SYMPOSIUM REPORT

Interaction between interstitial cells and smooth muscles in the lower urinary tract and penis

Hikaru Hashitani

Department of Regulatory Cell Physiology, Nagoya City University Graduate School of Medical Sciences, Nagoya 467-8601, Japan

Smooth muscles in the lower urinary tract and corporal tissue exhibit spontaneous contractile activity which depends on L-type Ca^{2+} channels. The mechanism underlying this activity is spontaneous electrical activity which shows varied form and property between these tissues. Recent studies revealed that interstitial cells (ICs) are widely distributed in the genitourinary system, and suggested their involvement in spontaneous muscle activity. ICs in the system are not a simple analogy of interstitial cells of Cajal (ICC) in the gut, which act as electrical pacemaker, but represent variability amongst tissues which may account for individual characteristics of each organ. In the bladder and corporal tissue, where smooth muscle cells are capable of generating spontaneous electrical activity, ICs may modulate smooth muscle activity. ICs in corporal tissue release prostaglandins via cyclooxygenase-2 (COX-2) activity and reinforce not only spontaneous but also nerve-mediated α-adrenergic contractions. In the bladder, their fundamental role in the integration of signals between populations of cells has been proposed, and thus changes in ICs may contribute to an overactive bladder, a pathological condition which results from increased excitability in detrusor smooth muscles. In the urethra, ICs may act as electrical pacemakers as do ICC. However, overall contractility of urethral smooth muscles does not necessarily rely on pacemaking of ICs, and thus some population of smooth muscles may also have their own excitability.

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Corresponding author H. Hashitani: Department of Regulatory Cell Physiology & Nephrourology, Nagoya City University Graduate School of Medical Sciences, Nagoya 467-8601, Japan. Email: hasitani@med.nagoya-cu.ac.jp

Interstitial cells (ICs) have been identified in the lower urinary tract and corporal tissue by their immunoreactivity against Kit receptor tyrosine kinase, a cell surface marker specific for ICC/ICs, or vimentin filament, a marker for cells of mesenchymal origin (Brading & McCloskey, 2005). Since smooth muscles in these tissues exhibit spontaneous excitation, ICs may drive smooth muscles as do ICC in the gut (Sanders, 1996). In the urethra, isolated ICs generate spontaneous electrical activity which is very similar to that recorded from intact urethral smooth muscles, suggesting that ICs may act as electrical pacemakers (Hashitani *et al.* 1996; Sergeant *et al.* 2000). In the bladder and corpus cavernosum, however, dispersed smooth muscle cells are capable of generating spontaneous electrical activity (Montgomery & Fry, 1992; Karkanis *et al.* 2003), and thus the functional role of ICs in these tissues may be different from that in the gut or the urethra. Therefore, besides intrinsic properties of smooth muscles and autonomic innervation, varied functions of ICs amongst these tissues may also contribute to individual characteristics of each tissue.

Whilst recognizing the variation of ICs in the lower urinary tract and corporal tissue, we may also need to consider the commonality of ICs in these tissues, particularly as a basis for pathological excitability of smooth muscles. Lower urinary tract symptoms (LUTS) and erectile dysfunction (ED) are extremely prevalent in ageing men. However, accumulated epidemiological surveys demonstrated the independent relationships of age to LUTS and to ED (McVary, 2006), and thus it is important to establish a reasonable pathophysiological basis to explain the association between LUTS and ED. Although several common factors have been already hypothesized for it, general distribution of ICs in the genitourinary system suggests that pathological changes in their property may also be crucial.

Corporal tissue

ICs have been identified in the corporal tissue by their immunoreactivity for Kit receptor tyrosinekinase

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(Hashitani & Suzuki, 2004). Spontaneous Ca^{2+} transients recorded from corpus spongiosum smooth muscle of the guinea-pig are readily blocked by pharmacological disruption of Ca^{2+} release from intracellular Ca^{2+} stores (Hashitani & Suzuki, 2004). Since spontaneous activity originating from ICs generally depends on Ca^{2+} store function, ICs in the corporal tissue could be primary pacemakers as with ICC in the gut. However, dispersed corporal smooth muscle cells are capable of generating spontaneous electrical activity by the opening of Ca^{2+}-activated Cl^- channels which are stimulated by Ca^{2+} release from intracellular stores (Karkanis *et al.* 2003; Craven *et al.* 2004). Therefore, ICs in the corporal tissue may have a functional role other than that of electrical pacemaker.

Spontaneous contractions of corporal smooth muscle are known to be inhibited by indomethacin, and thus background formation of prostaglandins may be involved in their generation (Christ *et al.* 1990). In the rabbit corpus cavernosum smooth muscle (CCSM), spontaneous contractions were largely attenuated by NS 398, an inhibitor for COX-2, suggesting that spontaneous production of prostaglandins via COX-2 may contribute to the generation of spontaneous activity (Fig. 1*A*; Hashitani *et al.* 2005). In addition, inhibition of COX-2 suppressed nerve-evoked α-adrenergic contractions. It should be noted that the COX-2 inhibitor reduced the amplitude of α-adrenergic contractions with a relatively small reduction in corresponding Ca^{2+} transients (Fig. 1*B–C*). Since PGF2α-induced contractions of CCSM are greatly suppressed by Y-27632 Rho kinase inhibitor, PGF2α may reinforce the α-adrenergic contractions through Ca^{2+}-independent mechanisms, presumably by the activation of Rho kinase.

Immunohistochemical studies showed that COX-2 was highly expressed in ICs in the corpus cavernosum and the corpus spongiosum (Hashitani *et al.* 2005). Haematoxylin & Eosin (HE) staining revealed that cells with COX-2 immunoreactivity were spindle-or star-shaped with some branches and were interconnected (Fig. 1*D*). COX-2-immunoreactive cells are scattered over corporal smooth muscle and background smooth muscle expresses no or very weak COX-2 immunoreactivity (Fig. 1*Ea* and *Fa*). COX-2-positive cells are also shown to express strong immunoreactivity against Kit (Fig. 1*Eb* and *c*) or vimentin (Fig. 1*Fb* and *c*) antibody. Therefore, ICs in CCSM may be a modulator of spontaneous activity, which originate from smooth muscle cells, by releasing prostaglandins via COX-2 activity.

Several studies indicated that the disturbed balance between spontaneously active NO–cGMP pathway and prostaglandins may be crucial for pathophysiology of erectile dysfunction (Fig. 2). The blockade of guanylate cyclase in CCSM has been reported to increase both resting tension and noradrenaline-induced contraction, suggesting that the tonic production of cGMP inhibits CCSM excitability (Minhas *et al.* 2000). Since the effects of guanylate cyclase inhibition were reversed by indomethacin, interaction and functional antagonism between cGMP and prostaglandins was suggested. Furthermore, increased cyclooxygenase activity is reported to account for ischaemia-induced increased contractions of CCSM in the rabbit (Azadzoi *et al.* 1992). It has also been reported that diminished NO production in diabetic CCSM is associated with increased Rho kinase activity, and that increased Rho kinase activity may suppress the production of NO (Bivalacqua *et al.* 2004). Since prostaglandins contract CCSM partly by increasing Rho kinase activity, increased Rho kinase activity in association with suppressed NO production may attribute to overexpression of COX-2 in ICs.

Urinary bladder

ICs are distributed throughout the bladder wall (Davidson & McCloskey, 2005). In the suburothelium region, a network of vimentin-positive ICs connecting thorough gap junction protein connexin43 (Cx43) has been identified (Sui *et al*. 2002; Wiseman *et al.* 2003). Suburothelial ICs form very close association with afferent nerves, and may play a modulatory role in the process of bladder sensation, i.e. signal transmission from urothelium to afferent nerves.

In detrusor muscle layer, ICs are found preferentially located on the boundary of muscle bundles from where spontaneous Ca^{2+} transients originate, suggesting that they may be crucial in generating spontaneous excitation (Fig. 3*A*; see also McCloskey & Gurney, 2002). However, spontaneous Ca^{2+} transients recorded from ICs in fact occurred independently of those of smooth muscles even when synchronous Ca^{2+} waves swept across muscle bundles (Fig. 3*B*; Hashitani *et al.* 2004). Therefore, although the location of boundary ICs seems to be ideal to drive the bulk of smooth muscles, they may not be electrical pacemaking cells. Indeed, electrophysiological recordings have demonstrated that isolated detrusor smooth muscle cells of the bladder are capable of generating spontaneous action potentials which are almost identical to those recorded from intact preparations (Montgomery & Fry, 1992; Hashitani *et al.* 2001). Unlike Ca^{2+} transients recorded from detrusor smooth muscles, those from ICs persisted in the presence of nifedipine (Fig. 3*C*) as did carbachol-induced Ca^{2+} oscillations in isolated ICs (McCloskey & Gurney, 2002), suggesting that voltage-dependent L-type Ca^{2+} channels are not involved in the generation of this activity.

ICs are closely associated with intramural nerves (Davidson & McCloskey, 2005). Following stimulation with sodium nitroprusside (SNP), an NO donor,

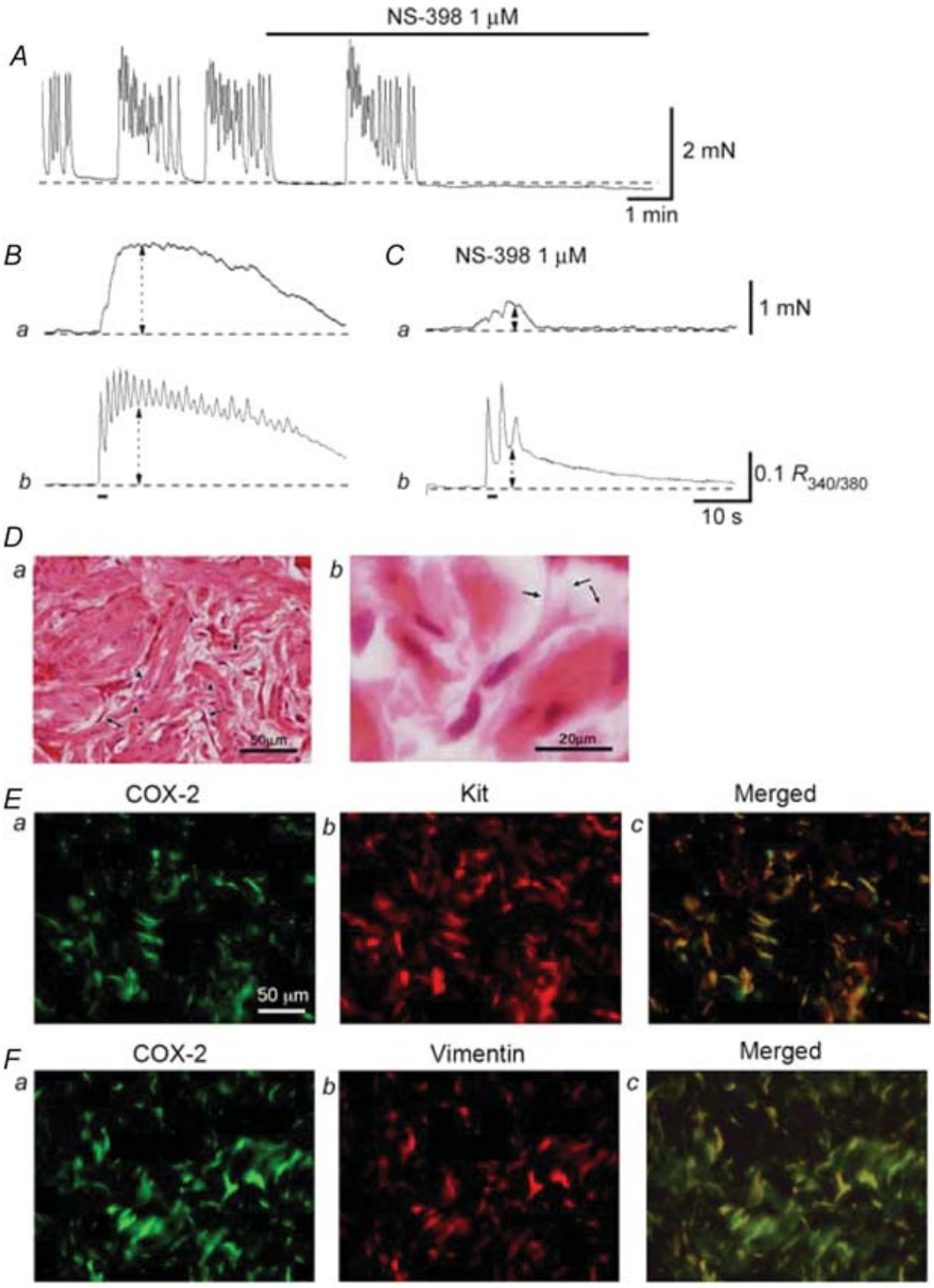

Figure 1. Function and distribution of COX-2-immunoreactive interstitial cells in corpus cavernosum
In a CCSM preparation that generated spontaneous contractions, NS-398 (1 μM) abolished the contractions (*A*). When changes in tension were measured simultaneously with changes in $[Ca^{2+}]_i$ in another preparation, transmural stimulation evoked phasic contractions (*Ba*) and corresponding increases in $[Ca^{2+}]_i$ (*Bb*). NS-398 (1 μM) strongly suppressed nerve-evoked contractions (*Ca*), and also reduced the amplitude of the initial phase of Ca^{2+} transients by about 50% (*Cb*). HE staining of the smooth muscle cells and ICs in corpus cavernosum (*Da*). Smooth muscle cells had a large, clear nucleus (arrowheads), while ICs were characterized by their smaller, darker nucleus (arrows). ICs typically had spindle- or star-shaped cell bodies and had some branches (arrows) which connected with neighbouring cells (*Db*). COX-2-immunoreactive cells, which had spindle- or star-shaped cells with some branches, were widely distributed throughout the corpus cavernosum (*Ea*). COX-2-immunoreactive cells were also stained for Kit antibody (*Eb* and *Ec*). COX-2-immunoreactive ICs in the corpus cavernosum (*Fa*) were also stained for vimentin (*Fb* and *Fc*).

ICs throughout the bladder demonstrated an intense induction of cGMP immunoreactivity, but detrusor muscle cells remained uniformly negative (Smet *et al.* 1996; Gillespie *et al.* 2004). Thus, ICs in the bladder may be involved in the neuromuscular transmission as do ICC in the gut (Hirst & Ward, 2003), and thus changes in ICs may account for increased excitability of detrusor smooth muscles. Our preliminary studies showed that sildenafil, a phosphodiesterase type 5 (PDE5) inhibitor, suppressed spontaneous contractions recorded from multiple detrusor smooth muscle preparations, whilst invariably unaffecting spontaneous activity in single muscle bundles. Interestingly, SNP rather enhanced spontaneous excitations in detrusor smooth muscles, and this was consistent with the results of whole bladder experiments (Gillespie & Drake, 2004). These results suggested that sildenafil may accumulate endogenous cGMP in ICs and diminish the synchronicity between muscle bundles.

Bladder overactivity has been suggested to result from increased coupling between detrusor smooth muscle cells (Brading, 1997). Increased connexin43-mediated intercellular communications in a rat model of bladder overactivity has been reported (Haefliger *et al.* 2002; Christ *et al.* 2003). Micromotion of the bladder wall, which may be attributed to spontaneous contractions of a unit of muscle bundles, has also been reported to be enhanced in a rat model of bladder overactivity (Drake *et al.* 2003). Therefore, either quantitative or qualitative changes in ICs may account for the increased excitability in the overactive bladder. The finding in human bladder that Kit-positive cells are increased in number in samples of bladder taken from patients with overactive bladder may support this idea (Biers *et al.* 2006). An unpublished observation from our group using a guinea-pig with bladder outlet obstruction demonstrated that populations of Kit/vimentin-positive ICs were dramatically increased in the obstructed bladder.

Urethra

In circular smooth muscles of the rabbit urethra, spontaneous excitation may originate from ICs by mean of the spontaneous release of Ca^{2+} from intracellular stores, which activates Ca^{2+}-activated Cl^- channels (Sergeant *et al.* 2000, 2001; Johnston *et al.* 2005). Resultant depolarizations may be transmitted to smooth muscle cells, and thus spontaneous transient depolarizations (STDs) recorded from intact urethral smooth muscle may attribute to spontaneous inward currents generated by ICs (Hashitani *et al.* 1996). Summed STDs result in larger depolarizations which reach the threshold for the opening of L-type Ca^{2+} channels (Hashitani & Edwards, 1999). The activation of L-type Ca^{2+} channels in smooth muscle cells contributes to the plateau phase of slow waves and leads to spontaneous contractions (Hashitani *et al.* 1996; Hashitani & Edwards, 1999). Therefore, spontaneous contractions are expected to be inhibited as a consequence of the inhibition of the primary step of spontaneous excitation, i.e. Ca^{2+} release from stores in ICs.

Cyclopiazonic acid (CPA is known to inhibit Ca^{2+} uptake into intracellular stores via SERCA pumps, and is known to suppress both spontaneous transient inward currents (STICs) in isolated interstitial cells and STDs in intact circular smooth muscle (Hashitani *et al.* 1996; Sergeant *et al.* 2001). Therefore, the blockade of SERCA pumps with CPA may suppress spontaneous contractions of the urethral smooth muscles. However, CPA increased the amplitude and duration of phasic contractions in 70–90% of preparations, although it largely reduced their frequency (Hashitani *et al.* 2006). CPA prevented the generation of spontaneous contractions in the remaining preparations. Since CPA abolished caffeine-induced Ca^{2+} transients in nominally Ca^{2+}-free solution, it may have effectively depleted intracellular Ca^{2+} stores. Thus, it is unlikely that CPA-treated preparations generated spontaneous contractions through a Ca^{2+} store-dependent pacemaker, i.e. through ICs.

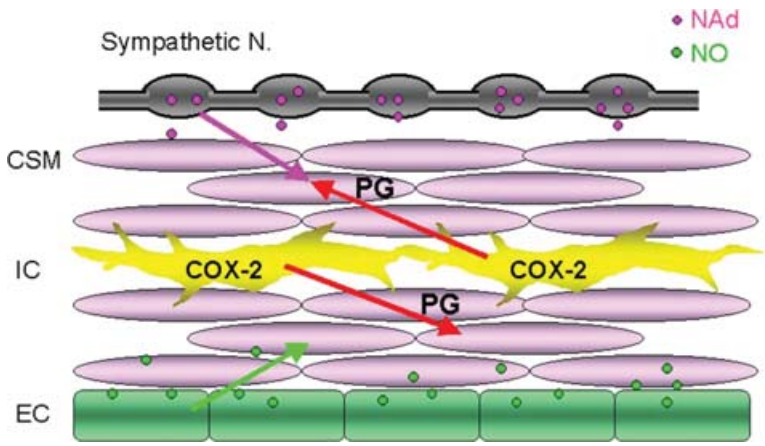

Figure 2. Role of interstitial cells in regulating cavernosal smooth muscle (CSM) tone
In corpus cavernosum, both spontaneous activity of CSM and neurally released noradrenaline (NAd; pink arrow) contract CSM. Spontaneously produced prostaglandins (PGs) via COX-2 activity in interstitial cells (ICs) not only reinforce spontaneous excitation of CSM (lower red arrow) but also facilitate nerve-mediated α-adrenergic contractions (upper red arrow). Conversely, spontaneously released nitric oxide (NO) from endothelium (EC) suppresses excitation (green arrow). Thus, the balance between spontaneously released PGs and NO is important in regulating CCSM tone to determine the contractile state of the penis.

Consistent with the results of contractile studies, CPA increased the amplitude and duration of spontaneous depolarizations and corresponding Ca^{2+} transients in some 40–60% of preparations, whilst suppressing their generation in the remainder (Hashitani *et al.* 2006). The frequency of spontaneous depolarizations and corresponding Ca^{2+} transients was again largely decreased in CPA-treated preparations. In preparations in which spontaneous depolarizations and Ca^{2+} transients were not abolished by CPA, nicardipine greatly diminished Ca^{2+} transients but not depolarizations. After the blockade of L-type Ca^{2+} channels, CPA invariably abolished

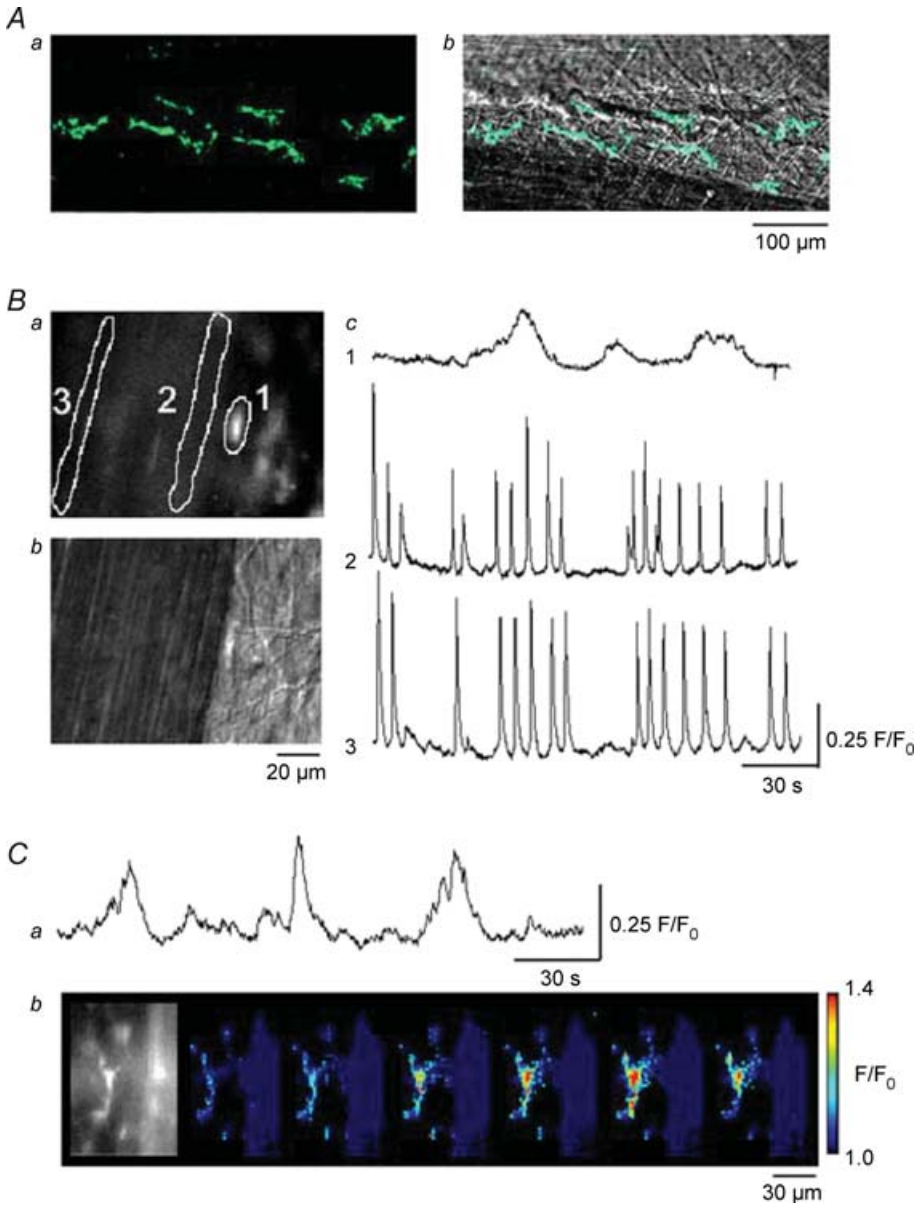

Figure 3. Ca^{2+} transients recorded from smooth muscles and interstitial cells in the guinea-pig bladder
Kit-positive ICs having spindle-shaped cell bodies, some 80 μm in length and less than 10 μm in width, are shown located adjacent to a pair of muscle bundles (*Aa*). The same images were superimposed on the plane images of the smooth muscle bundles (*Ab*). In a preparation loaded with fluo-4, IC located near the muscle boundary had a higher fluorescence intensity than that of smooth muscle (*Ba*). A plane image with Nomarski optics visualized the cell body of IC (*Bb*). When Ca^{2+} transients were recorded from the IC (area 1) and from two smooth muscle areas (areas 2 and 3), synchronous Ca^{2+} waves were detected at areas 2 and 3 (*Bc*). However, IC generated slow Ca^{2+} transients independently from those of smooth muscles (*Bc*). In another fluo-4-loaded preparation which had been exposed to nifedipine (10 μM) for 30 min, IC continued to generate slow Ca^{2+} transients (*Ca*). A series of frames with intervals of 2 s demonstrates a Ca^{2+} transient originating from the IC (*Cb*).

residual depolarizations and Ca^{2+} transients, suggesting that these events may result from Ca^{2+} store-dependent mechanisms. After all, this heterogeneous CPA sensitivity of spontaneous excitation of urethral smooth muscles may attribute to the existence of a secondary pacemaker which relies on L-type Ca^{2+} channels.

In longitudinal smooth muscle of the urethra, spontaneous action potentials are generated, and inhibited by nicardipine but not CPA, suggesting that L-type Ca^{2+} channels play a principal role in their generation (Hashitani *et al.* 2006). Thus, after disrupting primary pacemaking by ICs which depend on Ca^{2+} release, L-type Ca^{2+} channel-dependent pacemaking depolarizations arising from longitudinal smooth muscles may drive the circular smooth muscles. Alternatively, small populations of circular smooth muscles which are capable of generating spontaneous depolarization might dominate the pacemaking system.

To further explore the origin of CPA-sensitive spontaneous excitations, attempts were made to visualize Ca^{2+} transients from intact ICs in urethral smooth muscle preparations which had been loaded with fluo-4 AM. Spontaneous Ca^{2+} transients recorded from ICs occurred at the frequency of 1–10 min^{-1}, and had a longer duration (5–30 s) than those of urethral smooth muscle cells (1–3 s) which were measured in similar preparations (Fig. 4*A*, *Ba* and *Ca*). ICs were either distributed individually or several ICs were clustered, but did not compose an extensive network. Nicardipine (1 μM), which either abolished Ca^{2+} transients in smooth muscles or greatly reduced their amplitude (Fig. 4*Ba* and *b*), did not suppress Ca^{2+} transients of ICs (Fig. 4*Ca* and *b*). Although Ca^{2+} transients of ICs were virtually insensitive to nicardipine, their generation was prevented by switching from normal physiological saline to nominally Ca^{2+}-free solution. They were also readily abolished by CPA

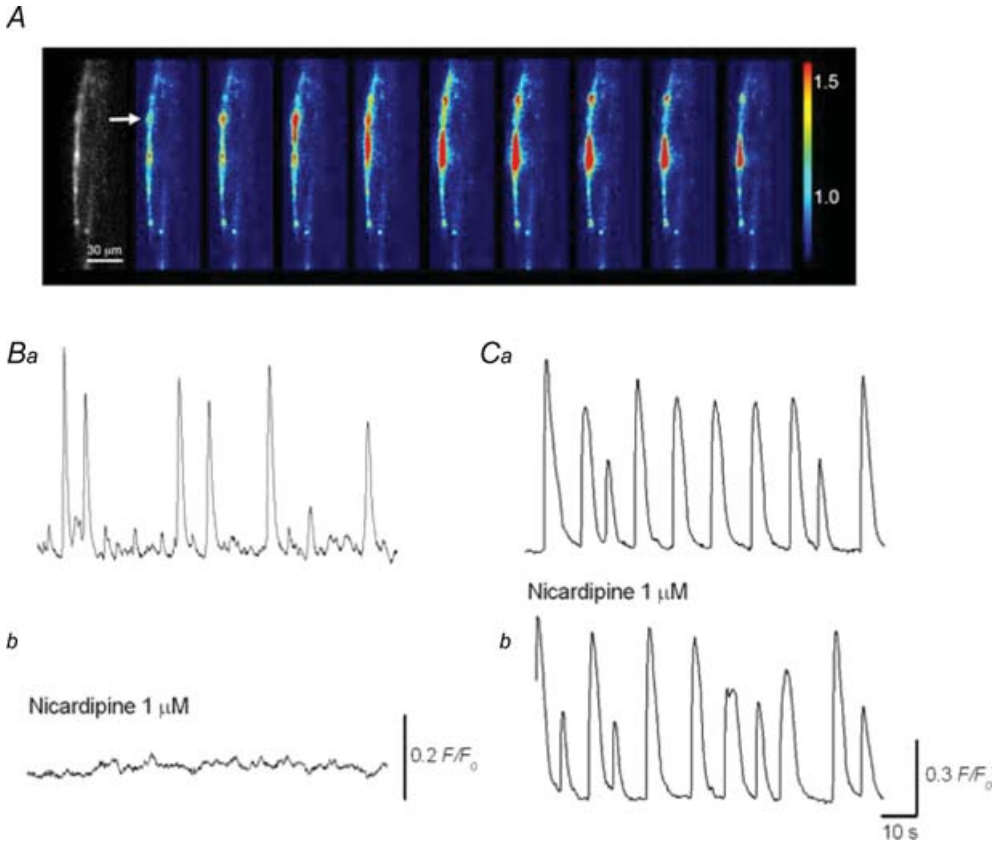

Figure 4. Ca^{2+} transients recorded from smooth muscles and interstitial cells in the rabbit urethra
In a preparation loaded with fluo-4, urethral IC located near the muscle boundary generated slow Ca^{2+} transients (*A*). A series of frames with intervals of 2 s demonstrates a Ca^{2+} transient originating from the IC (*A*). Note that Ca^{2+} transients were initiated at the point indicated by an arrow, and then propagated upward and downward. In a different preparation, Ca^{2+} transients recorded from urethral smooth muscles were largely attenuated by nicardipine (1 μM) (*Ba* and *C*). In another preparation, Ca^{2+} transients recorded from urethral IC were virtually insensitive to nicardipine (1 μM) (*Ca* and *b*). The scale bar in *Bb* also refers to *Ba*. The scale bar in *Cb* also refers to *Ca*.

(10 μM), ryanodine (30 μM) or caffeine (10 mM), and their amplitude was greatly reduced 2-APB (50 μM), suggesting that their generation can be attributed to the Ca^{2+} release from intracellular Ca^{2+} stores through both ryanodine-sensitive and $InsP_3$-sensitive Ca^{2+} channels. These results were consistent with properties of isolated urethral ICs (Johnston *et al.* 2005), and again confirmed that spontaneous activity of ICs relies on Ca^{2+} handling by intracellular Ca^{2+} stores.

Conclusion

ICs are widely distributed in the lower urinary tract and corporal tissues. Corporal ICs reinforce both spontaneous and nerve-mediated α-adrenergic excitations by releasing prostaglandins via COX-2 activity. In detrusor smooth muscle layer, ICs generate spontaneous excitations independent from those of smooth muscles. They may be targeted by nitrergic nerves and modulate communications between muscle bundles, and thus increasing their population may account for pathological excitability of detrusor smooth muscle. Urethral ICs may be the primary pacemaker to drive smooth muscles by a Ca^{2+}-store-dependent mechanism. However, there may also be L-type Ca^{2+} channel-dependent pacemaking presumably arising from smooth muscles. In the genitourinary system, overactivity of ICs may result in increased excitability of smooth muscles in individual organs, and thus may account for the association between LUTS and ED.

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