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Ca^{2+} phase waves - a fundamental mechanism underlying propagation of gastric slow waves

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Gastrointestinal motility is fundamentally dependent on rhythmic depolarisations termed slow waves, events that open voltage dependent Ca^{2+} channels and cause smooth muscle (SM) contraction. A key feature of slow waves is that they propagate relatively rapidly circumferentially near synchronously squeezing the gut but spread more slowly in an oral-anal direction to appropriately direct gastric contents. Yet key aspects such as how slow waves propagate remain in dispute with two primary hypotheses namely conventional action potential-based propagation contrasted against propagation occurring through phase waves generated by intracellular oscillators interacting as coupled oscillators (Daniel *et al.*, 1994).

Recently, we presented evidence for such an oscillator this being the intracellular ER/SR Ca^{2+} store and that slow waves propagated as phase waves resulting through these stores interacting as coupled oscillators (van Helden & Imtiaz, 2003). We termed these Ca^{2+} phase waves, a mechanism that represents a novel means for intercellular signalling that is very different to the conventional action potential. These studies were performed in single bundle strips from the gastric pylorus of the guinea-pig. In generalising this observation, we have investigated whether Ca^{2+} phase waves also underlie slow wave propagation across large multi-bundle sheets of gastric smooth muscle.

Animals were euthanased by exsanguination during deep isoflurane-induced anaesthesia (5-10% in air), a method approved by the University of Newcastle Animal Care and Ethics Committee. Tissue sheets of circular smooth muscle isolated from the gastric distal antrum dissected free of myenteric interstitial cells of Cajal (ICC-MY) showed robust slow waves and contractions. L-type Ca^{2+} channel blockade using nifedipine inhibited contractions without obvious effects on slow waves or slow wave propagation. Agents that inhibit store refill and/or inositol 1,4,5-trisphosphate receptors (IP3Rs) inhibited slow waves consistent with the view that slow waves are generated by (IP3R)-mediated Ca^{2+} release from intracellular Ca^{2+} stores. Slow waves exhibited much slower apparent conduction velocities ("CVs") across than along muscle bundles, this loosely paralleling electrical connectivity. T-type or other voltage dependent Ca^{2+} channels did not have a role in slow wave generation and/or propagation in this tissue. Importantly, the regenerative component of the slow wave was not essential for slow wave propagation, as pacemaker events that trigger the regenerative slow wave component, when subthreshold exhibited "CVs" that paralleled those of regenerative slow waves. Acetylcholine (ACh), a muscarinic agonist known to induce synthesis of IP3, enhanced synchronicity but at high concentrations decoupled slow waves. The findings support the hypothesis that slow waves propagate as Ca^{2+} phase waves, these arising through pacemaker-related Ca^{2+} stores interacting as coupled oscillators within and across the multibundle gastric tissue.

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Molecular and biophysical properties of smooth muscle-type voltage-gated Na^+ channels

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It is well-known that activation of voltage-dependent Ca^{2+} channels (Ca_V) and voltage-gated Na^+ channels (Na_V) is involved in the generation of action potentials in various types of excitable cells. Although voltage-gated Na^+ currents (I_{Na}) fail to be recorded in the vast majority of smooth muscle tissues, there are several reports regarding the existence of I_{Na} in vascular and visceral smooth muscles, suggesting that I_{Na} are involved in the generation of action potentials. Na_V appear to be selectively expressed in some, but not all smooth muscle, thereby questioning their significance in the physiology of the tissues. Furthermore, little attention has been given to the molecular properties of TTX-sensitive I_{Na} in smooth muscles.

Recent studies have revealed that Na_V consist of three subunits (expressed as a trimer): namely, an α subunit (260 kDa) which forms the core protein of the channel (possessing the TTX-binding sites) and two β subunits (30-40 kDa) which modify the channel function as an auxiliary subunit. To date, eleven isoforms of genes (*Scn1a-11a*) encoding TTX-sensitive and TTX-insensitive Na_V have been identified within a single family of Na_V, $Na_V1.X$ and four isoforms of genes (*Scn1b-4b*) encoding β subunits have been also detected.

The electrophysiological and pharmacological properties of Na_V in murine *vas deferens* smooth muscle cells were investigated using patch-clamp techniques. In whole-cell configuration, a fast, transient inward current was evoked in the presence of Cd^{2+}, and was abolished by TTX (K_d = 11.2 nM), mibefradil (K_d = 3.3 μM) and external replacement of Na^+ with monovalent cations (TEA^+, $Tris^+$ and $NMDG^+$). The fast transient inward current was enhanced by veratridine, an activator of voltage-gated Na^+ channels, suggesting that the fast transient inward current was a TTX-sensitive I_{Na}. The values for half-maximal (V_{half}) inactivation and activation of I_{Na} were -46.3 mV and -26.0 mV respectively. The molecular identity of the TTX-sensitive pore-forming subunits was revealed using RT-PCR analysis, *in situ* hybridization and immunohistochemistry. RT-PCR analysis revealed the expression of *Scn1a*, *2a* and *8a* transcripts, whilst *Scn1b* was only detected. The *Scn8a* transcript and the α subunit protein of $Na_V1.6$ were detected in smooth muscle layers. Furthermore, using $Na_V1.6$-null mice ($Na_V1.6^{-/-}$) lacking the expression of the Na^+ channel gene, *Scn8a*, I_{Na} were not detected in dispersed smooth muscle cells from the *vas deferens*, whilst TTX-sensitive I_{Na} were recorded in their wild-type ($Na_V1.6^{+/+}$) littermates. This study demonstrates that the molecular identity of the Na_V responsible for the TTX-sensitive I_{Na} in murine *vas deferens* myocytes is primarily $Na_V1.6$. (Zhu *et al*., 2008), co-expressed with β1 subunits.

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Spontaneous electrical and Ca^{2+} signals in the mouse renal pelvis that drive pyeloureteric peristalsis

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Pacemakers are ubiquitous throughout the body, setting all sorts of global rhythms, such as diurnal and circadian rhythms, as well as local rhythms that drive the heart and a number of smooth muscle organs. The upper urinary (pyeloureteric) system, consisting of the renal pelvis and ureter, is unique in physiology in that morphological, electrophysiological and Ca^{2+} imaging evidence suggests the presence of two putative pacemaker cell populations: atypical smooth muscle cells (SMCs) situated mostly in the proximal renal pelvis (Lang *et al.,* 2007a,b) and interstitial cells which are distributed throughout the pyeloureteric system and identified by their distinctive spindle- or stellate-shaped morphology and their immuno-reactivity to antibodies raised against the receptor tyrosine kinase, Kit (Pezzone, 2003).

We have recently established in excised strips (Lang *et al.,* 2007a) or single cells (Lang *et al.,* 2007b) of the mouse renal pelvis that:

i. nifedipine-sensitive action potentials and Ca^{2+} waves within the SMC wall are responsible for the peristaltic contractions that propagate the length of the pyeloureteric system.
ii. spindle-shaped atypical SMCs display high frequency spontaneous transient depolarizations (STDs) and Ca^{2+} transients that are reduced but not blocked by nifedipine or ryanodine. Under perforated-patch voltage clamp, a subpopulation of single SMCs displayed spontaneous transient inward currents (STICs) and long-lasting large inward currents (LICs) that could well be responsible for STD generation.
iii. interstitial cells with a morphology and distribution similar to Kit-positive cells display low frequency Ca^{2+} signals that are insensitive to nifedipine but readily blocked by ryanodine. These Ca^{2+} signals have time courses and frequencies similar to the long-lasting nifedipine-insensitive depolarizations recorded with intracellular microelectrodes. After enzymatic dispersal, two distinct populations of interstitial cells display STICS and LICs that are also little affected by 1 mM nifedipine and likely to be cation selective.

We have concluded that reduction but not blockade of STDs in atypical SMCs by nifedipine or ryanodine suggests that Ca^{2+} entry through L type Ca^{2+} channels and Ca^{2+}-induced release of Ca^{2+} (CIRCa) from internal stores is involved in the synchronization and propagation (entrainment) of STDs before they can provide a pacemaker drive to the SMC wall. However, the site of this entrainment that triggers a propagating contraction has yet to be established. Pelviureteric interstitial cells also generate their own non-propagating spontaneous electrical and Ca^{2+} signals albeit at a lower frequency than atypical SMCs. It is not yet clear, however, whether these spontaneously-active cells are indeed Kit-positive interstitial cell of Cajal-like cells and not other interstitial cells such as macrophages or fibroblasts, etc.

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Lang RJ, Zoltkowski BZ, Hammer JM, Meeker WF, Wendt I. (2007b) *Journal of Urology,* **177:** 1573-80.
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Do K^+ channels play a role in noradrenergic signalling in vascular smooth muscle?

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The mechanisms underlying depolarization of arterial smooth muscle by nerve-released noradrenaline (NA) remain largely unknown. In isolated vascular smooth muscle cells, applied NA produces an inward current by activating Ca^{2+}-activated Cl^- channels (Hogg *et al.*, 1994) and transient receptor potential (TRP)-like cation ion channels (Albert & Large, 2006). In rat iridial arterioles (Gould and Hill, 1996) and guinea-pig mesenteric veins (Van Helden, 1988), nerve-released NA produces a transient depolarization that is mediated by Ca^{2+}-activated Cl^- channels. However, there is no evidence that TRP-like cation channels contribute to nerve-evoked depolarization. In rat tail artery and guinea-pig mesenteric vein, nerve-released NA produces a slow phase of depolarization that is associated with a decrease in membrane conductance, indicating closure of K^+ channels (Cassell *et al.*, 1988; Van Helden, 1988). During ongoing nerve activity, this slow synaptic potential produces 15-20 mV of depolarization and contributes to constriction of the tail artery (Brock *et al.*, 1997). We have been investigating the mechanisms that underlie this depolarization.

Tail arteries were isolated from rats that had exsanguinated under deep anaesthesia (80 mg/kg pentobarbitone, i.p.). Artery segments were mounted in a recording chamber and the perivascular axons were electrically stimulated. Intracellular recordings were made from the smooth muscle cells. In rat tail artery, short trains of stimuli evoke both ATP-mediated excitatory junction potentials (EJPs) and a slow NA-mediated depolarization (NAD). Application of the α_1-antagonist prazosin (0.1 μM) slowed the rising phase of the NAD but did not change its amplitude. In contrast, the α_2-antagonist rauwolscine (1 μM) did not change the onset of the NAD but it did reduce its amplitude. In the presence of prazosin, the NAD was completely blocked by the K_{ATP} channel blockers, glybenclamide (10 μM, n = 6) and PNU 37883A (5 μM, n = 6). These agents also produced membrane depolarization. The α_2-adrenoceptor-mediated component of the NAD is produced by closure of K_{ATP} channels.

The NAD remaining when α_2-adrenoceptors were blocked with rauwolscine (1 μM) was increased in amplitude by glybenclamide (10 μM, n = 5). In rat tail artery, the time constant of decay of the EJP (τEJP) is determined by the membrane time constant (Cassell *et al.*, 1988). The τEJP of EJPs evoked at the peak of the rauwolscine-resistant NAD was prolonged (relative change 1.16, $p < 0.01$, n = 6). Similarly, the τEJP was prolonged during depolarization induced by the α_1-agonist, phenylephrine (0.5-1 μM, n = 5). These findings indicate a decrease in membrane conductance, suggesting that α_1-adrenoceptor-mediated depolarization is also produced by closure of K^+ channels. The rauwolscine-resistant NAD was unaffected by the Cl^- channel blockers, 9-anthracene carboxylic acid (100 μM, n = 5) and niflumic acid (10 μM, n = 5) or by the non-selective cation channel blocker, SKF 96365 (10 μM, n = 4).

Broad-spectrum K^+ channel blockers (tetraethylammonium, 4-aminopyridine, Ba^{2+}) did not inhibit the rauwolscine-resistant NAD. In CNS neurones, NA produces depolarization by closing the two-pore domain K^+ channel, TASK-1, but the selective blocker of these channels, anandamide (10 μM, n = 5), did not change the NAD. In heart, NA closes a Na^+-dependent K^+ channel that is blocked by quinidine. Quinidine (10 μM, n = 5) produced depolarization, slowed the τEJP and reduced the NAD. However, quinidine is reported to be an α_1-adrenoceptor antagonist.

These findings indicate that the NAD has two components: one of which is due to activation of α_1-adrenoceptors and the other to activation of α_2-adrenoceptors. The α_2-adrenoceptor-mediated component is produced by closure of K_{ATP} channels whereas α_1-adrenoceptor-mediated component is most likely mediated by closure of another type of K^+ channel.

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