

## SYMPOSIUM REPORT

# Origin of spontaneous rhythmicity in smooth muscle

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Rhythmic electrical activity is a feature of most smooth muscles but the mechanical consequences can vary from regular rapid phasic contractions to sustained contracture. For many years it was thought that spontaneous electrical activity originated in smooth muscle cells but recently it has become apparent that there are specialized pacemaker cells in many organs that are morphologically and functionally distinct from smooth muscle and that the former cells are the source of spontaneous electrical activity. Such a pacemaker function is well documented for the ICC of the gastrointestinal tract but evidence is accumulating that ICC-like cells play a similar role in other types of smooth muscle. We have recently shown that there are specialized pacemaking cells in the rabbit urethra which are spontaneously active when freshly isolated, readily distinguishable from smooth muscle cells under bright field illumination and relatively easy to study using patch-clamp and confocal imaging techniques. Recent results suggest that calcium oscillations in isolated rabbit urethral interstitial cells are initiated by calcium release from ryanodine sensitive intracellular stores, that oscillation frequency is very sensitive to the external calcium concentration and that conversion of the primary oscillation to a propagated calcium wave depends upon IP<sub>3</sub>-induced calcium release.

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## Introduction

Smooth muscle exhibits a wide variety of mechanical activity from rapid phasic contractions as in ureter (Shuba, 1977; Allen & Bridges, 1977; Lang *et al.* 2002) or lymphatics (McHale & Roddie, 1976; Kirkpatrick & McHale, 1977; Van Helden, 1993), slower rhythmic contractions as in the gastrointestinal tract (Bortoff, 1976), portal vein (Axelsson *et al.* 1967), or uterus (Bengtsson *et al.* 1984) to slow sustained contractions as in urethra (Brading *et al.* 2001). The first type of activity is usually associated with fluid propulsion while the last serves to alter resistance to flow or to cause a sphincter to remain tightly closed. Curiously, although mechanical activity is very variable, the electrical activity underlying it often shows remarkable similarities. Thus the electrical activity underlying two very different types of mechanical activity in lymphatics (sharp phasic individual contractions) and urethra (sustained tonic contraction) consists of a series of well-defined spike complexes in each case (Fig. 1).

Tomita & Watanabe (1973) argued that, although shape and amplitude of electrical activity varied from tissue to tissue, two basic types of electrical event, slow waves and spikes, could be discerned in spontaneously active smooth muscles. The frequency of firing of slow waves was little changed by changes in membrane potential while the frequency of spike discharge was very voltage dependent. They concluded that in all spontaneously active tissues an underlying basic slow wave exists which is the pacemaker potential. They reasoned that this was metabolically driven (mainly because of the high Q<sub>10</sub> observed in most tissues) and that it was modulated by the calcium concentration at the inside of the membrane. At that time smooth muscle was thought of as being a relatively homogeneous tissue and that pacemaking originated in typical smooth muscle cells. It was known, however, that separation of strips of gastrointestinal muscle into longitudinal and circular layers resulted in the longitudinal layer remaining spontaneously active while the circular layer became quiescent. (Bortoff, 1976). This was taken to mean that longitudinal muscle was inherently rhythmic and was thus responsible for pacemaking. This view was first challenged by Thuneberg (1982) who noted that local contractions correlated well with the distribution of ICC and that

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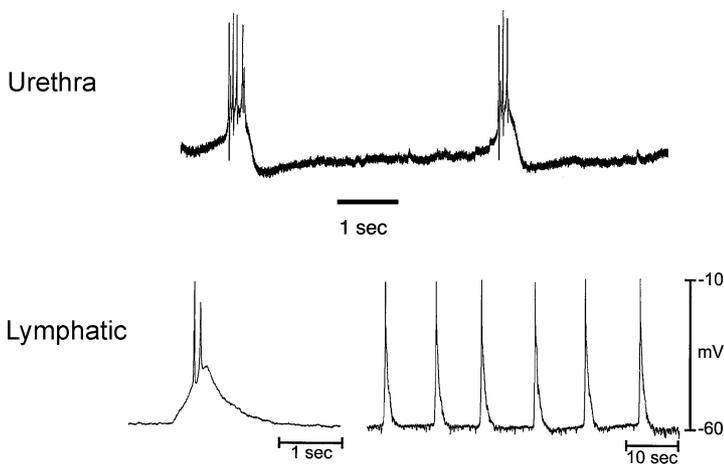
separation of circular and longitudinal layers resulted in ICC remaining attached to the longitudinal layer. He suggested that pacemaking might well arise in the ICC rather than the longitudinal smooth muscle. A large body of work has since confirmed the pacemaking function of ICC in the gastrointestinal tract (reviewed by Sanders, 1992, 1996).

More recently ICC-like cells have been found in a wide variety of smooth muscle tissues including blood vessels (Harhun *et al.* 2005), lymphatics (McCloskey *et al.* 2002), ureter (Klemm *et al.* 1999), urethra (Sergeant *et al.* 2000), bladder (McCloskey & Gurney, 2002), prostate (Exintaris *et al.* 2002), fallopian tube (Popescu *et al.* 2005) and uterus (Duquette *et al.* 2005). Some of these are thought to have a pacemaker function (such as those in portal vein, in lymphatics or prostate) but not those in arteries, uterus (where the influence is, if any, an inhibitory one) or bladder. In the case of the ureter the ICC-like cells described there appeared to have no role in pacemaking, a function that was ascribed instead to 'atypical' smooth muscle cells (Klemm *et al.* 1999). On the other hand Sergeant *et al.* (2000) described ICC-like cells that do represent an interesting model of pacemaking in non-gastrointestinal tract muscle. These are excitable, non-contractile, contain abundant vimentin but no myosin filaments and bear a striking resemblance to the cells isolated by Langton *et al.* (1989) from canine proximal colon. They have an abundance of calcium-activated chloride current, exhibit regular spontaneous depolarizations, which are increased in frequency by noradrenaline and blocked by perfusion with low calcium solution and by chloride channel blockers. The urethral smooth muscle cells, by contrast, are electrically quiescent and have very little calcium-activated chloride current. The fact that these freshly dispersed ICC are easily distinguishable from smooth muscle cells under bright field illumination and that they are reliably spontaneously active makes them relatively easy to study using patch-clamp and confocal imaging techniques.

### Pacemaker mechanism

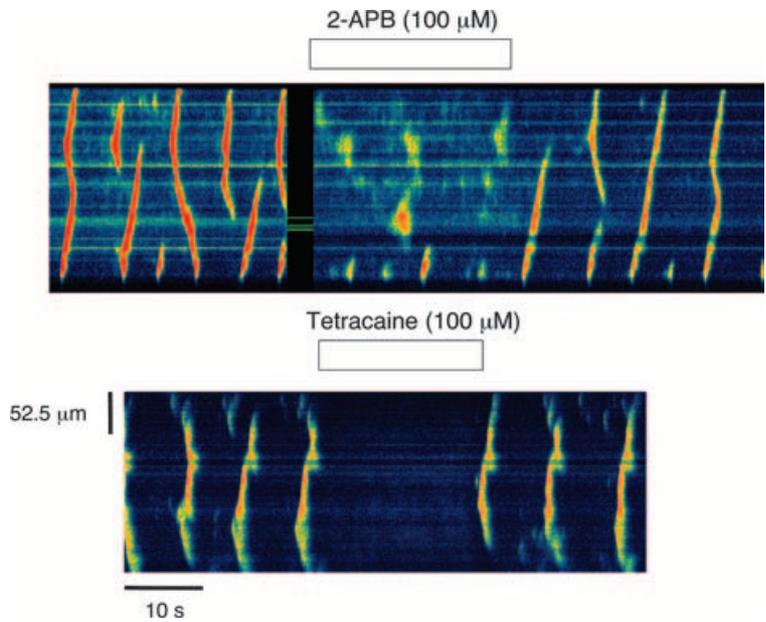
Current evidence suggests that the pacemaker in urethral interstitial cells is dependent both on  $\text{Ca}^{2+}$  release from intracellular stores and on influx of extracellular  $\text{Ca}^{2+}$  as either store depletion or brief exposure to  $\text{Ca}^{2+}$ -free bath solution rapidly abolishes electrical activity (Sergeant *et al.* 2001; Johnston *et al.* 2005). The role of stores in generating electrical activity in these cells is in little doubt. So far, two different membrane currents have been shown to be activated by store-released  $\text{Ca}^{2+}$ , namely the  $\text{Cl}^-$  current referred to above, and an outward current mediated by large conductance  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  (BK) channels. At physiological potentials opening of  $\text{Cl}^-$  channels produces 'spontaneous transient inward currents', or STICs, while the BK channels produce 'spontaneous transient outward currents', or STOCs. The question naturally arises as to how two opposing currents, both activated by a rise in intracellular  $\text{Ca}^{2+}$ , do not simply cancel each other out, but instead interact to produce co-ordinated electrical activity. One clue to answering this may be obtained by looking at the potentials at which STICs and STOCs occur. At slightly depolarized potentials of  $-40$  to  $-30$  mV both currents are observed but close to the membrane potential of  $-50$  to  $-60$  mV the STICs predominate. This is explained in part by the fact that the  $\text{Cl}^-$  channels are only weakly voltage dependent, while the BK channels show strong voltage dependence. Therefore, at more polarized potentials the open probability of the BK channels will remain low even if cytosolic  $\text{Ca}^{2+}$  is elevated, while the  $\text{Cl}^-$  channels will still be able to respond to  $\text{Ca}^{2+}$ . Because of these characteristics, spontaneous elevations in intracellular  $\text{Ca}^{2+}$  are likely to result in depolarization from the resting potential as a result of a preferential activation of the  $\text{Cl}^-$  channels.

There is now good evidence to support the view that spontaneous electrical events in the interstitial cells of the rabbit urethra are generated by the oscillatory release of calcium from intracellular stores (Sergeant *et al.* 2000; Sergeant *et al.* 2001; Sergeant *et al.* 2002). The 'prime



**Figure 1. Similarity of electrical activity in tonic and phasic smooth muscle**

The urethra normally maintains a tonic contraction (to prevent voiding of urine) while lymphatic vessels exhibit rapid phasic contractions (to act as lymph hearts). Nevertheless the electrical activity underlying these diverse mechanical manifestations is remarkably similar.

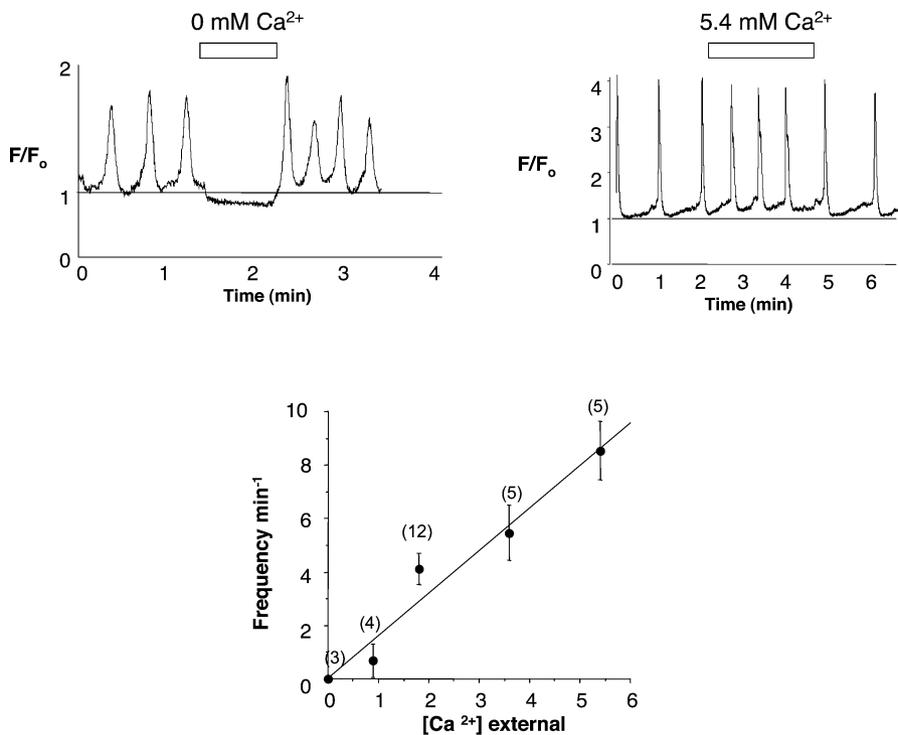


**Figure 2. Pseudolinescan or  $x, t$  plot of a propagating wave in a fluo-4 loaded interstitial cell, imaged with a Nipkow disk confocal microscope**

A continuous band of fluorescence is indicative of good propagation of the calcium wave as was the case before addition of  $100 \mu\text{M}$  2-APB. In its presence the well coordinated wave was lost but calcium oscillation continued in a localized fragmented pattern. In contrast  $100 \mu\text{M}$  tetracaine blocked oscillations completely (lower panel).

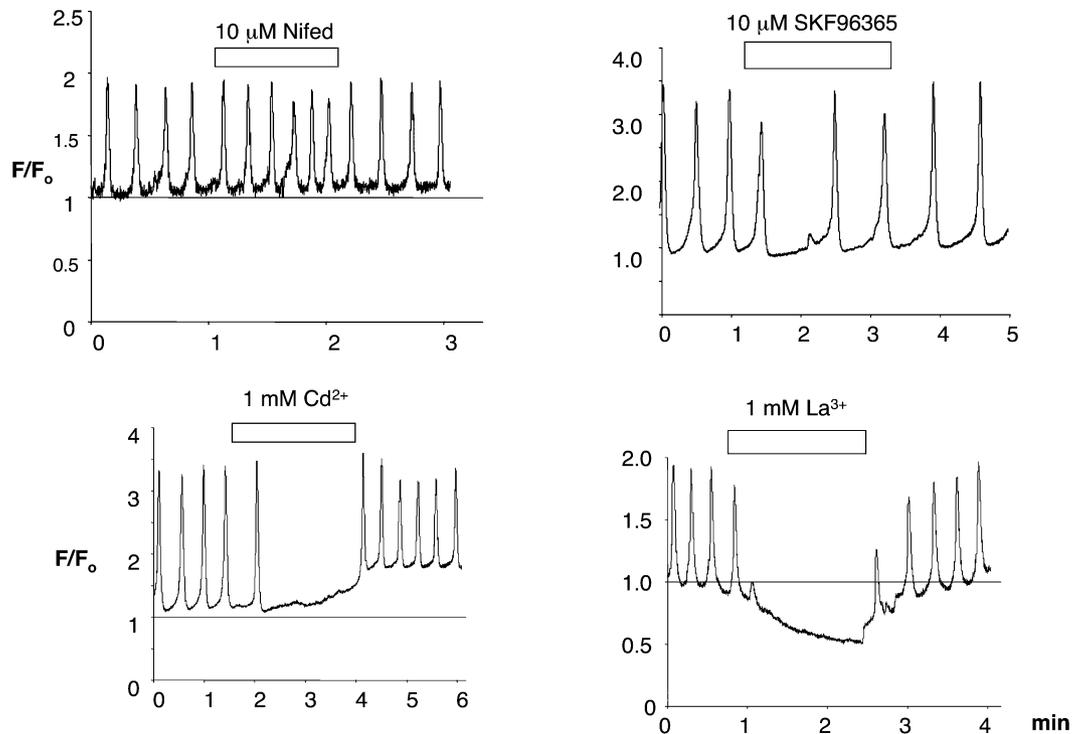
oscillator' would appear to be the ryanodine sensitive store rather than the  $\text{IP}_3$  sensitive store since blockade of the former with tetracaine or with ryanodine stopped oscillations completely whereas inhibition of the action or production of  $\text{IP}_3$  (Johnston *et al.* 2005) decreased the

amplitude or propagation of the calcium wave but did not entirely prevent calcium oscillations. This is illustrated in Fig. 2 which is a 'pseudolinescan' or  $x, t$  plot of a propagating wave in a fluo-4 loaded interstitial cell, imaged with a Nipkow disk confocal microscope. A continuous



**Figure 3. Oscillations ceased rapidly in  $0 \text{ mM Ca}^{2+}$  but increased above control values when external calcium was increased to  $5.4 \text{ mM}$**

The lower panel shows that there was an approximately linear relationship between external calcium concentration and frequency of oscillation.



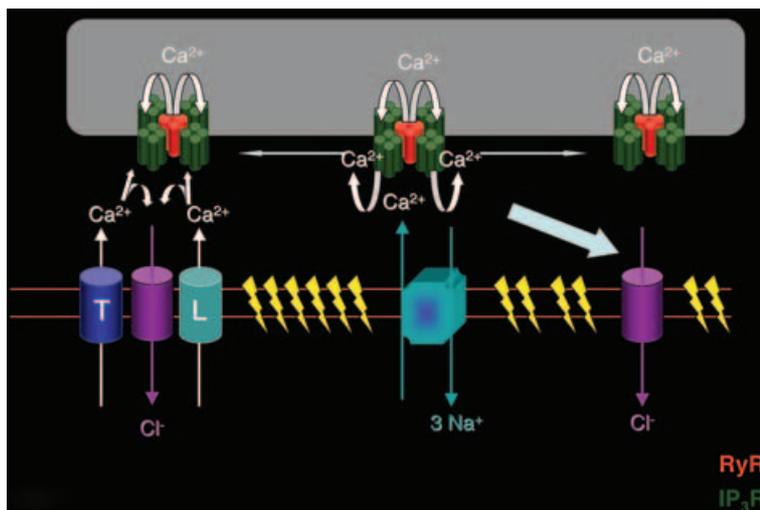
**Figure 4. The effects of four different calcium influx blockers**

At 1 mM cadmium and lanthanum abolished oscillations but these were little affected by 10  $\mu\text{M}$  nifedipine or SKF96365.

band of fluorescence is indicative of good propagation of the calcium wave as was the case before addition of 100  $\mu\text{M}$  2-APB (the main effect of which is to inhibit calcium release from IP<sub>3</sub>R modulated channels; this was a specific effect in the present context as discussed in detail in Johnston *et al.* 2005). The well-coordinated wave was lost but calcium oscillation continued in a localized fragmented pattern. In contrast 100  $\mu\text{M}$  tetracaine (known to inhibit calcium release from ryanodine sensitive stores in skeletal (Csernoch *et al.* 1999), cardiac (Lukyanenko

*et al.* 1996; Overend *et al.* 1997; Overend *et al.* 1998), and smooth muscle (Hyvelin *et al.* 2000; Cheranov & Jaggar, 2002)) blocked oscillations completely (lower panel).

Frequency of oscillation was very sensitive to external calcium concentration as illustrated in Fig. 3. Oscillations ceased rapidly in 0 mM Ca<sup>2+</sup> but increased above control values when external calcium was increased to 5.4 mM. The lower panel shows that there was an approximately linear relationship between external calcium concentration and frequency of oscillation. It is well known that external



**Figure 5. Proposed mechanism of spontaneous pacemaking in urethral interstitial cells**

The red and green clusters represent RyR and IP<sub>3</sub>R, respectively. Release from the ryanodine sensitive store is triggered by calcium influx via the sodium/calcium exchange. Release from the RyR causes further release from nearby IP<sub>3</sub> sensitive stores. This in turn sets up a regenerative wave raising calcium concentration in the vicinity of the calcium-activated chloride channels to a level sufficiently high to activate them. Efflux of chloride ions through these provides the depolarizing current underlying the pacemaker potential. This in turn may depolarize the membrane sufficiently to activate the T- and L-type voltage-operated channels.

calcium concentration affects oscillation frequency in many cell types (Kawanishi *et al.* 1989; Bootman *et al.* 1996; Shuttleworth & Thompson, 1996; Sneyd *et al.* 2004). For example Bootman *et al.* (1996) found that raising external calcium from 0 to 1.3 mM increased both frequency of oscillations and intracellular [<sup>3</sup>H]inositol phosphate levels in HeLa cells. When external calcium concentrations were increased above this level, oscillation frequency continued to rise even though the PLC response had already saturated. They took this to mean that external calcium (at least at levels above 1.3 mM) could control the release of calcium from intracellular stores by modulating the rate of store refilling between each calcium spike. Since PLC activity was essentially saturated they argued that the positive feedback responsible for calcium spiking must be generated by calcium-induced calcium release (CICR). The obvious mechanism for such an oscillator would be cyclical filling and emptying of a ryanodine sensitive store. We still do not fully understand the nature of the Ca<sup>2+</sup> influx pathway that sustains oscillatory activity in urethral interstitial cells, other than it is not nifedipine sensitive, is little affected by 10 μM SKF96365 (upper panels, Fig. 4) but is effectively blocked by 1 mM cadmium or lanthanum (lower panels). Sneyd *et al.* (2004) argued that the calcium influx pathway responsible for modulating frequency of oscillation did not depend on store depletion nor capacitative calcium entry since oscillations could occur in the absence of significant store depletion as shown by also by Park *et al.* (2000). This would accord with the above results where blockade of store-operated calcium entry with SKF96365, although it decreased frequency, did not abolish oscillations. We have recently conducted a detailed study of the capacitative calcium entry pathway in urethral interstitial cells (Bradley *et al.* 2005) and established that, while such a pathway clearly exists, it does not appear to be important in the modulation of STICs. The evidence presented so far makes it clear that a calcium influx pathway must exist if spontaneous oscillations are to be maintained but apart from lanthanum, cadmium and nickel, none of the blockers so far studied have been effective in inhibiting oscillations. Interestingly these three ions are known to block sodium/calcium exchange (Blaustein & Lederer, 1999) and this is known as a calcium influx pathway in catfish retinal horizontal cells (Micci & Christensen, 1998). It was therefore of interest to examine the possibility that the sodium/calcium exchanger might be implicated in modulation of calcium oscillations in urethral interstitial cells. Recently we have shown that frequency of calcium oscillations in urethral interstitial cells was significantly reduced ( $5.6 \pm 0.9 \text{ min}^{-1}$ , in control to  $0.13 \pm 0.13 \text{ min}^{-1}$  in the presence of drug,  $P < 0.05$ , Student's paired *t* test) by addition of 5 μM of the reverse mode Na<sup>+</sup>/Ca<sup>2+</sup> exchange inhibitor KB-R7943 (Bradley, Johnston, Hollywood, McHale, Thornbury & Sergeant, unpublished observation). Conversely reduction

in the external Na<sup>+</sup> concentration from 130 to 13 mM to promote reverse Na<sup>+</sup>/Ca<sup>2+</sup> exchange caused an increase in the frequency of STICs voltage clamped at -60 mV (from  $1.9 \pm 0.65 \text{ STICs min}^{-1}$  to  $5.5 \pm 1.25 \text{ STICs min}^{-1}$  ( $P < 0.05$ ). These results are strongly indicative of a role for the sodium/calcium exchanger as a calcium influx pathway in urethral interstitial cells.

## Conclusions

It is now generally accepted that spontaneous activity in the gastrointestinal tract originates, not in smooth muscle cells themselves, but in specialized pacemaker cells or ICC. This may also be true in many other smooth muscle types but the detailed experiments that would be required to confirm it have not yet been done. Our own studies of spontaneous electrical events in the interstitial cells of the rabbit urethra would suggest that the former are generated by the oscillatory release of calcium from intracellular stores. The 'prime oscillator' would appear to be the ryanodine-sensitive store rather than the IP<sub>3</sub>-sensitive store since blockade of the former with tetracaine or with ryanodine stopped oscillations completely whereas inhibition of the action or production of IP<sub>3</sub> did not. Figure 5 shows our proposed model for pacemaking in these cells. Influx of calcium across the plasma membrane (probably via the sodium/calcium exchanger) triggers calcium release from the ryanodine sensitive store which causes further release from nearby IP<sub>3</sub> sensitive stores. This sets up a regenerative wave raising calcium concentration in the vicinity of the calcium-activated chloride channels to a level sufficiently high to activate them. Efflux of chloride ions through these provides the depolarizing current underlying the pacemaker potential. This in turn will depolarize the membrane sufficiently to activate the T- and L-type voltage-operated channels.

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