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Spontaneous electrical activity in subpopulation of freshly isolated rat uterine myometrial cells

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Many types of smooth muscle including uterus exhibit spontaneous electrical and mechanical activity of myogenic origin. Elucidation of the mechanisms responsible for the uterine autorhythmicity is crucial for the understanding of labour. In recent years, pacemaking in GI tract has been attributed to the activity of interstitial cells of Cajal (ICC) (see Hirst & Ward, 2003 for review). A similar mechanism has also been found in some of the urogenital smooth muscles (Sergeant *et al.* 2000). In our experiments we found that cells resembling the ICCs' phenotype are present in the rat and human uterus. These cells, however, did not show spontaneous electrical activity attributable to their possible role as an intrinsic uterine pacemaker. In the present study we have investigated the ability of uterine smooth muscle cells to generate spontaneous action potentials.

Late pregnant (19–21 days) Sprague-Dawley rats were killed by cervical dislocation after CO_2 anaesthesia. Single cells were enzymatically isolated from the longitudinal layer of the myometrium and superfused with prewarmed (35°C) Krebs solution. Current clamp mode of the conventional patch clamp technique was used to measure transmembrane potential and to pass polarising current.

Most of the cells examined (26 out of 35) had stable membrane potential of -64 ± 12 mV (mean ± S.E.M.). All cells were capable of generating action potentials in response to supra-threshold (10–50 pA) depolarising current. The peak value of the action potential was $+12 \pm 9$ mV. Mean duration of the action potential at 90 % repolarisation was 86 ± 23 ms. Approximately 25 % of cells studied (9 out of 35) were spontaneously active. They generated spontaneous transient depolarisations of 10–20 mV amplitude, which lasted from 1.5 to 4 s. Many of these spontaneous depolarisations resulted in the generation of full sized action potentials with parameters similar to those of the evoked action potentials. Some of the spontaneous depolarisations remained sub threshold and did not lead to the generation of action potentials.

In conclusion, our data suggest that in the uterus, the pacemaking might be an intrinsic feature of smooth muscle cells rather than interstitial cells of Cajal or ICC-like cells.

Hirst GDS & Ward SM (2003). *J Physiol* **550**, 337–346.

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Possible roles for IGF-1 splice variants in the myometrium during pregnancy and following parturition

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The splice variants of the Insulin-like Growth Factor (IGF-1) gene, IGF-1Ea and MGF, are believed to have distinct roles in skeletal muscle hypertrophy and repair (Yang *et al.* 1996; Owino *et al.* 2001 and Hameed *et al.* 2003). Myometrial smooth muscle hypertrophy and repair are fundamental features of pregnancy and uterine involution following parturition, yet the molecular mechanisms involved in these processes are poorly understood.

15 female Sprague Dawley rats were used in the present study to determine the mRNA expression of the IGF-1Ea and MGF in the myometrium using real time quantitative PCR (Roche LightCycler, UK).

Measurements were made at day 18 of pregnancy ($n = 5$) and day 14 post parturition ($n = 5$) and compared to levels in the non-pregnant uterus ($n = 5$). Data was analysed using the ANOVA and Tukey HSD tests. During pregnancy mRNA expression levels of MGF and IGF-1Ea increased by 21 % and 310 % respectively compared to the controls. This increase was significant for IGF-1Ea ($P < 0.05$), but not for MGF. Interestingly, at day 14 post parturition, levels of MGF mRNA were markedly increased showing a 1536 % increase, whilst IGF-1Ea showed a 349 % increase compared to the non-pregnant controls. In both cases the increase was significant ($P < 0.01$ and $P < 0.05$ respectively) compared to the control. However, compared to day 18 gestation only the increase in MGF was significant ($P < 0.05$).

These results suggest that the two isoforms have distinct roles, with IGF-1Ea thought to be involved in myometrial hypertrophy during pregnancy. In contrast, it seems that MGF is involved later, in the repair and remodelling phase following parturition.

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All procedures accord with current UK legislation