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Distribution, expression and functional effects of small conductance Ca-activated potassium (SK) channels in rat myometrium

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ABSTRACT

Calcium-activated potassium channels are important in a variety of smooth muscles, contributing to excitability and contractility. In the myometrium previous work has focussed on the large conductance channels (BK), and the role of small conductance channels (SK) has received scant attention, despite the finding that over-expression of an SK channel isoform (SK3) results in uterine dysfunction and delayed parturition. This study therefore characterises the expression of the three SK channel isoforms (SK1–3) in rat myometrium throughout pregnancy and investigates their effect on cytosolic [Ca] and force and compares this with that of BK channels. Consistent expression of all SK isoform transcripts and clear immunostaining of SK1–3 was found. Inhibition of SK1–3 channels (apamin, scyllatoxin) significantly inhibited outward current, caused membrane depolarisation and elicited action potentials in previously quiescent cells. Apamin or scyllatoxin increased the amplitude of [Ca] and force in spontaneously contracting myometrial strips throughout gestation. The functional effect of SK inhibition was larger than that of BK channel inhibition. Thus we show for the first time that SK1–3 channels are expressed and translated throughout pregnancy and contribute to outward current, regulate membrane potential and hence Ca signals in pregnant rat myometrium. They contribute more to quiescence that BK channels.

1. Introduction

Changes in ion channel expression with gestation and labour are crucial to the control of uterine activity and hence successful pregnancy [1–5]. Calcium-activated potassium channels of large (BK), intermediate (IK) and small (SK) conductance are expressed in a variety of smooth muscles, where they are considered to contribute to excitability, via the membrane potential and hyperpolarisation. In the myometrium much previous work has focussed on the large conductance channels (BK), whose expression was found to decrease at the time of parturition in human myometrium, suggesting a role in the transition from quiescence to labour [1]. However pharmacological inhibition of BK channels was recently shown to have little or no functional effect in rat uterus [6]. Attention has recently focussed on SK channels after it was reported that in the mouse, over-expression of an SK channel isoform (SK3) results in uterine dysfunction and delayed parturition [7].

Three isoforms of SK (SK1–3) are produced by three different genes (KCNN1–3). They are all voltage independent and activated by Ca at around 0.1 μM, during influx on the action potential [8–11]. Thus it would be expected that, if they are expressed in the myometrium, they would be activated during the rise of Ca. All three isoforms are inhibited by apamin [12] and scyllatoxin [13]. A role for SK channels in regulating smooth muscle activity has been shown in a variety of preparations [14,15]. Treatment of smooth muscles with apamin has been reported to enhance slow waves and contractility in GI tract [16–18]. It has also been suggested that SK channels may be expressed in fibroblast-like pacemaker cells in the GI tract [19].

Data concerning SK channels in the myometrium are limited. Based on RT-PCR data, a down-regulation of SK2 and 3 in term, pregnant (but not labouring) human uterus compared with non-pregnant hysterectomy samples was suggested, although channel protein was not measured [20]. Expression of SK3 channel was down-regulated from mid-gestation in mice and the uterus from late gestation mice is less sensitive to apamin. However as apamin is a non-specific inhibitor of SK1–3, it is important to study the expression pattern of all three SK channels when relating pharmacology to function.

These data suggest that SK channel regulation may be an important component in the increase in uterine activity seen at term [21,22]. However no functional or electrophysiological investigation has been performed on the role of SK channels in the myometrium and no systematic study of their expression and pro-

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Table 1
Rat/mouse SK1–3 oligonucleotide primer pairs.

Gene	Oligonucleotide primer sequences (5′–3′ direction)	T_m (°C)	Amplicon size (bp)
SK1	(F) CTGTGGGAAGGGCGTGTGTCTG (R) CCGAACCCGGCTTTGGTCTGG	60	250
SK2	(F) ATGCCCCTTCCACAACCACTGC (R) TGCCACTACGGCTACCACCAAG	60	500
SK3	(F) CAAGAACGCTGCCGCCAATGTC (R) CCAGGCTGCCAATCTGCTTTTC	60	300

tein levels throughout gestation and labour has been conducted. The aims of the present study were therefore to determine (i) the expression pattern of all three SK channels in the myometrium, (ii) the functional significance of channel inhibition for myometrial contractility and excitability throughout gestation in the rat and (iii) how these functional effects compare with data from BK channel inhibition.

2. Methods

2.1. Tissues and isolated cells

Non-pregnant virgin rats and time-mated pregnant (day 0 is day of pairing, labour occurred overnight d22/23) and labouring rats were humanely killed by CO_2 inhalation and cervical dislocation. The data from pregnant animals were obtained on days 10–11, 16–17 and 20–21 and for simplicity these are referred to as days 10, 16 and 21 throughout. The uterus was removed and cleared of any fat and pups and small strips (1 mm × 4 mm) of longitudinal myometrium were dissected. All procedures were carried out according to UK Animals (Scientific Procedures) Act 1986 and local guidelines. To make isolated cells, previously published protocols were followed [23,24]. Briefly strips were incubated in low Ca (50 μM) Hank's balanced salt solution (HBSS, bought from Sigma) for 60 min at 36 °C. This was followed by enzymatic digestion using Liberase Blendzyme II at 0.42 Wünsch Units/ml of collagenase activity (Roche Applied Sciences, Indianapolis, IN) dissolved in low calcium HBSS. Digestion time varied between 1 h and 1 h 15 min. After digestion, fluffy strips were washed 2–3 times with low Ca HBSS and transferred into KB medium [25]. After 10–15 min incubation in KB medium, strips were triturated using fire polished glass pipette to release single cells. The suspension obtained was kept in KB medium at 4 °C and used within 8 h after isolation.

2.2. RNA reverse transcription and polymerase chain reaction

Tissue specimens of <1 cm^3 were immersed in RNAlater (Ambion) for 24 h before being snap-frozen in liquid nitrogen. Total frozen weight of tissue of 60 mg from each group was ground under liquid nitrogen using a mortar and pestle and homogenised with Qiashredder spin columns as directed by the manufacturer (Qiagen). Total RNA was extracted using the RNeasy™ Mini Kit and immediately assessed for degradation on 1% agarose gels containing 0.5 μg/ml ethidium bromide prior to reverse transcription. Purified RNA preparations (5 μg) were incubated with 0.6 μg oligo-(dT) primer (Invitrogen) for 10 min at 70 °C before cooling on ice for 5 min. Samples were incubated for 1 h at 37 °C in first strand buffer containing 50 mM Tris–HCl (pH 8.3), 75 mM KCl and 3 mM $MgCl_2$, 10 mM dithiothreitol and 0.5 mM dNTPs (both from Invitrogen), with the addition of 1.2 U RNase-free DNase I and 6 U RNasin RNase inhibitor (both from Promega). DNase I was inactivated by heating to 75 °C for 5 min before further addition of 5 U RNasin RNase inhibitor and 200 U of Superscript II™ (Invitrogen) for 1.5 h at 42 °C. The reaction was suspended by heating at 70 °C for 15 min.

Primers were synthesised by Proligo (France) at stock concentrations of 100 μM using published oligo sequences designed to amplify rat KCNN isoforms 1–3 [26]. Complementary DNA samples were assessed against negative RT reactions with β-actin primers to test for genomic DNA contamination prior to analysis with KCNN isoform specific primer pairs (Table 1). The β-actin reactions were also a comparative internal positive control for each PCR experiment.

Amplification of cDNA templates was performed using the following standard protocol for all primer pairs in a final volume of 25 μl; 0.625 U Hot Star Taq DNA polymerase, 10× PCR buffer (20 mM Tris–HCl pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$ both from Qiagen), 0.2 mM dNTP (AbGene) and 2 μM of each isoform specific primer. PCR was performed for 35 cycles, each consisting of denaturation at 95 °C for 20 s, primer annealing at an optimised temperature for 30 s and extension for 1 min at 72 °C prior to a 12 min completion step at 72 °C.

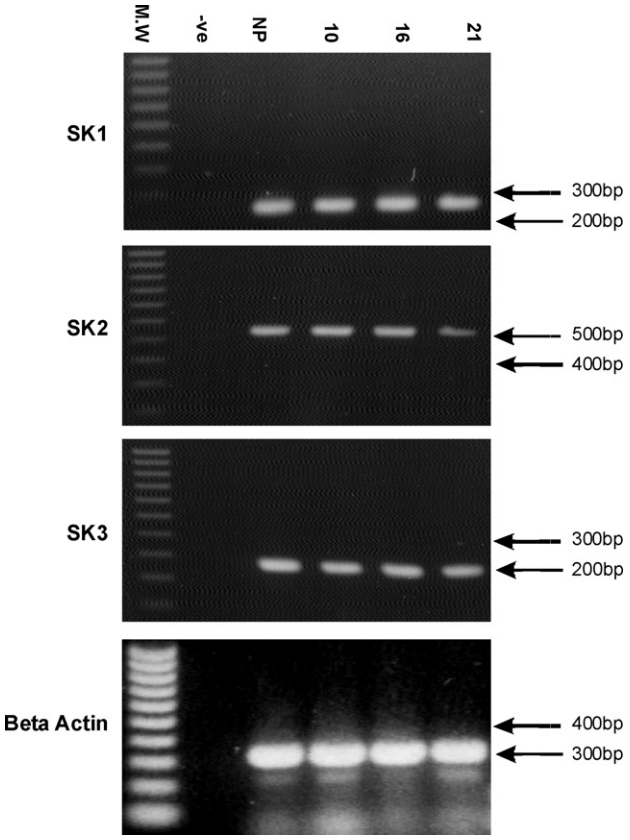

Fig. 1. Detection of KCNN channel isoform mRNAs in rat uteri at different stages of gestation by RT-PCR. The cDNAs generated from six uteri per group were analysed by PCR amplification for 35 cycles using isoform specific primers KCNN1–3. MW, DNA ladder (sized in base pairs); –, negative control (no template); NP, day 10, day 16 and day 21.