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Abstract

The isolated bladder shows autonomous micromotions, which increase with bladder distension, generate sensory nerve activity, and are altered in models of urinary dysfunction. Intravesical pressure resulting from autonomous activity putatively reflects three key variables; the extent of micromotion initiation, distances over which micromotions propagate, and overall bladder tone. In vivo, these variables are subordinate to the efferent drive of the central nervous system. In the micturition cycle storage phase, efferent inhibition keeps autonomous activity generally at a low level, where it may signal "state of fullness" while maintaining compliance. In the voiding phase, mass efferent excitation elicits generalized contraction (global motility initiation). In lower urinary tract dysfunction, efferent control of the bladder can be impaired, for example due to peripheral "patchy" denervation. In this case, loss of efferent inhibition may enable unregulated micromotility, and afferent stimulation, predisposing to urinary urgency. If denervation is relatively slight, the detrimental impact on voiding may be low, as the adjacent innervated areas may be able to initiate micromotility synchronous with the efferent nerve drive, so that even denervated areas can contribute to the voiding contraction. This would become increasingly inefficient the more severe the denervation, such that ability of triggered micromotility to propagate sufficiently to engage the denervated areas in voiding declines, so the voiding contraction increasingly develops the characteristics of underactivity~~becomes increasingly underactive~~. In summary, reduced peripheral coverage by the dual efferent innervation (inhibitory and excitatory) impairs regulation of micromotility initiation and propagation, potentially allowing emergence of overactive bladder and, with progression, detrusor underactivity.

Introduction

Urodynamic testing in a healthy human demonstrates low detrusor pressure during filling, with no large phasic contractions and overall high compliance. The voiding phase is characterised by an adequate flow, generated by an increase in pressure of suitable amplitude. Bladder emptying should be complete and achieved in a reasonable time scale. Detrusor overactivity (DO) is defined by the International Continence Society (ICS) as the presence of phasic bladder contractions during filling which may be spontaneous or provoked (1). Detrusor underactivity (DUA) is a contraction of reduced strength and/or duration, resulting in prolonged bladder emptying and/or a failure to achieve complete bladder emptying within a normal time span. The recognition that these may co-exist in the same person is established (2); originally this situation was termed "detrusor hyperactivity with impaired contractile function", but in ICS standardised terminology it signifies the presence of both DO (overactivity during storage) and DUA (underactivity during voiding) (Figure 1).

Attempting to explain the underlying pathophysiological processes for DO and DUA to co-exist in the same person requires an insight into how pressure is generated within the bladder, since both DO and DUA are observations characterised by their pressure changes seen during urodynamics (1). In DO, these are involuntary increases of pressure during storage, in DUA inadequate voluntary increases of pressure during voiding. Thus, the increases of intravesical pressure may have different origins in these two co-existing pathologies, the basis of which are potentially crucial when seeking to understand the pathophysiology of lower urinary tract dysfunction.

Autonomous bladder micromotility

The recognisable existence of autonomous bladder activity, and the associated micromotions (3, 4), suggests this is a physiological property, which may contribute to key storage phase symptoms such as urgency (5). Recent years have seen extensive research into the concept of autonomous bladder activity, and the associated micromotions (3, 4) and how they may contribute to key storage phase symptoms such

as urgency (5). Micromotions have now been comprehensively catalogued during experiments in which the bladder is isolated from the host animal and placed under physiological conditions. Several research groups haveThis has been evaluated this in small animal species (e.g. mouse, rat, guinea pig), using organ baths with transmural perfusion (6-8). Arterial perfusion methods have also been developed to evaluate the same properties in the bladder of large animals such as the pig (9, 10). It is also possible to detect discrete contractile units within the overall force generation using frequency analysis of contractions in isolated muscle strips (11), which can be altered by extrinsic pharmacological influences (12).

Micromotions in the isolated bladder have the following properties (Figure 2);

- They can be focal or propagating;
- Contractions may involve a varying amount of the bladder wall, in some cases over 50% of the total area (8);
- They generate phasic fluctuations of intravesical pressure, which are superimposed on any tonic contraction elicited by agonist exposure;
- They are enhanced by stimulation (either by increasing the intravesical volume or by extrinsic application of agonists);
- Exaggerated micromotions are seen in animal models of lower urinary tract pathologies, for example the isolated bladder from a rat which has previously had a period of partial bladder outlet obstruction (BOO) (8) and following spinal cord injury (13)
- The distortions resulting from the autonomous micromotions generate afferent nerve activity (14, 15).

Relationship of micromotions to intravesical pressure

A very pertinent observation is the fact that the amplitude of micromotion activity does not necessarily correlate with the amplitude of associated pressure change within the bladder lumen. Specifically, small movements can generate obvious pressure changes (9). Conversely, quite large movements may be associated with only small pressure fluctuations, which would be below the threshold considered detectable within the clinical setting (e.g. 0.5 cm H_2O) (9) .

We hypothesised that three fundamental properties determine how intravesical pressure is affected by intrinsic bladder micromotility.

- If micromotions are absent they clearly cannot influence pressure, so the first key property is the initiation of autonomous contractile activity.
- Secondly, if the movements are initiated but confined to a very small area, their effect on pressure is likely to be negligible. Thus the extent and rate of micromotion propagation is a key factor.
- Thirdly, it is clear that non-contracting parts of the bladder with low intrinsic tone could dissipate wall tension generated from adjacent micromotions. This would mean that micromotion contractions would in effect be damped, and thus attenuate intravesical pressure changes, even for relatively large localised movements.

In summary, the ability of micromotility to generate intravesical pressure changes is determined by the initiation and propagation of the micromotions, and the overall contractile tone of the bladder wall.

It is important to understand how micromotions are initiated and there is now clear recognition of several functional influences within the bladder wall potentially able to generate or modulate detrusor activity, including interstitial cells and the urothelium. The suburothelium, which includes a functional syncytium of interstitial cells connected by gap junctions, expresses Ca^{2+} waves which influence the spontaneous contractions of subjacent muscle (16, 17). The presence of niflumic acid-sensitive Ca^{2+}-activated Cl^- channels (Anoctamin-1) on interstitial cells modulates the frequency of muscle contractions in young rodents (18). Interstitial cell inhibition may thus be a means to reduce initiation of micromotility. Adrenergic stimulation to lower the overall muscle tone of the bladder wall reduces the phasic pressure fluctuations in the bladder (19), possibly by reducing overall tone of the bladder wall. Finally, propagation of electrical activity is a well-characterised feature of isolated bladder physiology (20), which is probably mediated by gap junctions (21). gGap junction blockade, with agents such as 18β-glycyrrhetinic acid, attenuates propagation and consequently leads to reduction of amplitude of the micromotions and associated pressure fluctuations (22). This congruent reduction in the intravesical pressure changes detected with individual

micromotions by manipulation of micromotion initiation, microcontraction propagation and general bladder tone at any given moment are key factors determining whether autonomous bladder activity results in proportionate intravesical pressure fluctuations.

In several species, phasic pressure changes can be seen during the storage phase of normal healthy animals. These are generally termed non-voiding contractions (NVCs) (23), or non-micturition contractions, and altered frequency or amplitude of such activity is seen with bladder filling, especially at higher bladder volumes (24). Alterations are clearly evident in animal models. For example, NVCs have been evaluated in spinal canal stenosis (a model of detrusor underactivity (25)), and irritant instillation (a model of detrusor overactivity (26)). Furthermore, changes in expression of NVCs can influence the transition from storage to voiding phase (27). Thus, insight into the intracellular pathways (for example role of protein kinase C (28)) and intercellular communication (for example electrical propagation (20, 21)) potentially provide direct insight into urodynamic properties and lower urinary tract dysfunction.

Regulation and dysregulation of bladder micromotility in vivo

Normal regulation of micromotility

In the intact animal, the autonomous behaviour of the lower urinary tract is subordinate to the imposed behaviour determined by the efferent innervation. Clearly, excitatory efferent innervation is responsible for generating a globalised bladder contraction at the time of voiding. In a well-innervated bladder, the simultaneous activation of all efferent nerves results in synchronised contraction of all parts of the bladder wall. Thus, micromotion initiation, and by inference the bladder wall tone, are maximal for efficient expulsion of urine.

Rodents exhibit ~~NMCs~~ NVCs during the storage phase, which can be observed urodynamically in conscious, anaesthetised and decerebrate animals (19, 29, 30). Importantly, the ~~NMCs~~ NVCs become substantially enhanced in amplitude when the brain-stem becomes non-functional, and this appears to be a result of loss of tonic central inhibition of autonomous activity arising in the bladder wall (19). This is probably the explanation for the high prevalence of DO in many patients with neurological disease. Thus, understanding of the expression of storage phase micromotions requires

consideration of the efferent inhibitory CNS influences that reduce peripheral micromotility. Precisely where such influences may be active needs further research, and may be within the peripheral ganglia or the bladder wall, where they may serve to reduce initiation and/or propagation of micromotility, or general bladder tone. Overall, efferent inhibition appears to down-regulate the extent of localised activity without eliminating it altogether.

In theory, varying the strength of efferent inhibition could be a feature of the intermittent awareness of bladder filling ~~state~~ people normally experience during the storage phase. Generally, an individual is not aware of the bladder filling state, but sporadically they perceive sensations which are categorised as first sensation of filling, normal desire to void and strong desire to void in urodynamic terminology (31). This could reflect a general suppression of micromotions by efferent inhibition, corresponding with the overall time where people are not specifically aware of their bladder, and occasions in which a transient reduction of efferent inhibition could allow slightly increased expression of motility. These occasions of transient increase in motility might then generate afferent stimulation (14, 15) proportionate to the bladder volume (32), and hence enhanced sensory reporting of filling state. Since this low level motility would be asynchronous, there would be little detectable pressure change, and the compliance of the bladder would be unaffected.

Overactive bladder and detrusor overactivity

A common feature of many lower urinary tract dysfunctions is the presence of denervation. In fact, the patchy denervation pattern seen in DO was suggested to be a general defining feature (33), and has been described in idiopathic (34), obstructive (35) and neurogenic (36) detrusor overactivity. This patchy nerve loss pattern has been mapped in three-dimensional image reconstructions and shows a consistent anatomical arrangement throughout the length of detrusor muscle bundles (37). Implicitly, the loss of local innervation would prevent the effects of efferent influences during storage and voiding (Figure 3). Where efferent inhibitory influence is impaired, denervated areas would be better able to express spontaneously their autonomous micromotility during the storage phase. This may lead to DO if the condition of the bladder wall in terms of

motility propagation and tone support the spread of excitation and transmission of force. Alternatively, increased amplitude of localised motility with minimal effect on pressure may be another pattern, as has been described in some women with urinary urgency in the absence of DO (38). This situation may signify increased initiation, with short propagation and/ or low tone.

The symptoms associated with altered micromotions would be affected by the extent of preservation of afferent nerves. If afferents are well preserved, the increased micromotility would result potentially in increased filling sensation. If the afferent innervation is impaired along with the efferent, then reduced sensation may occur.

In prenatal and neonatal rodents, large amplitude micromotions help expel urine from the bladder when neuronal innervation is not yet complete (16). This activity can contribute to a spinal cord to bladder reflex where voiding can be initiated by perigenital stimulation (39, 40). In infants, voiding is also partly reflexive, occurring when the bladder is full (41). After neuronal innervation is complete, reflex activity and micromotions disappear but can re-emerge in pathologies including spinal cord injury and chemical cystitis. This has been demonstrated in whole bladder sheet preparations where single-unit afferent nerve firing and tension are simultaneously recorded (Figure 4). With spinal cord transection, supraspinal inhibition is removed (42) and large amplitude micromotions develop that can stimulate afferent nerves (15) to trigger reflex voiding as well as the local release of neuropeptides. These large amplitude micromotions require overdistension, as they are prevented by urinary diversion (17), and an intact mucosa, as its removal abolishes this activity (43). In chemical irritation with acetic acid, where overdistension does not occur and large amplitude micromotions do not develop, afferents are sensitized such that low amplitude micromotions can stimulate afferent firing (Figure 4B-C). A safe and effective treatment to inhibit micromotions has not yet been demonstrated. However, there are indicators that the main drugs used clinically to treat overactive bladder can influence this type of activity. For example, β3-adrenergic receptor agonists (44, 45) and antimuscarinics (11, 44) reduce non-voiding contractions in a rodent model of storage overactivity. However, the sensitivity of afferent nerves can be dampened with botulinum neurotoxin (46) and β3-

adrenergic receptor agonists (A. Kanai, unpublished observations), and these can be surmised to treat micromotion-driven bladder overactivity.

Micromotions in patients with detrusor underactivity

DUA is a complex and poorly understood urodynamic observation, but it is worth considering how the initiation and propagation of bladder micromotions are likely to be relevant factors for pressure generation during voiding. In a normal bladder with full efferent innervation, voiding is associated with powerful excitation and reduced inhibition, so that initiation and tone will be high in the entire organ. For the fully-innervated bladder, propagation may not be a particular consideration in voiding. However, in those individuals with denervation, the ability to initiate contractions will be reduced in the denervated areas; thus denervated areas may only contract if there is propagation of excitation from a neighbouring innervated area, possibly via interstitial cells that are also present between detrusor muscle bundles. The potential for propagation of micromotions to recruit contraction in denervated areas during voiding may allow compensation for some loss of innervation. Thereby, people with the patchy denervation pattern characteristic of DO could maintain seemingly normal voiding, even though compensatory mechanisms are involved. Voiding in such people would comprise direct efferent stimulation of innervated areas, and indirect stimulation of denervated areas, as a result of propagation of excitation from neighbouring innervated areas (Figure 3). Consequently, the effect of a small extent of denervation will be modest, since denervated areas could deliver some contribution to a voiding contraction, even if it is less than that expected with full innervation. Nonetheless, there must be some constraints on the extent to which this is possible. Propagation of micromotions appears to be relatively slow; in an extensively denervated area, it is likely that the time taken to generate a rise in tone and contraction of a denervated area would be too long for effective contribution to voiding. Thus, increasing severity of denervation will be associated with a reduction in contraction response, and hence detrusor underactivity.

These processes have not been studied in humans with DUA, but in an ageing mouse model, there is a clear-cut loss of phasic pressure fluctuations (7), which is associated with bladder distension *in vivo.*

Summary

It is proposed that the generation of small intravesical pressure transients depends on the initiation and propagation of bladder wall micromotions coupled to a significant overall bladder tone. These processes are modulated by the phase of the micturition cycle, with efferent inhibition preventing excessive activity during the storage phase and efferent excitation ensuring synchronous activation of all areas during voiding. In situations where innervation is impaired, the influence of central nervous efferents on this activity is altered; the emergence of autonomous activity is seen during urine storage, due to loss of efferent inhibition. During voiding, loss of efferent excitation may be partly compensated by propagation of micromotions from the innervated areas, but not where denervation is ~~too~~ extensive (or ~~if~~ gap junctions are sparse). The extent to which aberrant micromotions generate sensation may be determined by whether afferent nerves are preserved.

Acknowledgement

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Figure legends

Figure 1; End of the filling cystometry and the pressure flow study (PFS) from a 38 year old man where DO with DUA is present. 1. Detrusor overactivity; 2. Time of maximum flow rate in PFS (maximum flow rate is 11 ml/sec, detrusor pressure 30 cm H_2O, so bladder contractility index is 85, indicating impaired contractility); 3. The subject undertakes abdominal straining to supplement the poor contractile function); 4. Cough-subtraction at the end of the void is adequate. Overall duration of voiding phase was 100 seconds, and a post void residual was present.

Figure 2; A schematic approach to representing a 3-dimensional bladder in 2-D. Much like the earth's globe (A) can be represented as a 2-D map (B), the intact bladder (C) can be portrayed schematically in 2-D (D), incorporating landmarks (trigone, urethra, ureters and nerve trunks. This illustration conveys the propagating pattern of micromotility, yellow indicating motile, and stars indicating initiation points of the microcontractions.

Figure 3; Two-dimensional representation of the possible impact of denervation on the bladder in an intact animal. TOP ROW; The innervation shown heading towards the trigone is marked with a minus (-), to signify efferent inhibition as present in the storage phase. A. Where innervation is complete, the entire bladder is effectively inhibited to facilitate storage. B. Where there is mild, patchy denervation, the affected areas are able to express some autonomous activity, and consequently OAB/ DO. C. Severe denervation increases the propensity to autonomous micromotility. BOTTOM ROW; The same bladders, but during the voiding phase (innervation +). A. The fully innervated bladder shows consistent, coherent contraction of the entire bladder wall simultaneously. B. The presence of partial denervation is compensated by the triggering from the adjacent edges where (innervated) patches are stimulated by the innervation. C. The sheer extent of denervation prevents the triggering from innervated areas generating sufficient contraction of the denervated areas to contribute usefully, resulting in detrusor underactivity.

Figure 4. Schematic diagram of an *in vitro* recording chamber for the measurement of rat bladder tension and afferent nerve activities. The urinary bladder is isolated with its associated L6-S2 spinal roots and lumbar splanchnic nerve which carry the pelvic and hypogastric afferent nerves, respectively. Detrusor contractile activity is monitored through a tension transducer which is connected to a stepper motor to stretch and elicit mechanical stimulation. Nerves are split in the oil baths to allow recording of mechanosensitive afferents that respond to micromotion-induced bladder stretch, as well as nociceptors that fire spontaneously, with single-units (at the arrows) determined by off-line analysis. B. Under normal conditions, micromotions do not generate significant changes in tension as reflected by the lack of afferent nerve firing from the S1 spinal nerves. C. Addition of 0.1% acetic acid to the bladder bath enhanced micromotions which, combined with sensitization of the afferent nerves, triggered robust firing.