AuPS/ASB Meeting - Adelaide 2010

Free comunications: Smooth muscle

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Chair: Nick Spencer

Temporal relationships between intraluminal manometry and actual gut movement in the isolated rabbit small intestine

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The content of the intestine is propelled by coordinated movements of the muscle layers involving both myogenic and neurogenic mechanisms. The relation between motions of the gut wall and the resulting intraluminal pressure has not been well established. We have investigated this relationship in isolated segments of rabbit small intestine taken from 4 animals killed by i.v. injection of lethobarbital (0.5ml/Kg). Segments of 40 cm were placed into an organ bath containing warm (36°C) oxygenated Krebs solution, constantly bubbled with carbogen gas. Krebs was infused *via* the cannulated oral end at 2-4ml/minute and the contents could be expelled and measured at the anal end *via* a non-return valve. Motions of the intestinal wall was recorded by a video camera placed above the bath. Spatio-temporal maps of changes in diameter (Dmaps) were constructed from the video recordings (Hennig *et al.*, 1999). Spatiotemporal maps of intraluminal pressure (Pmaps) were constructed from high-resolution fibre-optic manometry recordings (Arkwright *et al.*, 2009). The relation between movements and intraluminal pressure were compared during periods of motor activity that occurred spontaneously or elicited by slow distension or by erythromycin (10-6M). A total of 85 minutes of combined Dmaps and Pmaps were analysed. During this period 813 longitudinal muscle contractions, 288 circular muscle contractions in the diameter maps and 1049 pressure events were identified in the pressure maps. All antegrade propagating circular muscle contractions were associated with high pressure waves and with outflow at the anal end indicating propulsive motor activity. Incomplete propagating circular muscle contractions were also associated with pressure waves. In the segment of intestine not invaded by these contractions, peaks of pressure were recorded simultaneously indicating a common cavity. Pressure waves of a much lower amplitude were also associated with spontaneous pendular longitudinal muscle contractions. These results will enable a more appropriate interpretation of manometry *in vivo*.

Hennig GW, Costa M, Chen BN, Brookes SJ. (1999) *Journal of Physiology* **517:** 575-590.

Arkwright JW, Underhill ID, Maunder SA, Blenman N, Szczesniak MM, Wiklendt L, Cook IJ, Lubowski DZ, Dinning PG. (2009) *Optics Express* **17:** 22423-22431.

Gap junction coupling between smooth muscle cells modulates responses to inhibitory motorneurons and exogenous ATP

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We have previously reported that electrophysiological responses of gut smooth muscle cells, including inhibitory junction potentials, are absent during the first 30-60 minutes after setting up preparations *in vitro*. Here, we investigated the mechanisms that underlie this temporary unresponsiveness.

Methods: Segments of guinea pig ileum, with mucosa and submucosa removed, were used for intracellular recording under current clamp conditions. Circular smooth muscle cells were impaled with glass micropipettes filled with carboxyfluorescein (5%) and KCl (1M), in Krebs solution (34°C) containing 1μM hyoscine and 1μM nicardipine to inhibit smooth muscle contractions. We recorded resting membrane potential (RMP), inhibitory junction potentials (IJPs) evoked by single shot stimuli, and responses to ATP applied locally by pressure ejection (140kPa nitrogen pulses, 50-100ms duration, 10mM). Cells were dye-filled by 0.5nA hyperpolarising pulses (50% duty cycle for 2 minutes, followed by 1 minute diffusion). Dissections were carried out in cool Krebs solution (14°C). The start of the equilibration period was considered as being the moment when warmed Krebs solution (35°C) first reached the recording chamber.

Results: The recording chamber warmed to 34-35°C within the first 5 minutes of the equilibration period (n=3), however IJP amplitude was typically less than 1mV for the first 30 minutes (n=12). IJP amplitude then increased gradually so that by 90-120 minutes, IJP amplitude averaged -11.5mV±1.6mV (n=12). Cells with IJPs less than 1mV were classed as ‘unequilibrated’ and cells with IJPs greater than 10mV were classed as ‘equilibrated’. ‘Unequilibrated’ cells were significantly hyperpolarised compared to equilibrated cells where RMPs were -58.8±1.4mV and -47.2±0.4mV respectively (cells=23 and 64, n=12, $p<0.0001$). Input resistance was significantly greater in ‘unequilibrated’ cells (15.5±1.9 MΩ) than ‘equilibrated’ cells (8.7±0.7 MΩ, n=12, $p<0.0001$). ‘Unequilibrated’ cells showed significantly less dye coupling (mean=2.1±0.3 carboxyfluorescein-filled profiles) compared to ‘equilibrated’ cells (mean=4.4±0.3, n=12, $p<0.0001$). Addition of gap junction blockers carbenoxolone (100μM) and 18β Glycyrrhetinic acid (10μM) made “equilibrated” cells change their characteristics back to a state similar to “unequilibrated” cells. In the presence of carbenoxolone, circular smooth muscle cells had a more negative resting membrane potential, smaller IJP amplitude, increased input resistance and reduced dye coupling. Glycyrrhetinic acid similarly reduced IJP amplitude, increased input resistance and reduced dye coupling between ‘equilibrated’ cells. These results suggests that gap junction coupling between smooth muscle cells may increase during the equilibration period and may underlie the changes in IJP amplitude. In other cell types, gap junction permeability is reduced by high cytostolic [Ca^{2+}]. We tested whether influx of calcium, during the set-up procedure, might cause uncoupling during the ‘equilibration’ period. Preparations were dissected in low [Ca^{2+}], high [Mg^{2+}] Krebs solution (mM: Ca^{2+} 0.25; Mg^{2+} 2.5 at 14°C), and transferred into normal Krebs solution at 35°C for recording. There was no significant reduction of the equilbration period in these preparations. Lastly we tested whether the loss of IJPs during the equilibration period was associated with a loss of sensitivity by smooth muscle cells to ATP; the transmitter of the fast IJP. Hyperpolarisations evoked by local application of 10mM ATP were significantly smaller in ‘unequlibrated’cells (0.3±0.3mV) compared to ‘equilibrated’ cells (-13.0±1.1mV, n=6, $p<0.0001$)), paralleling the change in amplitude of the IJP. This suggests that the loss of neuronal input may be due to a change in electrophysiological properties of the postsynaptic cell during equilibration.

In conclusion, following set-up of dissected gut preparations *in vitro*, smooth muscle cells show significant changes in electrophysiological properties that recover during the “equilibration period”. Smooth muscle cells are hyperpolarised and have a reduced response to inhibitory stimulation either by inhibitory motorneurons or by exogenous application of ATP. During this period gap junction coupling is suppressed. Pharmacologically blocking gap junctions mimics the change in electrophysiological properties during the equilibration period. The change in gap junction coupling does not appear to be due to influx of calcium into smooth muscle cells during the set-up procedure.

KV4 and ANO1 / TMEN16A chloride channel expression profiles distinguish between atypical and typical smooth muscle cells in the mouse renal pelvis

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Previous intact preparation experiments and calcium imaging have suggested that atypical smooth muscle cells (SMCs) in the proximal renal pelvis are likely to be the pacemaker cells that drive pyeloureteric peristalsis (Lang *et al*., 2007). However, their electrical characteristics, location and mechanisms of pacemaker generation remain obscure. Standard perforated-patch patch clamp, intracellular microelectrode recording and immunohistochemistry techniques were used. In some experiments a transgenic mouse with enhanced yellow fluorescent protein (eYFP) exclusively expressed in cells containing α-smooth muscle actin (α-SMA) were used.

Single atypical eYFP-α-SMA$^+$ SMCs could be distinguished electrophysiologically from typical eYFP-α-SMA$^+$ SMCs by the absence of a voltage-operated transient 4-aminopyridine-sensitive ('A-type' KV4) K^+ current (I_{KA}) and the absence of spontaneous transient outward currents (STOCs) arising from the opening of large conductance Ca^{2+}-activated K^+ (BK) channels. Both typical and atypical SMCs displayed spontaneous transient inward currents (STICs) flowing through niflumic acid (NFA)-sensitive Cl^- channels. However, 25% of typical SMCs also displayed a large NFA-sensitive Cl^- current which displayed relatively slow kinetics of activation and deactivation, presumably reflecting a relatively high internal Ca^{2+} concentration. Atypical SMCs also fired prolonged large inward currents (LICs), which were cation-selective and blocked by La^{3+} or ryanodine. Immunostaining for ANO1/ TMEN16A Cl^- channel subunits was found predominately in the distal regions of the renal pelvis, co-localizing with intense immunostaining for α-SMA. In contrast, α-SMA$^-$ interstitial cells (ICs) were distinguished by the presence of a Xe991-sensitive KV7 current, a small I_{KA} current, tetraethylammonium-sensitive BK channel STOCs and Cl^--selective STICs blocked by NFA. Intense TMEN16A immunostaining also located to a population of Kit$^-$ α-SMA$^-$ ICs in the proximal and mid regions of the renal pelvis.

We conclude that (i) KV4$^+$, BK STOC$^+$, α-SMA$^+$ SMCs are the typical SMCs that facilitate muscle wall contraction, (ii) TMEM16A or KV7 immunoreactivity may be useful makers of Kit$^-$ ICs in the urogenital tract, and (iii) KV4$^-$ α-SMA$^+$ atypical SMCs firing cation-selective LICs are likely to be the pelviureteric pacemakers.

Lang RJ, Hashitani H, Tonta, MA, Parkington, HC & Suzuki H. (2007) Spontaneous electrical activity and Ca^{2+} transients in typical and atypical smooth muscles and interstitial cells of Cajal-like cells of mouse renal pelvis. *Journal of Physiology* **583**, 1049-1068

Controlling uterine contractions: the role of interstitial cells

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During labour, the smooth muscle cells (SMCs) in the wall of the uterus generate strong, rhythmic contractions that are necessary for vaginal delivery. Such contractions are kept in abeyance before the onset of labour and the transition into labour involves the activation of contraction associated processes. The processes recruited in this switch are incompletely understood. Rhythmic, controlled SM contractions are exquisitely exemplified in the functioning of the gastrointestinal tract. In this location "interstitial cells" (ICs), described initially over 100 years ago by Cajal, play a major role in organ rhythmicity. In the last decade, ICs have also been identified within the wall of the uterus, but their role remains elusive. Here we investigated the role of uterine ICs in late pregnant and labouring human and mouse uterus using a variety of approaches.

Tissues were obtained from women undergoing caesarean delivery. Mice were studied 24 hours before delivery and in labour. Either membrane potential or cytoplasmic Ca^{2+} was recorded simultaneously with tension. In single SMCs and ICs isolated with collagenase, ionic currents were recorded and molecular fingerprinting assessed using single-cell RT-PCR. Cellular localizations of proteins of interest were made using immunohistochemical techniques.

Uterine ICs stained with vimentin but since this can stain several cell types, in human uterine tissue distinction was made between ICs, fibroblasts and immune cells by a co-staining approach. Prolyl 4-hydroxylase, which identifies fibroblasts, co-localized with vimentin in cells that had a small volume around the nucleus, and 2 long slender projections. Unphosphorylated connexin 43 and c-Kit identify ICs, and cells co-staining with these had a large cell volume with 3-5 projections emanating from the nuclear region. CD45, which identifies macrophages, did not co-stain with vimentin. Vimentin staining, in cells located amongst the SMCs, doubled in human tissues obtained during labour (2.4±0.3%) compared with those in late pregnancy but not in labour (1.3±0.2%). Isolated SMCs had a robust large-conductance, Ca^{2+}-activated K^+ current, which was absent from ICs. This was verified in pairs of SMC and IC from 20 women using single-cell RT-PCR.

In mice, vimentin staining did not occur amongst the SMCs in the outer, longitudinal muscle layer, although vimentin staining was observed between SMC bundles in this layer. In contrast, strong vimentin and c-Kit staining occurred amongst the SMC of the inner circular muscle layer, and in the loose tissue between the two muscle layers. This segregation provided as close as might be possible to a natural "IC knock-out" preparation, which we exploited to further probe the role of ICs in uterine pacemaking. Isolated circular strips were always spontaneously contractile, and the SMC had "resting" potentials of −57±3mV. In contrast, longitudinal strips were always quiescent, with resting potentials of −71±2mV. In view of the high density of vimentin-staining cells in the loose intermediate region between the two muscle layers, we made circular and longitudinal strips that had: (1) as much intermediate material as possible removed; and (2) as much intermediate material as possible retained. Circular strips with intermediate material attached were spontaneously contractile, while removing this material led to the abolition of this activity. In contrast, longitudinal strips remained quiescent, whether or not intermediate material was present. However, longitudinal strips with intermediate *and* circular muscle remaining attached always contracted spontaneously. These spontaneous contractions in fully intact longitudinal strips were abolished by imatinib (2×10^{-4}M), which putatively blocks c-Kit.

In conclusion, ICs may well play a role in the generation of spontaneous uterine contractions. In mouse uterus, interaction between ICs and circular SMCs appears to be required for full development of pacemaking. In addition, vimentin/c-Kit staining cells appear critical for the spread of contractions between the two muscle layers. The longitudinal muscle is the more important for successful vaginal delivery, while the circular layer appears to play a greater role in generating contractions. In human uterus, the smooth muscle layers are more diffuse and a circular/longitudinal distinction is not evident. The tissue is arranged in thin layers throughout the wall, with the SMC within each layer in the same orientation but with a different orientation in adjacent layers. Here, cells that link the SMCs within this intricately layered network are likely to be critical for organized contraction of the organ, such as is necessary for timely vaginal delivery. ICs may fulfil this role.