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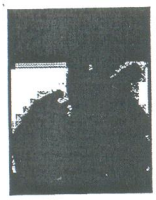

Review

The physiological function of lower urinary tract smooth muscle

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ABSTRACT

The lower urinary tract is a muscular system composed of the urinary bladder and the outflow tract. During filling with urine the bladder is relaxed and the outflow tract offers a high resistance; during emptying the outflow resistance falls and the bladder wall generates a high wall tension to raise intravesical pressure. The coordination of these responses is organized in the brainstem and sacral spinal cord to control the activity of autonomic and somatic efferents to the smooth muscle of the bladder (detrusor) and the smooth and skeletal muscle of the bladder base and urethra. Detrusor contraction is predominantly controlled by parasympathetic fibres releasing acetylcholine and ATP; the outflow tract is controlled by parasympathetic and sympathetic fibres to the bladder base (trigone) and urethral smooth muscle (including a nitregic component) and somatic fibres to the urethral rhabdosphincter. The smooth muscles also develop spontaneous contractions that determine the tone of the musculature. The cellular signaling pathways that evoke contraction due to neurotransmitter release and the origin of spontaneous activity are discussed, as well as the electrical properties of the smooth muscle relevant to the propagation of electrical signals. Finally the interaction of muscle cells with other cell types (epithelium and interstitial cells) is considered, relevant to their ability to regulate muscle contractility. Throughout, the basic physiological processes are considered in relation to pathological developments that are prevalent in the human lower urinary tract, in particular the overactive bladder and urinary incontinence, and the identification of drug targets to manage these conditions.

Contents

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1. Introduction: Functions of the lower urinary tract (LUT) in health and disease

The LUT comprises two regions: the urinary bladder and the outflow tract (bladder neck, or base, and urethra — Fig. 1). For most of the time the LUT stores urine as it fills via the ureters. To achieve this the bladder is a highly compliant vessel (low wall stress), coupled to a high outflow resistance due to the presence of internal and external sphincters. In women the outflow tract is shorter than in men. Therefore a reduction of sphincter function has more important consequences than in men to permit involuntary loss of urine (incontinence). However, loss of sphincter function in men, as may occur after radical prostatectomy, can also result in incontinence (Dalkin et al., 2003). During periodic voiding a reversal of properties occurs: bladder wall stress is high to raise intravesical pressure, coupled to a low outflow resistance. Changes to bladder wall stress and outflow resistance are governed largely by alterations to the contractile state of the smooth and skeletal muscle components of these regions, and these in turn are regulated by autonomic and somatic innervation to the region. Most of this review will be concerned about the regulation of the contractile state of detrusor smooth muscle in the bladder wall and its relevance to the common condition of detrusor overactivity. However, a brief consideration of outflow tract muscles is important to put in context the complex regulation necessary to maintain a properly functioning LUT. There will be some omissions from this brief review, in particular the physiological basis of some other functional disorders such as detrusor underactivity, and the role of other muscular tissue such as that found in the prostate gland.

Lower urinary tract symptoms (LUTS) of urgency, nocturia and frequency, with or without incontinence (Abrams et al., 2002) are extremely common in the population (estimated between 10 and 20%) and increase with age. In many of these cases LUTS are associated with detrusor overactivity (DO) where there are uncontrollable contractions of the bladder (Irwin et al., 2006; Oelke et al., 2008; Coyne et al., 2009). DO is associated with several conditions including bladder outlet obstruction (in older men often due to prostate enlargement), or spinal cord injury, but in most cases the cause is idiopathic. However, because of the immense social cost and financial burden to the health services there is considerable effort to understand and manage the condition and this provides much of the motivation to study LUT musculature.

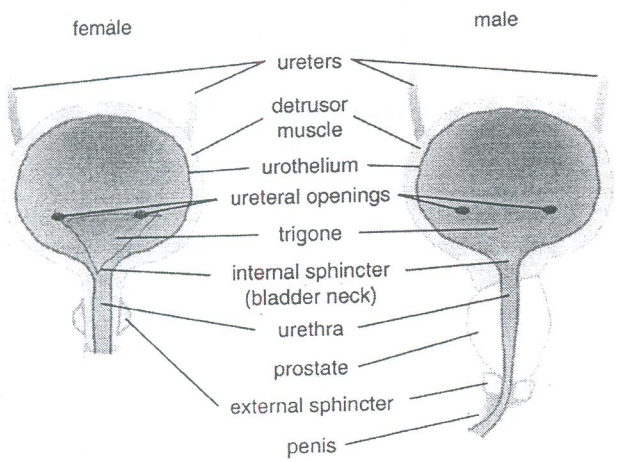

Fig. 1. The lower urinary tract in women and men. The dome of the bladder is lined by urothelium and detrusor smooth muscle to form the largest part of the bladder. The bladder base (trigone and bladder neck) forms a functionally separate part of the bladder. The urethra is shorter in women than in men and is surrounded by smooth and skeletal muscle. In men the prostate gland also surrounds the urethra but has no functional control over outflow tract resistance, except when it enlarges as a benign or metastatic growth.

2. Detrusor smooth muscle: contractile proteins and intracellular Ca^{2+}

The body of the bladder – the dome – consists of smooth muscle bounded on the inner face by a urothelium/suburothelium and on the outer face by serosa (Fig. 2). Although beyond the scope of this review, the urothelium/suburothelium does more than separate the detrusor muscle from the urine in the bladder lumen, it is the site of afferent sensation and also can exert a direct influence on detrusor function itself (see below) — (reviewed in Birder and de Groat (2007) and Hanna-Mitchell and Birder (2008)). Detrusor muscle cells are spindle-shaped single nucleated cells organized into bundles separated by connective tissue. Thin filaments of α- and β-actin are attached to dense bodies on the cell membrane, to provide binding sites for myosin thick filaments, mainly of the SM1B and SM2B subtypes (Martin et al., 2007). An increase of the sarcoplasmic $[Ca^{2+}]$, $[Ca^{2+}]_i$, from a basal level of 50–100 nM initiates contraction, half maximal activation is achieved at about 1 μM (Wu et al., 1995). The source of Ca^{2+} can be extracellular, via L- and T-type Ca^{2+} channels (Montgomery and Fry, 1992; Sui et al., 2003b) or from intracellular stores (Fry et al., 1994) released by Ca^{2+}-induced Ca^{2+} release, or separately by activation of IP_3 receptors (Wu et al., 2002). The increase of $[Ca^{2+}]_i$ is transient and Ca^{2+} is either removed from the cell via Na^+/Ca^{2+} exchange (Wu and Fry 2001) and an ATP-dependent Ca-pump (Liu et al., 2006), or re-accumulated in intracellular stores via a SERCA pump; the activity of the latter is modulated by intracellular proteins, such as phospholamban (Nobe et al., 2001).

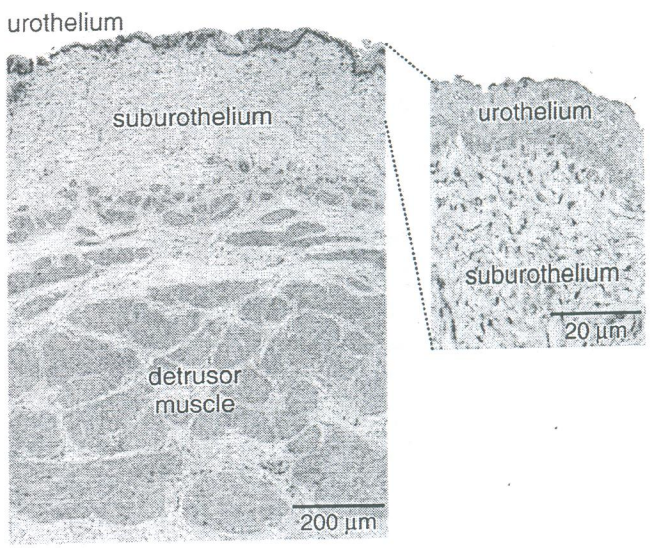

Fig. 2. Cross-section of the bladder wall of a neonatal sheep. It shows the urothelium uppermost, separated from the detrusor muscle layer by a suburothelium. The inset shows an enlargement of the urothelium and adjacent suburothelium, separated by a basal lamina. The suburothelium is filled with a network of interstitial cells (myofibroblasts) seen as the darker objects in the layer.

As with other smooth muscles, the contractile proteins are activated by myosin phosphorylation via a myosin light chain kinase (MLCK), which in turn is activated by a $Ca^{2+}{}_4$–calmodulin complex. Dephosphorylation of the myosin light chain induces relaxation, via a myosin light chain phosphatase (MLCP, Fig. 3). The sensitivity of the contractile system can therefore be altered by modulating the activities of MLCK or MLCP. Intracellular messengers that modulate the activity of these enzymes can therefore modulate contractile activity. MLCK activity itself is decreased by phosphorylation with several kinases including: CaM kinase II, mitogen-activated protein (MAP) kinase, cAMP-dependent kinase (PKA) and p21-activated kinase (Yamaguchi, 2004). An example is the action of caffeine, application of which causes near maximum release of Ca^{2+} from intracellular stores, but evokes little or no sustained tension in isolated muscle strips. The effect of a release of Ca^{2+} on myofibrillar activation is offset by inhibition of phosphodiesterase (PDE) activity, which enhances intracellular cAMP and hence PKA activation.

MLCP activity can also be reduced by phosphorylation, which increases the Ca^{2+}-sensitivity of the contractile system. Of importance is inhibition of MLCP activity by *rho*-associated kinase (ROK or ROCK), which in turn is activated by G-proteins of the *rho*-family, ROCK I/II in detrusor (Kimura et al., 1996). Inhibitors of ROCK activity, e.g. Y-27632 and HA-1077, attenuate agonist-induced contractions, but do not affect depolarisation-mediated (with high [KCl]) contractures (Durlu-Kandilci and Brading, 2006). This suggests that the *rho*-kinase pathway plays a role in regulating the contractile state of the bladder.

3. Detrusor smooth muscle: nerve-mediated contractions

Preganglionic parasympathetic fibres originate in the sacral (S2–S4) spinal cord and relay in pelvic ganglia near the bladder base and throughout the bladder wall. *In vivo*, detrusor contraction is controlled by a dense network of parasympathetic motor nerves. Nerve fibres expand at intervals into varicosities that contain small vesicles of neurotransmitters, so that nearly every detrusor myocyte is in functional contact with a motor nerve. These nerves stain for acetylcholinesterase, an extracellular enzyme that degrades acetylcholine, and are therefore designated as cholinergic fibres. Functionality of these nerves can be determined *in vitro* using muscle strips. Tetanic stimulation with short pulses ($\leq$0.1 ms) generates phasic contractions that are completely abolished by the neurotoxin tetrodotoxin (TTX; Sibley, 1984). TTX blocks Na^+ channels, which are lacking in detrusor muscle but present in motor nerves. With detrusor muscle from most animals, such nerve-mediated contractions are partially blocked by the muscarinic receptor antagonist, atropine. The remainder is mainly blocked by pre-treatment with the non-hydrolysable ATP

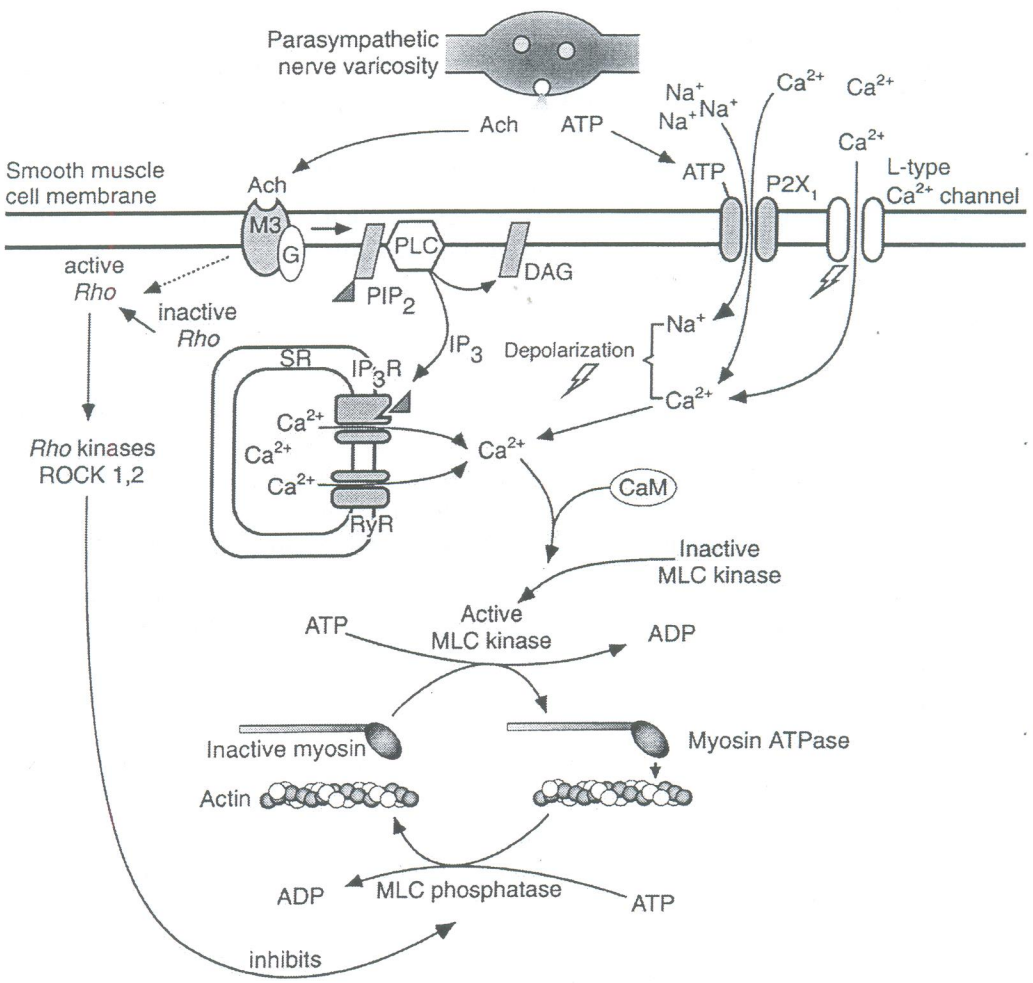

Fig. 3. A diagram of some of the important intracellular signaling pathways in the detrusor muscle cell. Shown are two surface receptors: acetylcholine (Ach) M_3 receptor and the $P2X_1$-ATP receptor. The M_3 receptor initiates the formation of inositol trisphosphate (IP_3) and diacylglycerol (DAG) from membrane phospholipids (PIP_2) by the action of phospholipase-C (PLC). IP_3 initiates Ca^{2+} release from intracellular Ca^{2+} stores (SR). Ca^{2+} binds to calmodulin (CaM) to activate myosin light chain (MLC) kinase and phosphorylate and activate myosin to bind to actin. M_3 receptor activation may also activate the *rho*-kinase pathway, which phosphorylates and reduces the activity of myosin light chain phosphatase. Shown also is the influx of cations through the $P2X_1$ receptor ion channel and the L-type Ca^{2+} channel.

analogue, ABMA (α,β-methylene ATP), which initially activates but subsequently desensitises purinergic (P2X) receptors. Thus, acetylcholine (Ach) and ATP are functional neurotransmitters that initiate detrusor contraction.

With detrusor muscle samples from human bladders that lack any contractile pathology (and old-world monkeys), nerve-mediated contractions are completely abolished by atropine implying that Ach is the sole activating neurotransmitter. Of interest however, is that with detrusor samples from human bladders displaying pathological contractile overactivity, atropine-resistance emerges, with the residual contraction blocked by ABMA (Sjögren et al., 1982; Palea et al., 1993; Bayliss et al., 1999). One hypothesis for the emergence of ATP as a functional transmitter in human bladder pathologies is that ATP is released along with acetylcholine from parasympathetic nerve varicosities. In the normal bladder ATP is completely broken down in the neuromuscular junction by extracellular ectoATPases so that none activates the smooth muscle. In bladders exhibiting DO either excess ATP is released, or ATP is less effectively broken down in the junction. Evidence for the latter possibility was obtained when it was shown that ectoATPase activity is reduced in detrusor samples from human DO bladders (Harvey et al., 2002), corroborated by other observations that show enhancement of the nerve-mediated contraction in the presence of ectoATPase inhibitors such as ARL 67156 (Westfall et al., 1996, 1997). This observation provides one target for reducing contractions in the overactive bladder, but has yet to be clinically exploited.

4. Receptor targets for motor neurotransmitters and intracellular pathways

4.1. Muscarinic mechanisms

There are five subtypes of muscarinic receptors from molecular (m_{1-5}) and pharmacological (M_{1-5}) characterisation. In detrusor, m_2 and m_3 subtypes are expressed, with m_2 receptors expressed three- to nine-times in excess to m_3 receptors (Wang et al., 1995). However, pharmacological studies, using subtype selective antagonists also regularly show that in human detrusor from stable bladders this less numerous M_3-receptor fraction is responsible for contractile activation (Hegde, 2006). More recently, it has been proposed that M_2 receptors do exert a more significant role in some pathological bladders, such as denervated or hypertrophied organs, or during M_3-receptor desensitisation (Braverman et al., 2006a). Antimuscarinic agents are the current mainstay for the clinical treatment of LUTS and there is current debate about the superiority of M_3-selective agents (e.g. darifenacin) or those that have a broader subtype profile (e.g. oxybutynin). Clinical studies show little difference between the effectiveness of the selective and broader-spectrum types of antagonists (Chapple et al., 2008), so that the significance of the possible shift of functional receptor subtype in pathological conditions remains unresolved.

M_3 receptors are coupled to $G_{q/11}$-proteins, which activate the enzyme phospholipase-C (PLC) to generate the second messengers inositol trisphosphate (IP_3) and diacylglycerol (DAG) from membrane phosphoinositides (PIP_2). In turn, IP_3 binds to a receptor on intracellular Ca-stores to release Ca^{2+} for contraction (see Fig. 3). A rise of $[Ca^{2+}]$ near the surface of Ca^{2+} stores releases further Ca^{2+} through ryanodine receptors (RyR) by a process of Ca^{2+}-induced Ca^{2+} release (CICR) to accelerate the increase of the sarcoplasmic $[Ca^{2+}]$. Experimental evidence supports the significance of this pathway in detrusor: muscarinic agonists increase inositol phosphate production and tension over the same agonist concentration range in detrusor strips (Harriss et al., 1995); the rise of $[Ca^{2+}]$ generated by muscarinic agonists is independent of membrane potential; intracellular Ca^{2+} release is blocked by several IP_3-receptor blockers, including heparin (Wu et al., 2002; Braverman et al., 2006b). DAG may play a supplementary role by activating protein kinase-C, which in turn phosphorylates MLC phosphatase, reducing its activity and hence increasing the Ca^{2+}-sensitivity of the contractile proteins. However, the exclusivity of this intracellular pathway, mediated by PLC, has more recently been challenged as some inhibitors of PLC activity are relatively ineffective in attenuating contractions mediated by musarinic agonists such as carbachol. One proposal is that the Ca^{2+}-sensitivity of the contractile proteins is enhanced through activation of the *rho*-kinase pathway; the rise of intracellular $[Ca^{2+}]$ is attributed to activation of non-specific cation channels that in turn depolarise the cell to open L-type Ca^{2+} channel (Frazier et al., 2007a). There have been a number of attempts to reconcile the controversy surrounding the importance or not of the PLC-mediated pathway; and it may be that there are considerable species differences (Wuest et al., 2007).

M_2 receptors are coupled to G_i-protein that reduces cAMP production by its influence on adenylate cyclase activity. cAMP in turn activates protein kinase-A which also phosphorylates MLC phosphatase and so increases the Ca^{2+}-sensitivity of the contractile proteins. Several reviews consider in more detail the role of muscarinic-dependent pathways in generating detrusor contaction (Hegde, 2006; Frazier et al., 2007b).

Muscarinic receptors are also desensitised after prolonged exposure to Ach, mediated by phosphorylation of the muscarinic receptor by guanosine phosphate binding G-protein coupled receptor kinase (GRK: Pals-Rylaarsdam et al., 1995); m_2 and m_3 GRK mRNAs have been described. Expression of GRK_2 is reduced in detrusor from patients with obstructed bladders (Furuya et al., 2006) and may therefore contribute to consequent detrusor overactivity.

4.2. Purine receptor mechanisms

In most mammalian species ATP is co-released with Ach from parasympathetic nerves and in the bladder activates purinergic receptors to initiate contraction. The significance of this in the overactive human and animal bladders has been described above. ATP binds to purinergic, P2, receptors that are in turn divided into P2X and P2Y families (Ralevic and Burnstock, 1998: Burnstock, 2007). P2X receptors are ionotropic, ligand-gated non-specific cation channels while P2Y receptors are metabotropic G-protein coupled. Seven P2X receptors subtypes have been cloned and characterized ($P2X_{1-7}$) and $P2X_1$ labelling is present on detrusor muscle (Elneil et al., 2001). The $P2X_1$ receptor is a non-specific cation channel and activation generates an inward, depolarising current of Na^+ and Ca^{2+} sufficient to activate L-type Ca^{2+} channels to generate an action potential and further Ca^{2+} influx (Wu et al., 1999, Fig. 3). The influx of Ca^{2+} can either activate directly the contractile proteins, or more effectively further raise the intracellular $[Ca^{2+}]$ by CICR from Ca^{2+} stores.

There are eight subtypes of P2Y receptors ($P2Y_{1,2,4,6,11-14}$) linked either to $G_{q/11}$ ($P2Y_{1,2,6}$), G_i ($P2Y_{12-14}$) or several proteins: $G_{q/11}/G_i$ ($P2Y_4$); $G_{q/11}/G_s$ ($P2Y_{11}$) (Abbracchio et al., 2006; http://www.iuphardb.org/index_ic.jsp). P2Y receptors, although not a specific subtype, have been implicated in relaxation of smooth muscle, possibly via cAMP-dependent PKA activity (McMurray et al., 1998).

Hydrolysis of ATP ultimately yields adenosine that can exert its own effects on detrusor through P1 receptors, of which four subtypes have been described; A_1, A_{2A}, A_{2B}, and A_3, all of which are coupled to G-proteins, positively (A_2) or negatively ($A_{1/3}$) regulating adenylyl cyclase activity. Less is known about their actions in detrusor; however adenosine relaxes detrusor via A_2 receptors (Nicholls et al., 1992; Gopalakrishnan et al., 2002), and could also reduce transmitter release via A_1 receptors.

4.3. Adrenergic mechanisms

Direct sympathetic innervation of the detrusor is less important than parasympathetic activation, although hypogastric fibres modulate parasympathetic bladder ganglia. However, all three β-receptor subtypes are expressed in detrusor muscle, β3 the most

abundant (Yamaguchi, 2002; Nomiya and Yamaguchi, 2003). Receptors are G_s-protein-coupled receptor and activation elevates smooth muscle cAMP and causes detrusor relaxation (Badawi et al., 2005). Downstream effectors activated via cAMP include not just PKA-dependent MLC phosphatase inactivation, but also activation of large-conductance, Ca^{2+}-activated K^+ (BK_{Ca}) channel — see also Michel and Vrydag (2006). There is currently active interest in developing β-receptor agonists suitable for clinical use as a treatment for bladder overactivity (Malmgren et al., 1987; Hicks et al., 2007; Leon et al., 2008).

5. Spontaneous activity

The bladder is capable of generating considerable spontaneous contractile and electrical activities, and this is reflected *ex vivo* in isolated bladders, multicellular detrusor preparations or even isolated cells (Sibley, 1984; Sui et al., 2009, Fig. 4). Moreover, such activity is greater in such isolated preparations taken from animals and patients with overactive bladders (Mills et al., 2000; Ikeda et al., 2007; Sui et al., 2009). Spontaneous activity can take the form of contractions involving the temporal coordination of many muscle bundles, or can be uncoordinated when it manifests as changes to muscle tone. Spontaneous activity is resistant to TTX, but is abolished by agents that limit transmembrane Ca^{2+} influx, such as L-type Ca^{2+}-channel blockers and reduction of extracellular Ca^{2+} itself (Guarneri et al., 1991). The implication of all these observation is that it arises from within the bladder itself. Several hypotheses have been advanced to account for spontaneous activity, which are not mutually exclusive. Two of these relate to altered activity of the nervous control to the bladder and whilst they cannot be the exclusive explanation, they could contribute to the phenomenon and so therefore deserve inclusion.

5.1. Neurogenic hypothesis

Reduced central nervous inhibition to the sacral parasympathetic motor nuclei increases the gain of the micturition reflex and augments motor activity to detrusor muscle (de Groat, 1997). This may represent a distinct population of patients with overactive bladders.

5.1.1. Transmitter leak

This occurs from motor fibres to generate small local contractions or increases of tone (Andersson, 2004).

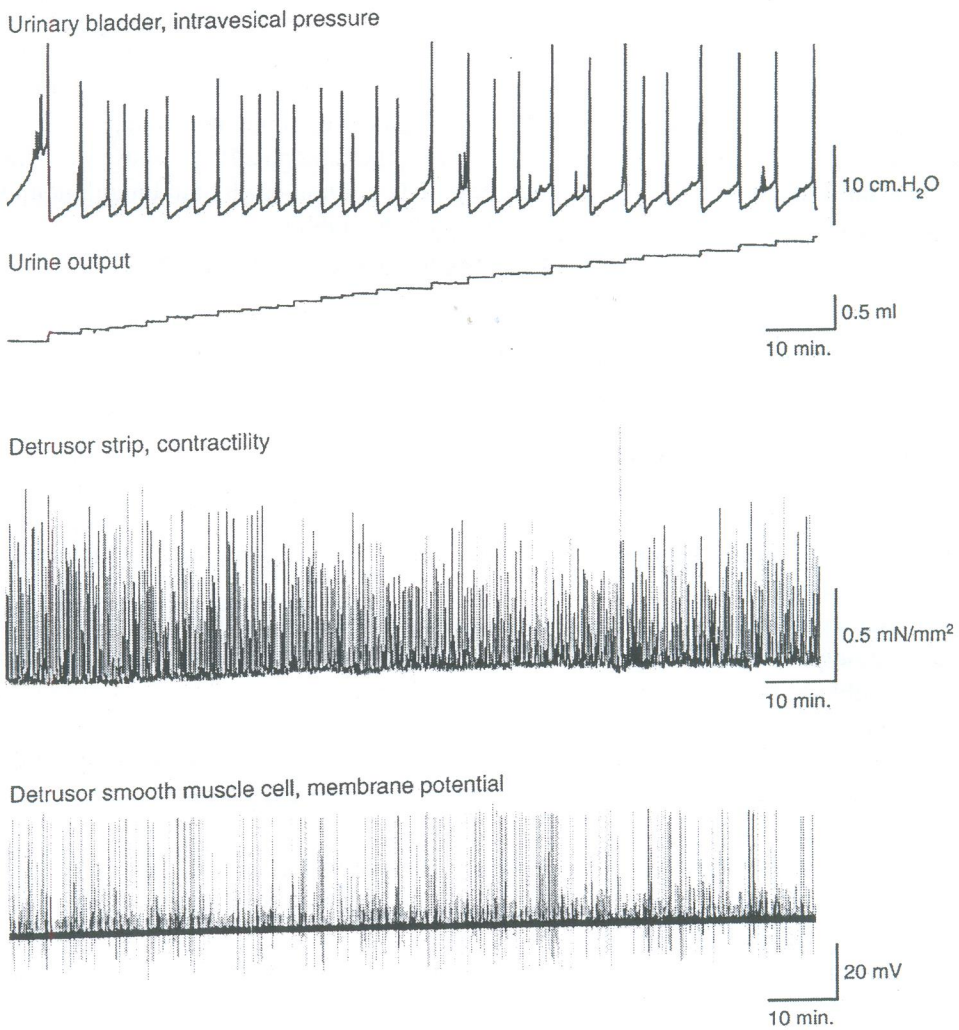

Fig. 4. Spontaneous activity in the urinary bladder. Top tracings. Intravesical pressure in an *in vivo* mouse bladder during filling (rate: 1 ml h^{-1}). Transient pressure rises – 'voiding contractions' – cause the periodic output of urine. Middle tracing. Isometric tension recordings of spontaneous activity from an isolated, superfused and unstimulated strip of guinea-pig bladder. Lower tracing. Spontaneous action potentials from an isolated mouse bladder strip as recorded with an intracellular microelectrode. The resting potential (−44 mV) is indicated by the thicker horizontal line. The APs show a depolarising phase and after hyperpolarisations.

5.2. Myogenic hypothesis

Changes to the excitability and intercellular coupling of smooth muscle cells with other myocytes (Brading, 1997) or interstitial cells lead to the larger contractions (Hashitani, 2006). Two factors are important for this hypothesis: an increase of myocyte spontaneous activity and also better coupling between cells to generate coordinated contractions originating from several muscle bundles.

Increased spontaneous activity is recorded in isolated cells from overactive bladder samples (Sui et al., 2009). The origin of this activity may reside in altered Ca^{2+} channel populations. Both L-type and T-type Ca^{2+} currents can be recorded from detrusor muscle. However, the proportion of T-type current is increased in cells from bladders showing DO (Sui et al., 2003a). Because T-type channels are opened at more negative membrane potentials an increase in density would increase spontaneous electrical activity (Yanai et al., 2006). Low concentrations of $NiCl_2$, which would preferentially block T-type Ca^{2+} channels, reduced spontaneous activity, indicating their functional role (Chow et al., 2003).

Gap junction blockers reversibly inhibit large spontaneous contractions in overactive bladders, using animal models (Ikeda et al., 2007). Detrusor smooth muscle cells are electrically connected via gap junctions composed of the connexin subtype Cx45 (Sui et al., 2003a). The expression of connexins in detrusor is lower than in tissues such as myocardium indicating that detrusor does not form such an effective electrical functional syncitium. Moreover Cx45 decreases in detrusor from overactive bladders, so that increased intramuscular functional connectivity could not lead to enhanced, larger spontaneous contractions. However, between muscle bundles a network of interstitial cells can be identified, which label for Cx43 and also the tyrosine-kinase receptor protein c-*kit*. Interstitial cells generate spontaneous and carbachol-evoked Ca^{2+} and electrical activity (McCloskey and Gurney, 2002). It is hypothesized that these cells modulate and coordinate activity of detrusor bundles facilitating the large, slower overactive-bladder contractions (Hashitani et al., 2004). Interstitial cells may also be a control point for regulation of spontaneous activity; they are innervated by afferent nerves labelling for nitric oxide synthase (Smet et al., 1996), and they also express cGMP activity. Our understanding of these cells is in its infancy, but they potentially form an important target to modulate overactive bladder activity.

5.3. Urotheliogenic hypothesis

Activity of the urothelium/suburothelium layer (often referred to as the mucosa) influences detrusor function (Ikeda and Kanai, 2008). The urothelium is a transitional epithelium interfacing directly with the bladder lumen. Below this is a suburothelium containing a dense network of capillaries and afferent nerves, and also a network of interstitial cells (myofibroblasts) connected by Cx43 gap junctions (Fig. 1, inset) that show several differences to their detrusor layer counterpart (Sui et al., 2004; Davidson and McCloskey, 2005). The number of these cells is greatly increased in overactive bladders (Ikeda et al., 2007, Kubota et al., 2008).

The urothelium/suburothelium can both increase and decrease detrusor contractile modalities. With isolated detrusor preparations the magnitude of nerve-mediated and carbachol-evoked contractions is attenuated if the urothelium/suburothelium is left intact. The negative inotropic agent has been demonstrated to be a diffusible agent but its identity is at present unknown (Hawthorn et al., 2000; Murakami et al., 2007).

On the other hand, spontaneous activity is increased if the mucosa is left intact. Optical imaging experiments of bladder cross-sections show that spontaneous activity often arises in the suburothelium and spreads to the detrusor layer (Kanai et al., 2007). Using bladder sheets with the urothelial surface uppermost, optical imaging measurements show propagating Ca^{2+} waves over the sheets, but only in those sections where the mucosa had not been removed (Sui et al., 2008). These waves were initiated by local application of muscarinic agonists or UTP, or focal mechanical stimulation. The use of UTP was of interest as this purine evokes excitatory responses in suburothelial interstitial cells, but not in detrusor myocytes. Taken together these experiments are consistent with the hypothesis that spontaneous activity can originate in the suburothelial layer of interstitial cells and then propagate to the detrusor where spontaneous contractions are generated. The increase of suburothelial interstitial cells in bladders showing detrusor overactivity is consistent with their role in generating increased activity.

6. Electrical activity in detrusor smooth muscle

6.1. Function of electrical activity

Detrusor smooth muscle is electrically excitable and capable of generating action potentials (APs). APs are normally generated in bursts and are associated with contraction. Although the major motor transmitter, acetylcholine, probably acts through mechanisms independent of altering the membrane potential, electrophysiological properties are important. For example control of the resting membrane potential is essential to regulate Ca^{2+} influx and efflux via ion channels and ion exchangers such as Na^{+}–Ca^{2+} exchange, and action potentials can initiate and sustain spontaneous activity. Furthermore, ionotropic neurotransmitters such as ATP are functionally significant in human pathologies (Bayliss et al, 1999). In mouse detrusor, spontaneous excitatory junction potentials (sEJPs) are generated by release of ATP (Young et al., 2008). In this paper it was hypothesized that ATP release from parasympathetic terminals can trigger spontaneous APs when sufficient cation influx through $P2X_1$ channels opens L-type Ca^{2+} channels.

The extent to which APs can propagate between functionally connected detrusor cells is unclear. The presence of Cx45 gap junctions provides a mechanism for intercellular electrical coupling and a finite electrical conductance of the intracellular space can be measured (Sui et al., 2003b). Early experiments recording electrical activity with the sucrose-gap technique (Creed et al., 1983; Fujii, 1988) pre-suppose that electrical current flows between cells. Furthermore direct measurement of the cable properties of detrusor demonstrated a space constant of more than 1 mm (Seki et al., 1992; Fry et al., 1999). This long value, which means that intracellular current can flow several cell lengths, is made possible by the high membrane resistance of detrusor myocytes, confining the weak intracellular electrotonic currents to this compartment. AP propagation is thus possible, at least within muscle bundles, albeit with a fairly slow conduction velocity. The slow conduction is not detrimental to this tissue however, as contractile activation itself is relatively slow. This analysis is further evidence that the basic contractile element in the detrusor muscle mass is the muscle bundle.

6.2. Ion channels: resting and action potentials

The membrane potential is sufficiently negative (−40 to −50 mV) to permit initiation of a regenerative AP. The upstroke phase is carried by Ca^{2+} influx, predominantly through L-type Ca^{2+} channels, and repolarisation is mediated by K^{+} efflux through several K^{+} channels (Montgomery and Fry, 1992; Imaizumi et al., 1998). The Ca^{2+} influx is sufficient to elicit further release from intracellular stores to sustain contractions. Detrusor L-type Ca^{2+} channels show a prolonged-open state induced by large depolarisations (Nakayama and Brading, 1993), which would enhance Ca^{2+} influx. T-type Ca^{2+} channels have also been described in detrusor muscle and their contribution to total inward current is enhanced in myocytes from bladders showing DO (Sui et al., 2007: see above: Spontaneous activity and Myogenic

hypothesis), with the proportion of total inward Ca^{2+} current increased in cells from overactive bladders (Sui et al., 2007). Several receptor modulators that alter detrusor contractility also affect the L-type Ca^{2+} current. Antimuscarinic agents such as propiverine, attenuate L-type Ca^{2+} current (Zhu et al., 2008), an effect probably mediated via M_3 receptors. β-agonists also attenuate Ca^{2+} current by a cAMP/protein kinase A-dependent mechanism (Kobayashi et al., 2003).

Outward current is predominantly carried through Ca^{2+} activated large-conductance K^+ (BK_{Ca}) and small conductance (SK_{Ca}) channels. These channels determine the resting membrane potential, AP repolarisation (Herrera et al., 2001; Hashitani and Brading, 2003) and determine myocyte contractility (Herrera et al., 2000). Ca^{2+} entry through voltage-dependent Ca^{2+} channels activates both BK_{Ca} and SK_{Ca} channels, but whereas SK_{Ca} channels are modulated by Ca^{2+} entry itself, BK_{Ca} channels are regulated by Ca^{2+} release from intracellular stores via ryanodine receptors (Herrera et al., 2001; Herrera and Nelson, 2002). More recently it has been demonstrated that the SK2 member of the SK family is the functionally significant subtype in detrusor (Thorneloe et al., 2008). Physiologically, coupling of L-type channel influx to outward current activity is linked to regulation of Ca^{2+} influx into the myocyte (Wu et al., 2002), whereby decreased Ca^{2+} influx activates less outward current, depolarises the cell and hence enhances L-type channel opening.

Alteration to BK_{Ca} current density may contribute to myogenic bladder overactivity. Deletion of the *slo*-gene that encodes the channel protein enhances muscle sensitivity to cholinergic and purinergic agonists (Werner et al., 2007), conversely injection of *slo*-cDNA reduced overactivity (Christ et al., 2001). BK_{Ca} channel activity is regulated by phosphorylation of the pore-forming α-subunit (Tian et al., 2008), and offers a mechanism whereby cyclic nucleotides and PKC activation can regulate channel function. Conversely, the Ca^{2+}-dependent phosphatase, calcineurin, decreases BK_{Ca} conductance (Loane et al., 2006).

Intracellular ATP-gated K^+ channels (K_{ATP}) have also been described in detrusor smooth muscle, and channel openers hyperpolarise the cell and reduce spontaneous activity (Bonev and Nelson, 1993). A problem with the use of these channel modulators is that of tissue specificity, as many are as potent, if not more, in generating similar responses in vascular smooth muscle. Recently K_{ATP} channel openers that show selectivity for detrusor over vascular smooth muscle cells have been described (Yunoki et al., 2008).

Stretch-activated channels are present in the detrusor myocyte membrane and could serve a dual purpose: to permit cation influx to depolarise the cells and thus cause contraction to counter an initial stretch; and to initiate intracellular signaling cascades that may initiate cellular reconfiguration or growth (Adam et al., 2004). Physical stretch of detrusor myocytes opens non-selective cation channels, depolarises the cell and augments Ca^{2+} influx through Ca^{2+} channels (Wellner and Isenberg, 1994). However, stretch also increases K^+ channel activity (Baker et al., 2008). The net effect of these two actions would be to increase spontaneous activity.

7. The bladder base

The bladder base consists of the trigone, vesicoureteric junction (i.e. where the ureters drain into the bladder) and the anterior bladder wall (Fig. 1). It may be considered to form a functional entity that controls urine outflow during filling and voiding and also provides an effective anti-reflux mechanism to prevent urine being forced back into the ureters (Tanagho, 1982). It therefore plays a vital role in maintaining continence and aiding micturition. Anatomically it is demarcated as the triangular region between the ureteral openings and the bladder outlet. The region was believed to have a mesodermal origin, but more recent studies suggest rather that the trigone is formed predominantly from bladder muscle; and the contribution from ureteral longitudinal fibres is more limited (Viana et al., 2007).

In general, the bladder base musculature exhibits greater spontaneous activity within individual myocytes, compared to detrusor and lays down more connective tissue. Spontaneous activity is mainly driven by ion fluxes through membrane L-type Ca^{2+}-channels and Ca^{2+}-activated Cl^--channels. Extensive gap junction coupling ensures electrical propagation and coordination of function throughout the tissue (Roosen et al., 2009a). In all the functional properties of this region, a stable structure is provided against which the bladder dome can contract and relax during the micturition cycle.

The area is one of dual sympathetic/parasympathetic innervation and both systems significantly control contractile function (Michel and Vrydag, 2006; Roosen et al., 2008), as demonstrated from experiments using adrenergic and muscarinic receptor agonists and antagonists. α_{1D}-adrenoceptors are the most abundant subtype in humans; muscarinic receptor distribution is similar to that the in detrusor (Michel and Vrydag, 2006). The contrast between the bladder base and dome to sympathetic stimulation reflects the different receptor profiles in these two regions. Animal studies show that during the storage phase contraction is generated by sympathetic activation, which when coupled to similar actions on urethral smooth muscle generates an effective closure mechanism for the bladder outlet and minimises vesicoureteral reflux. The role of the parasympathetic innervation is more controversial; acetylcholine generates contraction of the smooth muscles but its contribution is less well-defined.

More recently, considerable synergy of action, with respect to contractile activation, has been demonstrated between the adrenergic and muscarinic systems in the trigone. When both are activated together the end result is significantly greater than if the two are independently activated. The two processes act through different cellular pathways; muscarinic-dependent activation is a $[Ca^{2+}]_i$-dependent process whereas the adrenergic system primarily operates through Ca^{2+}-sensitisation of the contractile machinery (Roosen et al., 2008; Roosen et al., 2009b). This synergistic mechanism may contribute to the prevention of urinary reflux into the ureter and increase outflow closure pressure, as the trigone is sited to regulate both functions. This synergy may explain why combination therapies of antimuscarinic and α-receptor blocker agents are particularly effective in reducing LUTS symptoms, even in men with outflow tract obstruction (Athanasopoulos et al., 2003; Lee et al., 2004; Kaplan et al., 2007; Rovner et al., 2008), where there has been a reluctance to prescribe antimuscarinic agents for fear of urinary retention.

During micturition the trigone may relax and facilitate the passage of urine into the urethra. Generation of NO has a vital role in relaxation of the bladder base, as it does in the urethra (Andersson and Wein, 2004). NO relaxes isolated smooth muscle preparations from the outflow region, suggesting that it may be involved in the decrease of intraurethral pressure that precedes and accompanies the start of normal micturition. This NO-based relaxation provides an effective mechanism to allow the bladder base and trigone to switch from a closed to open state, especially in combination with the autonomic control of the contractile machinery.

8. Muscles of the urethra

8.1. Role of the musculature

It has been stated above that during voiding, relaxation of the bladder outlet precedes detrusor contraction to facilitate emptying. During filling the outlet is contracted to maintain continence. The tissues of the urethra (urethral smooth and skeletal muscle as well as the mechanical properties of the lamina propria) combine their functionality with the bladder base to bring about efficient filling and continence. Urethral smooth muscle consists of an inner longitudinal layer and an outer circular layer. The longitudinal muscle shortens during micturition and circular smooth muscle contracts to maintain

continence, especially in the longer male urethra. A skeletal muscle component forms an incomplete ring around the urethra to contribute to the external sphincter mechanism (Strasser et al., 2000a; Ho et al., 1997; Burnett and Mostwin, 1998). It should be remembered that the levator ani (puborectalis, pubococcygeus and iliococcygeus) and coccygeus muscles of the pelvic floor also provide considerable support, but they will not be considered here. Loss of a continence mechanism through poor function of outflow tract musculature (pelvic floor, bladder base or urethral function) can lead to urine leakage when abdominal pressure rises and transmits to the bladder. Thus coughing, straining or even movements can evoke urine loss, termed 'stress urinary incontinence', and this is especially prevalent in women.

8.2. Urethral smooth muscle

The urethra is innervated by both the sympathetic and parasympathetic systems. Activity in pudendal nerve parasympathetic fibres relaxes urethral smooth muscle, especially in the proximal portion, and therefore the outflow region; hypogastric sympathetic fibres (T10–L2) generate contraction. Sympathetic control is mediated by α_1 receptors, mainly the $\alpha_{1A/L}$ subtype (Nishimatsu et al., 1999; Bagot and Chess-Williams, 2006) and about half of the urethral pressure is maintained by adrenergic receptor activation (Andersson and Wein, 2004). Partial $\alpha_{1A/L}$ agonists, such as Ro 115–1240, have been proposed as agents that could manage stress urinary incontinence without significant cardiovascular side-effects (Musselman et al., 2004).

Urethral smooth muscle also exhibits spontaneous electrical and mechanical activity that contributes to overall muscular tone. Electrical activity occurs in bursts of APs superimposed on a slower rhythmic oscillation of membrane potential, and can be initiated by autonomic transmitters (Callahan and Creed, 1981). Both L-type and T-type Ca^{2+}-currents have been recorded in isolated myocytes. Blockade of the former type reduced the number of APs in each burst; the frequency within bursts was attenuated by blockade of T-type current (Bradley et al., 2004). However, both channels represent targets that may modulate spontaneous activity. The muscle cells are closely associated with interstitial cells that may at least modify their activity (McHale et al., 2006), as they may do in detrusor muscle. Interstitial cells are closely associated with NOS-synthase containing nerves and this suggests that they may be intermediaries between nerves and urethral smooth muscle (Lyons et al., 2007).

Urethral relaxation may be initiated by β-receptor (possibly β_2) activation (Takeda et al., 2003; Yamanishi et al., 2003), but the action is less significant than in detrusor (Michel and Vrydag, 2006). More significant is the control exerted by gases such as NO and CO. Pudendal nerve stimulation induces relaxation, an effect partially blocked by atropine and inhibitors of NO production (le Feber and Els van Asselt, 1999; Persson et al., 2000). Although the pudendal nerve is considered to contain myelinated, somatic fibres, it also contains parasympathetic fibres, both of which release acetylcholine. NO may be co-released from nerve endings with acetylcholine, or from urethral tissues close to nerve fibres (Persson et al., 1997). NO-mediated relaxation is due to production of cGMP and activation of cGMP-dependent protein kinase. CO can also exert relaxation through a rise of cGMP, which may be equivalent in magnitude to the effect of NO (Schroder et al., 2002). Dysfunction of the NO system is present in several disorders associated with lower urinary tract function, such as diabetes, bladder outlet obstruction or bladder inflammation (Ho et al., 2004; Yang et al., 2007).

The loss of urethral tone after menopause has led to the suggestion that lack of oestrogen is a contributory factor. Initial studies indicated that oestrogen supplementation may be beneficial. However, more recent clinical studies show that supplemental oestrogen may actually worsen stress incontinence as collagen content in the periurethral connective tissue is lowered. This decreases urethral closure pressure, i.e. the positive pressure in the urethra during bladder filling, and so increases the likelihood of incontinence (Jackson et al., 2002; DuBeau, 2005).

8.3. Urethral skeletal muscle

Onuf's nucleus is a group of neurons in the ventral part (laminae IX) of the anterior horn of the sacral region of the human spinal cord and is the origin of the pudendal nerve that carries both somatic and autonomic fibres. Pudendal somatic fibres innervate urethral skeletal muscle – the rhabdosphincter – which forms a very effective continence mechanism because contraction kinks the urethra, thereby greatly increasing its resistance. In the human rhabdosphincter fast-twitch fatigue-sensitive, fast-twitch fatigue-resistant and slow-twitch muscle fibres have been described, with the majority fatigue-resistant (Creed and van der Werf, 2001). Increasing age and greater parity in women are associated with a loss of muscle mass and may contribute to stress incontinence (Strasser et al., 2000b). Damage to motor nerve function may also contribute to stress incontinence: vaginal distension in an animal model had no effect on muscle mass, but reduced the number of urethral nerves as well as leak point pressures (i.e. intravesical bladder pressure at which urine leakage occurs due to increased abdominal pressure in the absence of a detrusor contraction (Lin et al., 2008)). The decline of sphincter, and striated muscle, function has motivated the development of myoblast implants to improve continence (Furuta et al., 2007), or the use of basic fibroblast growth factor to facilitate muscle cell generation (Takahashi et al., 2006). The β_2-agonist clenbuterol also increased urethral skeletal muscle contraction (Morita et al., 1995), suggesting an alternative approach. Dysfunction of the rhabdosphincter has been proposed as a cause of urinary retention in some young women when there is failure of the sphincter to relax during micturition, Fowler's syndrome (Fowler et al., 1988). The syndrome is diagnosed by a characteristic electromyogram recording from the rhabdosphincter of patients suggestive of inappropriate skeletal muscle activity. The origin of the condition is unknown and there is no known associated neurological disorder (although about 50% of patients have polycystic ovaries), but one hypothesis is that it is due to ephaptic electrical transmission between cells, much as can occur between nerve axons under certain conditions (Sanders, 1989). Neuromodulation may be effective in restoring voiding activity but there remain significant complication rates (Datta et al., 2008).

9. Conclusion

The above review has shown that the lower urinary tract is a complex muscular system that relies on an interaction between different components to maintain its storage and voiding functions. A loss of coordinated activity of any part – through over- or underactivity – can lead to profound clinical symptoms that at best are socially debilitating and at worst can lead to organ failure (urinary retention will raise pressures in the ureter and kidney, potentially leading to failure of the latter). An understanding of the cellular and tissue processes that control normal and abnormal activity is essential to provide a basis for the development of effective therapeutic and other treatments that can manage these conditions.

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