Activation of at least three classes of ion channels by β-adrenoceptor activation in pregnant uterine smooth muscle

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Preterm labour complicates 5-10% of births, has significant repercussions for neonatal morbidity and mortality and may have consequences for lifelong health. Agents that stimulate β-adrenoceptors are commonly used to suppress preterm uterine contractions, yet despite considerable effort, a complete understanding of the mechanisms involved is lacking. We have previously shown that activation of β-adrenoceptors in sheep myometrium markedly reduces the sensitivity of the contractile apparatus to Ca^{2+} and induces large hyperpolarization that is inhibited by blockade of ATP-sensitive K^+ (K_{ATP}) channels (Parkington *et al*., 2000). Stimulation of β-adrenoceptors shifts the activation curve for large-conductance, Ca^{2+}-activated K^+ (BK_{Ca}) channels to the left in human myometrium (Zhou *et al*., 2000).

In the present study we probed the effects of β-adrenoceptor activation in late pregnant sheep myometrium using a variety of approaches: (1) simultaneous recording of membrane potential and tension in myometrial strips; (2) patch clamp recordings of single channel and whole cell currents in freshly isolated cells from these same ewes; and (3) simultaneous recording of extracellular electrical (EMG) and contractile activity in the uterus of conscious ewes at days 130-140 of pregnancy (term ~145 days). Under general halothane anaesthesia and using full sterile techniques, EMG electrodes and transducers were attached to the uterus and catheters implanted into a branch of the uterine artery and the jugular vein for local and general drug infusion, respectively. A fetal jugular catheter was implanted to monitor fetal well being. Isolated tissues were obtained during surgery and again at *post mortem*. Labour was induced preterm in 5 ewes by infusion of RU486 (0.5 mg/kg) (Hirst *et al*., 2005). Salbutamol was used to stimulate β-adrenoceptors.

The hyperpolarization (15 mV in tissues from 20 ewes) evoked in cells in myometrial strips by salbutamol (10-300 nM) was blocked by glibenclamide (1 μM, n=16) and PNU-37883A (10 μM, n=4) but not by iberiotoxin (60 nM, n=3) or charybdotoxin (60 nM, n=6), indicating activation of K_{ATP} but not BK_{Ca} channels. However, salbutamol induced a prominent activation of BK_{Ca} channels in isolated cells and caused a leftward shift of the activation curve that was similar to raising the intracellular Ca^{2+} concentration (n=12). Blockade of BK_{Ca} channels with iberiotoxin revealed that salbutamol also activated two small channels, a K_{ATP} channel of 62pS and a channel (conductance 14 pS) that reversed near −20 mV. In strip preparations continuously superfused with glibenclamide-containing solution, the amplitude of the action potential was reduced by salbutamol, and this was blocked by iberiotoxin. In addition, in the presence of PNU-37883A, salbutamol induced a small depolarization.

Infusion of salbutamol (100 μg/kg/h) into conscious ewes, 7 days after surgery, caused immediate cessation of the bursts of EMG activity and associated contraction that occurs 3-4 times per hour. Following 30 min infusion of glibenclamide (1mg/kg/h), salbutamol failed to suppress uterine activity before labour (n=7), during normal spontaneous labour (n=3), and following induction of labour preterm (n=5).

These results demonstrate a significant activation of K_{ATP} by salbutamol in pregnant sheep myometrium at the single channel and tissue levels and in the intact ewe. BK_{Ca} channels are also activated, and their main effect is to reduce the amplitude of the action potential and not to cause membrane hyperpolarization. An intriguing and unexpected action of salbutamol was the activation of an inward conductance, but its role in the excessive "rebound" uterine activity observed following salbutamol withdrawal *in vivo* awaits further investigation.

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