Controlling uterine contractions: the role of interstitial cells

H.C. Parkington,[1] Q. Li,[1] M.A. Tonta,[1] J. Iqbal,[1] M.M. Davies,[1] K.W. Taylor,[1] S.P. Brennecke,[2] H.A. Coleman,[1] R.J. Lang[1] and P.J. Sheehan,[2] [1]Department of Physiology, Monash University, Clayton, VIC 3800, Australia. and [2]Royal Women's Hospital, Corner Grattan Street and Flemington Road, Parkville, VIC 3052, Australia..

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.