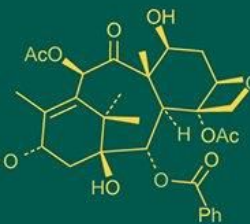
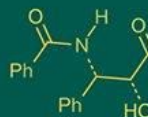
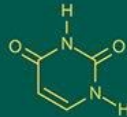


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Characterization of TRPC4 channel in nonpregnant and pregnant mouse uterus

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Abstract

Uterine myometrium shows versatile function ranging from quiescent state during pregnancy to highly contractile state during parturition. It can also exhibit tonic contractions under the influence of contractants. Influx of Ca^{2+} is central to the effect of various uterotonic agents like oxytocin, prostaglandin etc. Among the various Ca^{2+} permeating channels, TRP channels may play role in myometrial contraction by modifying Ca^{2+} homeostasis. The present work shows the expression of TRPC4 channel in the myometrium of pregnant and non-pregnant mouse and we propose that it has important role in maintaining basal and agonist-induced uterine contraction.

Keywords: TRPC4 channel, pregnant, non-pregnant, uterus

Introduction

Uterus is an amazing organ as it maintains a delicate balance of contraction and relaxation during pregnancy and labor. Uterine myometrium is predominantly a phasic muscle which remains quiescent during pregnancy and highly contractile during parturition. It can also exhibit tonic contractions when exposed to high concentrations of contractants.

Myometrial smooth muscle undergoes significant changes in morphology and in basal contractile activity and responsiveness during gestation, labor and after parturition (Sanborn, 2007) [20]. Uterus contracts spontaneously following waves of electrical activity that result in membrane depolarization, a rise in intracellular calcium and the generation of force (Bernal, 2007) [4].

The uterus is relatively quiescent during gestation and its contractility increases as labor progresses. The mechanisms underlying the switch from uterine quiescence to contractile activity in labor are not clearly understood, although the pathways affecting myometrial calcium homeostasis play an important role in it. It has been evident that extracellular calcium is essential for the generation of spontaneous and agonist-induced contractile activity in myometrium at term (Szal *et al.*, 1994; Tribe, 2001) [23, 27] and during labor (Parkington *et al.*, 1999) [17].

During pregnancy myometrial tissues undergo mechanical stretch. Stretch of myometrial tissue, *in vivo* and *in vitro*, stimulates expression of a range of labor-associated proteins, such as COX-2 and prostaglandin as well as oxytocin receptors (Loudon *et al.*, 2004; Sooranna *et al.*, 2004; Terzidou *et al.*, 2005) [15, 22, 24]. Transient Receptor Potential (TRP) channels are the Ca^{++} permeating channels (den Dekker *et al.*, 2001; Yang *et al.*, 2002) [7, 31] subdivided into 7 subfamilies: TRPA (Ankyrin), TRPC (Canonical), TRPM (Melastatin), TRPML (Mucolipin), TRPN (No mechanoreceptor potential C or NOMPC), TRPP (Polycystin) and TRPV (Vanilloid). TRPC4 is widely expressed in endothelium (Freichel *et al.*, 2001; Tiruppathi *et al.*, 2002) [10, 25], smooth muscle (Beech *et al.*, 2004) [3], intestinal pacemaker cells (Walker *et al.*, 2002; Torihashi *et al.*, 2002) [29, 26], brain (Hofmann *et al.*, 2000) [12] and adrenal gland (Philipp *et al.*, 2000) [18].

Expression of TrpC4 mRNA and proteins in term human myometrial tissue and primary cultured human myometrial smooth muscle (HMSM) cells has been reported (Dalrymple *et al.*, 2002; Yang *et al.*, 2002) [6, 31].

TRP channels may play role in myometrial contraction by modifying Ca^{2+} homeostasis. Therefore this work was done to characterize TRPC4 channels in mouse uterus.

Materials and methods

Healthy adult female Swiss Albino mice (30-35 g) were used for the study. Animals were provided with standard feed mixture boiled in dry milk powder (5%) and water *ad libitum*. Uteri from pregnant and non-pregnant mouse were used for the study. Non-pregnant animals after acclimatization and pregnant animals in advanced stage of

pregnancy (day 17-19) were sacrificed by cervical dislocation. Uterus, after collection, was immediately transferred into cold (4°C) aerated Modified Kreb's Henseleit Solution (MKHS). Adhering fat and tissues were cleaned and uterus was stored in refrigerator at 4°C till further experiments.

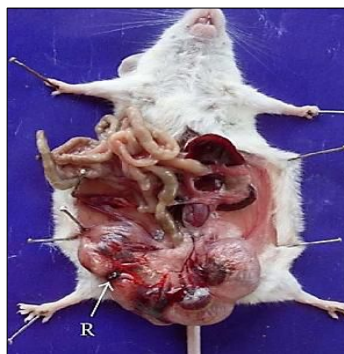


Fig 1: Sacrificed pregnant mouse with gravid uterus

Molecular characterization of TRPC4 channel in pregnant and non-pregnant mouse uterus was done by using Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) and sequencing. Uterine tissues from late pregnant and non-pregnant mice were isolated and stored in RNA Later (100 mg in 1 ml) at -20 °C. Approximately 20 mg tissue was snap frozen in liquid nitrogen. Total RNA was isolated with Total RNA Mini Kit (IBI Scientific, USA) as per the manufacturer's instructions. The purity of the RNA was

checked in a NanoDropR spectrophotometer (ND-1000 spectrophotometer; Coleman Technologies Inc) by A_{260}/A_{280} and A_{260}/A_{230} ratio and quantitated as follows:

A_{260} OD = 40 μ g/ml

The RNA samples showing A_{260}/A_{280} ratio 1.95-2.05 and A_{260}/A_{230} ratio >1.5 were used for cDNA synthesis. It was carried out from the mRNA present in the total RNA using First Strand cDNA Synthesis kit (Fermentas, USA).

Table 1: Description of primer

Type of Gene	Primer sequence	Size	Accession No.
TRPC4	F5'-TCTGCAGATATCTCTGGGAAGGATGC-3' R5'-AAGCTTTGTTTCGAGCAAATTTCATTC-3'	411 bp	NM_016984

Primers for TRPC4 gene were taken from published literature (Kunert-Keil *et al.*, 2006) [33]. The cDNA was subjected to amplification with the primers at specific annealing temperature to optimize the PCR conditions. The cDNA preparations were checked for quality by performing PCR with standard GAPDH primers. The polymerase chain reaction was standardized for TRPC4 genes using cDNA from uterus using standard protocol (Sambrook and Russel,

2001) [19]. DNA amplified by PCR was subjected to agarose gel electrophoresis as described by Sambrook and Russel (2001) [19].

Results

Primers for TRPC4 gene were taken from published literature and they produced a product of 411 bp in non-pregnant as well as pregnant uterine tissues of mice (Fig.2).

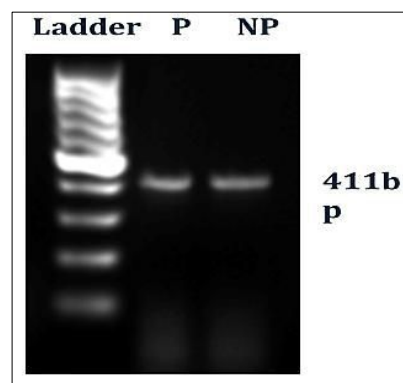


Fig 2: Agarose gel electrophoresis of RT-PCR product of TRPC4 using RNA extracted from mouse uterus (Lane 1; 100 bp DNA ladder, Lane 2; 411 bp transcript of TRPC4 from pregnant mouse uterus, Lane 3; 411 bp transcript of TRPC4 from non-pregnant mouse uterus)

Histological examination of pregnant and non-pregnant mouse uterus was done using H & E staining. The

representative images of H&E-stained uterine tissues are presented in Fig. 30 (10x). The tissue architecture was

compact in case of non-pregnant uterus. Thickness of the endometrial stroma, luminal epithelium, myometrium and glandular epithelium was increased in pregnant uterus as

compared to non-pregnant uterus. The density of stromal glands was also increased in pregnancy.

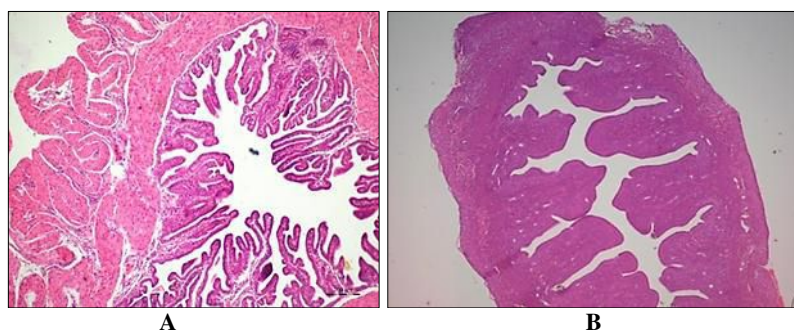


Fig 3: Histological sections of (A) pregnant and (B) non-pregnant uterine tissues of mouse (H & E stained)

Discussion

Uterine contractions occur throughout the estrous cycle in the non-pregnant state and throughout gestation in pregnancy. Contractile activity in the non-pregnant uterus appears to be fundamentally different than in the pregnant organ (Aguilar and Mitchell, 2010) [1]. Non-pregnant uterus, unlike the early pregnant one, is not quiescent (Dickinson, 1937) [8] and endometrial wave like activity has been observed in them (Gestel *et al.*, 2003) [34]. The uterotonic like oxytocin, prostaglandin, CRH, endothelin, connexin-43 etc. influence the contractile state of uterine smooth muscle cells (Fuchs, 1995) [11]. Agonist-induced increase in $[Ca^{2+}]_i$ and myometrial contraction involve a variety of Ca^{2+} entry channels. TRPC4 channels make important pathways for Ca^{2+} entry. These are non-selective cation channels, expressed in almost all cells and preferentially permeable to Ca^{2+} (Filosa *et al.*, 2013) [9].

This study showed the presence of TRPC4 channels in pregnant and non-pregnant mouse uterine tissues as revealed by the gene expression. A single band of 411 bp was obtained by RT-PCR by the same primer used by Kunert-Keil *et al.* (2006) [33], who reported expression of TRPC4 gene in mouse brain and aorta. TRPC4 mRNA has been shown to be expressed in the greatest abundance in rat (Babich *et al.*, 2004) [2] and human myometrium (Dalrymple *et al.*, 2002; Ulloa *et al.*, 2009) [6, 28] and play a major role in regulating Ca^{2+} entry in myometrial cells. Our study confirms its presence in pregnant and non-pregnant mouse uterus. TRP channels make important pathways for Ca^{2+} entry. Ulloa *et al.* (2009) [28] demonstrated a store-independent contribution of TRPC4 channels to receptor-activated Ca^{2+} entry in human myometrium. Normal reproductive activities require intimate structural and functional synchronization between uterine endometrium and myometrium. A rise in intracellular calcium mediated via stretch activated channels and release from intracellular calcium stores has been described in cardiac myocytes and vascular smooth muscle cells (McCarron *et al.*, 1997; Zou *et al.*, 2002; Calaghan *et al.*, 2003; Liao *et al.*, 2003) [16, 32, 5, 14]. Uterine stretch modulates uterine growth and contractility in pregnancy via alterations in calcium signaling (Dalrymple *et al.*). We suggest that the TRPC4 channels have major contributions in the phasic contraction of myometrium under the influence of agonists viz. oxytocin and $PGF_{2\alpha}$.

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The work has been done in the Division of Pharmacology IVRI, Bareilly, UP. The support in the form of chemicals, equipments and lab animals were also provided by the Division of Pharmacology IVRI, Bareilly, UP.

Conflict of Interest

There is no conflict of interest.

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