**What are the origins and relevance of spontaneous bladder contractions? ICI-RS 2017**


## Abstract

*Introduction*

Storage phase bladder activity is a counter-intuitive observation of spontaneous contractions. They are potentially an intrinsic feature of the smooth muscle, but interstitial cells in the mucosa and the detrusor itself, as well as other muscular elements in the mucosa may substantially influence them. They are identified in several models explaining lower urinary tract dysfunction.

*Methods*

A consensus meeting at the International Consultation on Incontinence Research Society 2017 congress considered the origins and relevance of spontaneous bladder contractions by debating which cell type(s) modulate bladder spontaneous activity, whether the methodologies are sufficiently robust, and implications for healthy and abnormal lower urinary tract function.

*Results*

The identified research priorities reflect a wide range of unknown aspects. Cellular contributions to spontaneous contractions in detrusor smooth muscle are still uncertain. Accordingly, insight into the cellular physiology of the bladder wall, particularly smooth muscle cells, interstitial cells and urothelium, remain important. Upstream influences, such as innervation, endocrine and paracrine factors, are particularly important. The cellular interactions represent the key understanding to derive the integrative physiology of organ function, notably the nature of signalling between mucosa and detrusor layers. Indeed, it is still not clear to what extent spontaneous contractions generated in isolated preparations mirror their normal and pathological counterparts in the intact bladder. Improved models of how spontaneous contractions influence pressure generation and sensory nerve function are also needed.

*Conclusions*

Deriving approaches to robust evaluation of spontaneous contractions and their influences for experimental and clinical use could yield considerable progress in functional urology.

***Key words***; detrusor overactivity, LUTS, detrusor underactivity, overactive bladder, physiology

## Introduction

The urinary bladder muscle is not inactive during the storage/ filling phase of the micturition cycle. Many different terms have been used to describe storage phase bladder activity, based on visible contraction or associated effects on vesical pressure (e.g. non-micturition contractions, micromotions, autonomous or intrinsic bladder activity, spontaneous contractions, low amplitude rhythmic contractions). Interest in such activity is driven by implications for the clinical contexts, such as overactive bladder syndrome (OAB) [1-4] and impaired detrusor compliance. Such contractions have been described in the literature for several decades, in various experimental contexts. Similar large amplitude spontaneous contractions are seen in neonates in some species- perhaps to help empty the bladder before neural control is fully established. More recently, the theoretical relevance to voiding dysfunction has been discussed [5]. Spontaneous contractions exist not only *in vivo*, but also in isolated bladders and isolated muscle preparations, therefore in part at least, they are intrinsic to bladder tissue. Where spontaneous contractions are seen in the presence of nerve blockade (e.g. tetrodotoxin) and antagonists of muscarinic and purinergic receptors, a “myogenic” or “autonomous” basis is likely to be contributory. In urodynamic recordings, non-voiding contractions are also considered to be spontaneous or autonomous, and concurrent video recordings demonstrate associated micromotions. During the International Consultation on Incontinence Research Society (ICI-RS) 2017 meeting, a discussion was held on the origins and relevance of spontaneous bladder contractions. The phenomenon was considered by addressing the following questions:

1. Which cell type(s) modulate bladder spontaneous activity?
2. Are the methodologies sufficiently robust?
3. What implications does spontaneous activity have in healthy and abnormal lower urinary tract function?

## Which cell type(s) modulate bladder spontaneous activity?

The bladder wall incorporates several cell types, including urothelium, interstitial cells (IC), vascular smooth muscle (VSM), muscularis mucosa (present in humans, other large species and guinea-pigs, but not rats and mice), detrusor smooth muscle (DSM), nerves and a number of other cells, including fibroblasts and immune cells. There is compelling evidence that DSM (and also muscularis mucosa) [6,7], generates spontaneous action potentials, spontaneous intracellular calcium transients and spontaneous contractions. The origin of spontaneous activity in DSM is not fully understood, but it appears to be an intrinsic property of the smooth muscle cells. Dispersed smooth muscle cells studied with patch clamp exhibit spontaneous changes in membrane potential and also fire action potentials [8]. Studies of DSM preparations with microelectrodes demonstrated spontaneous action potentials when the cells were in a more physiological arrangement with e.g. gap junction coupling [6,9]. In bladder strip preparations, spontaneous contractions are insensitive to tetrodotoxin, confirming their non-neurogenic origin. L-type calcium channels are responsible for the upstroke of the DSM action potential; nifedipine blocks the spontaneous action potentials and spontaneous contractions [10]. Moreover, inward $Ca^{2+}$ currents in dispersed DSM cells are reported for several species [11-13]. A study of transgenic mice, with knockout of the CaV1.2 channel, showed that this channel is essential for normal bladder contraction in the mouse [14]. The ability of DSM cells to fire spontaneously, rather than be driven by a pacemaker cell, contrasts with the rabbit urethra, where the smooth muscle cells are electrically quiescent and the IC appear to act as pacemakers via a depolarizing calcium activated chloride conductance [15].

The activity in DSM may be modulated by neighbouring cells, as shown in myography experiments [6,7,16]. DSM activity is apparently enhanced by apposition with the mucosal layer, in intact preparations [17] or with the mucosal layer dissected off but left in close proximity (CH Fry, D Kitney; unpublished observations). The involvement of the mucosa was also demonstrated when its partial removal from intact bladder sheets of spinal cord injured rats abolished the large amplitude spontaneous contractions in the denuded area. The enhancement in amplitude is accompanied by a decrease in frequency, when compared to control bladders, and may be due to a decrease in the number of focal initiation/pacemaker sites [18]. Imaging of $Ca^{2+}$ signaling

across the bladder wall suggests that this activity may be initiated by $Ca^{2+}$ transients that originate in the mucosa [19]. Spontaneous contractions are enhanced by the mucosa, particularly if hypertrophied, for example in animals with neurogenic overactivity [20]. An unresolved issue is the nature of communication between mucosa and detrusor, this may be due to diffusion of contractile agonists or via cell-to-cell contacts. Optical imaging experiments demonstrate conduction of electrophysiological signals between two layers suggesting the latter mode of connectivity.

Besides DSM, isolated mucosa of some species (e.g. guinea-pig and pig) develops spontaneous phasic contractions [17,21]. In these species, as in the human bladder, a muscularis mucosa forms a discontinuous layer in the suburothelium, that expresses α-smooth muscle actin immunoreactivity, and is clearly distinct from the DSM [6,7]. It is thus tempting to hypothesise that the muscularis mucosa contributes to mucosa spontaneous activity. This is consistent with the fact that mucosa preparations with the urothelium and larger blood vessels removed, still develop large spontaneous contractions [6,7].

The involvement of IC in modulation of detrusor spontaneous activity has been suggested in studies of $Ca^{2+}$-signalling in bladder tissue sheets. It has been hypothesised that activating interstitial cells initiates smooth muscle contraction [20], possibly via direct electrical coupling through gap junctions. However, $Ca^{2+}$-events in detrusor IC were of markedly lower frequency than in the neighbouring DSM cells, showing that it was unlikely that the detrusor IC were acting as a primary pacemaker, but they may modulate DSM activity [10,22]. The spread of non-micturition activity is thought to be through the ICs, which form a network throughout the bladder wall. They express phosphodiesterase-5 (PDE5) enzyme activity [23] and prostaglandin receptors (EP1 and EP2) [24]. PDE5 enzyme activity in guinea-pig ICs raises the suggestion that after PDE5 inhibition a rise of cGMP occurs in ICs, and that this effect might be spread through the functional network of ICs. In addition, prostaglandin receptor stimulation in these cells may be involved in the modulation of the rapid generation of coordinated contractile responses [24-26]. *Ex vivo* experiments with isolated guinea pig bladders have shown that inhibition of PG production reduces non-micturition activity frequency and suggested that PG is necessary for the normal increase in autonomous activity following cholinergic stimulation [27]. Patients with

OAB have increased urinary prostaglandins [27] which is thought to affect bladder activity directly by effects on smooth muscle and/or indirectly via effects on neurotransmission and IC activity [28].

The innervation would be expected to influence DSM contractions, and a basal β3-adrenoceptor mediated sympathetic tone has been identified in rat [29], with an inhibitory effect on spontaneous DSM activity. Inhibition of neuronal acetylcholine release by presynaptic β3-adrenoceptors has been reported in human and rat bladders [30,31], which may modulate spontaneous contractions and afferent activities of the bladder. However, in most other species including human, sympathetic nerve fibres predominantly innervate the blood vessels but not DSM. This anatomical evidence is consistent with the fact that sympathetic nerve-mediated, β-adrenergic relaxations of DSM strips are hardly detected, while sympathetic α-adrenergic constrictions of submucosal blood vessels are readily detected in in vitro studies. The expression of β-receptors in these species may indicate an endocrine influence. During filling of the bladder, there is no activity in the parasympathetic nerves innervating the bladder, and direct evidence for spontaneous release of acetylcholine from nerves during the filling phase of the bladder has so far been lacking [32]. However, there is release of acetylcholine from autonomic cholinergic nerves in guinea pig and rat bladders under *in vitro* and *in vivo* conditions [33] that model the filling phase, and this is also seen in pig bladder *in vitro* [14]. These findings support a hypothesis that antimuscarinic drugs used to treat bladder dysfunction can act by inhibiting a myogenic afferent pathway during the filling phase [32]. During the storage phase, acetylcholine may be released from neuronal and non-neuronal sources, and directly or indirectly excite afferent nerves in the suburothelium and within the detrusor.

### Are the methodologies sufficiently robust?

Spontaneous contractions are most commonly recorded from strips of bladder wall, cut in longitudinal or circular orientation, with or without the mucosal layer (urothelium and lamina propria). Strips studied with in vitro myography [34] typically develop spontaneous activity during the equilibration phase after being mounted; this activity is typically maintained for several hours during experiments and is not blocked by neuromuscular transmitter receptor antagonists or the nerve blocker, tetrodotoxin, showing its myogenic origin. However, a

problem with the use of bladder strips lies with the unique orientation of DSM [35] which does not form discrete layers as per gastro-intestinal or vascular smooth muscle, but comprises bundles of smooth muscle cells that apparently interlock with large spaces between bundles, facilitating expansion for urine storage. Cutting strips in either a longitudinal or circular fashion, therefore provides preparations with some smooth muscle bundles lying perpendicular or oblique to the long axis of the strip and therefore contributing little to the overall force generated by the strip. This issue may result in tension recordings somewhat under-representing the activity of the smooth muscle in the preparation to an unpredictable extent. An alternative lies with recording activity in the intact organ, in the *ex vivo* setting. Various groups have studied micromotions of the bladder wall through video recordings and correlated them with spontaneous changes in intravesical pressure (pressure transients)[36,37]. Such an approach facilitates investigation of activity in the bladder wall and has shown that many of the micromotions do not correlate with pressure transients. Measurements of bladder pressure transients therefore are limited by under-representation of activity in the smooth muscle of the bladder wall.

The different methodologies have their benefits and limitations and we therefore recommend that investigators take this into account when designing new programmes of research. *In vitro* myography of bladder strips is a powerful means of testing pharmacological modulators of receptors, ion channels and signal transduction pathways. Furthermore, the option of removing the mucosal layer, or indeed, sharp removal of the urothelium, leaving the lamina propria layer intact, enables elucidation of activity in the layers of the bladder and how these interact to generate activity in intact strips. A more reductionist approach using cells enzymatically dispersed from fresh bladder tissue (e.g. urothelium, smooth muscle cells, ICs) enables investigation of mechanisms at the cellular and molecular level, though these methods can influence cellular phenotype in a short time frame. The use of patch-clamp electrophysiology and live-cell $Ca^{2+}$-imaging has demonstrated spontaneous changes in membrane potential and spontaneous $Ca^{2+}$-transients in DSM [8] and IC [38,39], which show that individual cells have the ability to generate activity in the absence of their native microenvironment or stimulation from transmitters released from nerves or the circulation. The conclusions of such experiments

should be tested on *ex vivo* preparations to determine their relation to the development of spontaneous contractions in the whole bladder.

### What implications does spontaneous activity have in healthy and abnormal lower urinary tract function?

Evidence from several studies indicates potential roles for spontaneous contractions during normal bladder filling. Spontaneous contractions may contribute in setting baseline tone [1]. They increase in amplitude with increased muscle strain [40,41], transiently increase in frequency following rapid stretching [42] and are associated with enhanced afferent nerve activity [43,44]. These studies suggest that spontaneous contractions may play a role in providing bladder filling information to the central nervous system and also be the response to incoming information from afferent and efferent nerves, as well as neuromodulator release from the urothelium [45]. The lamina propria is heavily innervated, so that signal transduction from the bladder may draw heavily on spontaneous motility in mucosa.

A normal bladder can accommodate a broad range of volumes at relatively low filling pressures while simultaneously providing sufficient wall tension to maintain an efficient shape in the presence of abdominal loads (i.e. a low bladder wall surface area to volume ratio). To accomplish these functions the bladder exhibits viscoelastic, reversibly plastic behavior [46,47] and acutely adjustable preload tension [48,49]. Bladder contractions, including spontaneous rhythmic contractions, can modify preload by adjusting resting muscle strain [47,50]. Adjustable preload is due to cross-bridges and slippage of these cross-bridges during the relaxation phase of a spontaneous contraction that may allow accommodation during filling [47]. Adjustable preload has been quantified as dynamic elasticity during urodynamics [51]; however, the effect of spontaneous contractions on this behavior has yet to be studied clinically.

Spontaneous contractions do not occur uniformly throughout the bladder wall, but can be coordinated and propagated as waves [41]. Furthermore, detrusor cells can be stretched substantially during filling and must be able to contract to void efficiently over a broad volume range. This may be accomplished by acute length adaptation[48,50,52], which may be regulated by rhythmic contractions. For an efficient voiding contraction, the wall should not have any slack

or “wrinkles”, and a role of spontaneous contractions during filling may be to keep out the wrinkles.

The precise physiological role of spontaneous contractions in the muscularis mucosa is not well understood. A morphological relationship between muscularis mucosa and suburothelial blood vessels may indicate that spontaneous contractions of the muscularis mucosa prevent the vessels from stretching upon bladder wall distension. Similarly, contraction of muscularis mucosa may also effectively maintain the folding of the urothelium during bladder filling. In addition, the anatomical location of muscularis mucosae indicates it may have a larger impact on suburothelial sensory nerves than DSM. Thus, it may have an important role in sensing bladder fullness in normal and pathological bladders.

### *Extrinsic influences*

There is a high prevalence of phasic urethral pressure variations in women with OAB [53]: urethral pressure changes greater than 15 $cmH_2O$ are considered to reflect a potential contribution to the symptom pathophysiology. Urethral pressure variations may cause involuntary loss of an important source of inhibition for Onuf’s nucleus in the sacral spinal cord, reflecting a reciprocal relationship between the voiding reflex, the external sphincter and the pelvic floor muscle tonus [56]. Thus, a sudden involuntary pelvic floor relaxation could lead to reduced detrusor inhibition, which may facilitate emergence of DSM contractions. In men, the emergence of bladder outlet obstruction secondary to benign prostate enlargement represents an additional potential source of pathophysiological challenge whose implications for DSM micromotions is yet to be established. For women, pelvic organ prolapse may represent an analogous situation, with prevalence of OAB reportedly higher in prolapse patients [55]. Blood flow is another potential extrinsic influence that should be considered, noting that moderate ischemia increases the frequency spontaneous contractions in animal bladder models [56].

## Research priorities

The cellular contributors to spontaneous contractions in DSM are still uncertain. It will be important to identify what the contractile elements in the mucosa are. Moreover, the cellular interactions are still poorly understood yet represent the key understanding to derive the

integrative physiology of organ function. A notable example is the need to identify the nature of signaling between mucosa and detrusor layers. Indeed, it is still not clear to what extent spontaneous contractions generated in isolated preparations equivalent processes in the intact bladder. Given the importance of pressure generation in voiding and its relevance in pathophysiology (detrusor overactivity), we still need to generate more appropriate models of how spontaneous contractions influence pressure generation. For example, adjustable preload has been quantified as dynamic elasticity during urodynamics, but the effect of spontaneous contractions on this behaviour has yet to be studied clinically.

The range of observations of cellular- behavior, interactions and influences made in the experimental context, evidently have substantial implications for human physiology, clinical diagnostics and therapy. Deriving approaches to tolerable evaluation of spontaneous contractions and their influences in humans represents a challenge, which could yield considerable progress in functional urology.

Ultimately, the driver behind appreciating the mechanisms underlying this activity is to ascertain their contributions to clinical issues, such as OAB, impaired detrusor compliance and detrusor underactivity. Thus, identifying pharmacological control, and hence therapeutic target identification are potentially valuable. In addition, urodynamic techniques to identify this activity in situ in a clinical setting are key to translating the physiological insights into therapeutic possibility.

## Conclusions

The spontaneous contractions of detrusor smooth muscle reflect several challenges to identify sources, influences and consequences. There are many cell types which are likely to be directly relevant, and which could therefore serve as subjects of valuable investigation. Both cellular and integrative physiological approaches are needed. Insights into the implications of this activity for generation of both bladder sensory nerve activity and intravesical pressure, are likely to yield developments in the clinical setting of lower urinary tract dysfunction.

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