are limited, implying that pharmacological treatments may only be effective for mild disturbances of urethral function, such as SUI. Despite the stagnant success rate in the search for novel therapies to improve treatment, as noted by Pipitone *et al.* [2], there remains hope that "advancing our knowledge of urethral function and failure may uncover novel therapeutic targets." Significant knowledge gaps persist regarding the relative contributions of striated and smooth muscle, the rich vascular plexus, and the largely unstudied connective tissue within the urethra. Addressing these gaps may or may not lead to substantial clinical improvements in pharmacological strategies. Nevertheless, further investigation into the structures supporting normal urethral function is warranted, especially from a physiological perspective. Such studies could also provide a foundation for developing regenerative medicine techniques applicable to treatment. Promising approaches may include not only stem cell injections, which have shown positive results in preliminary studies, but also the use of stem cell-derived components, such as secretomes and chemokines [42-44].

7. CONCLUSION

During exercise and physical stress, the external urethral striated sphincter modulates urethral pressure. At rest and during bladder filling, maximal urethral pressure is primarily determined by the activity of the urethral smooth muscles.

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