Special Article

Ion Channels: New Targets for the Next Generation of Tocolytics Agents

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Ion channels are interesting molecules since they mediate not only uterine contraction but also uterine relaxation. We have examined the expression, function, and correlation between the large conductance calcium-activated potassium (BKCa) channel, β2 adrenoceptor (AR), and long-lasting (L) type calcium (Ca2+) channel. These are the main channels and receptor that are involved in the uterine contraction/ relaxation process. Our evidence has shown that BKCa channel is closely correlated with β2 AR in mediating uterine relaxation. Both proteins are situated in close proximity on the plasma membrane of human myometrium and are downregulated approximately 50% after the onset of labor. Interestingly, L type Ca2+ channel, which involves in the contraction pathway, seems to be in the same compartmentation as BKCa channel/ β2 AR macromolecular complex. Further studies are now being conducted to identify the signaling complex components that could potentially be a target for new tocolytic agents.

Keywords: *Ion channel, Preterm, BKCa channel, Pregnancy, Uterus*

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Preterm birth is a major clinical problem associated with perinatal mortality and serious neonatal morbidity in both developed and developing countries[1-3]. Despite the number of research that has been performed to find the treatment for preterm labor, the incidence of preterm delivery is still rising[2,4,5]. Searching for the safe and effective tocolytic agents is frustrating. This is due to the lack of understanding about the mechanisms that initiate uterine contraction.

Uterus

The uterus and the upper part of vagina are developed from the fusion of the caudal end of the paramesonephric ducts. The uterine wall consists of three layers; serosa, myometrium and endometrium. Myometrium consists predominantly of smooth muscle cells. The uterus increases in size throughout pregnancy. Uterine growth begins after implantation involving both myometrial cells hypertrophy and hyperplasia. Myometrial cells increase from the length of 50 mm and the width of 5 mm at 12 -16 gestational weeks to the length of 500 mm and the width of 15 mm at term.

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Parturition

Labor is a physiologic mechanism when the uterus changes from quiescence state to regular contraction to deliver the baby. Parturition is a multifactorial process involving multiple interconnected positive feed forward, and negative feedback loops. The parturition mechanisms occur as a serial change of myometrium, cervix, and decidua. Once the uterus and cervix are ready, the endocrine, paracrine, and autocrine system will initiate uterine contraction. Unfortunately, we still do not know how these signaling pathways join and how to effectively stop them.

A variety of molecules has been linked to the parturition pathway but we do not know which ones trigger the whole process. We are interested in exploring the functions and roles of ion channels, particularly, potassium (K^+) channels and calcium (Ca^{2+}) channels that are the end targets of most signaling pathways and mediate uterine relaxation and contraction,

respectively.

Ion channels

Ion channels are macromolecular protein pores (Fig. 1) in the plasma membrane that allow specific ion to pass in and out the cells *e.g.*, K^+ channels allow K^+ ion to pass through, Ca^{2+} channels allow Ca^{2+} ion to pass through, etc. They play an important role in transmitted electrical signal in muscle, nerve and synapse[6]. The unequal distribution of ions across plasma membrane creates cell resting membrane potential. The major ions in the extracellular compartment are sodium (Na^+) (140 mmol/l), Ca^{2+} (1.5 mmol/l) and chloride (Cl^-) (135 mmol/l) while K^+ (150 mmol/l) levels are higher inside the cells. Direction of the current flow is determined by the concentration gradient and the membrane potential difference.

Na^+ channels have been found in rat and human myometrium[7-9]. The level of Na^+ channels in rat myometrium increases shortly before the onset of labor[8,10]. Cl^- channels increase Ca^{2+} entry through Ca^{2+} channels by mediating depolarization causing smooth muscle contraction[11,12]. The number of Ca^{2+} channels including transient (T) type and long-lasting (L) type Ca^{2+} channels are expressed in myometrium[8,10,13,14]. Evidence have shown that L type Ca^{2+} channels are progressively increased during pregnancy[8,15,16]. K^+ channels are the largest family of ion channels and are widely distributed in smooth muscle including myometrium[17-19] and are involved in mediating smooth muscle relaxation[17,20,21].

Our recent studies[18,21-24] are concentrated on K^+ channel, particularly on the large conductance calcium-activated potassium (BK_{Ca}, Maxi-K) channel, and Ca^{2+} channel, particularly on L type Ca^{2+} channel since they play major roles in mediating relaxation and contraction, respectively. The current guideline by the Royal College of Obstetricians and Gynaecologists (RCOG) also recommends the use of nifedipine, an L type Ca^{2+} channel blocker, as the first line drug for the treatment of preterm labor.

BK_{Ca} channel is a subtype of calcium-activated potassium channel family that is activated by both voltage and intracellular Ca^{2+} level. When the level of intracellular Ca^{2+} reaches 100 nmol/l, BK_{Ca} channel will shift to less voltage and more calcium regulated mode[25]. This channel consists of α and β subunits (Fig. 2). The a subunit is a pore-forming part whereas the β subunit modulates Ca^{2+} sensitivity of this channel[26-29].

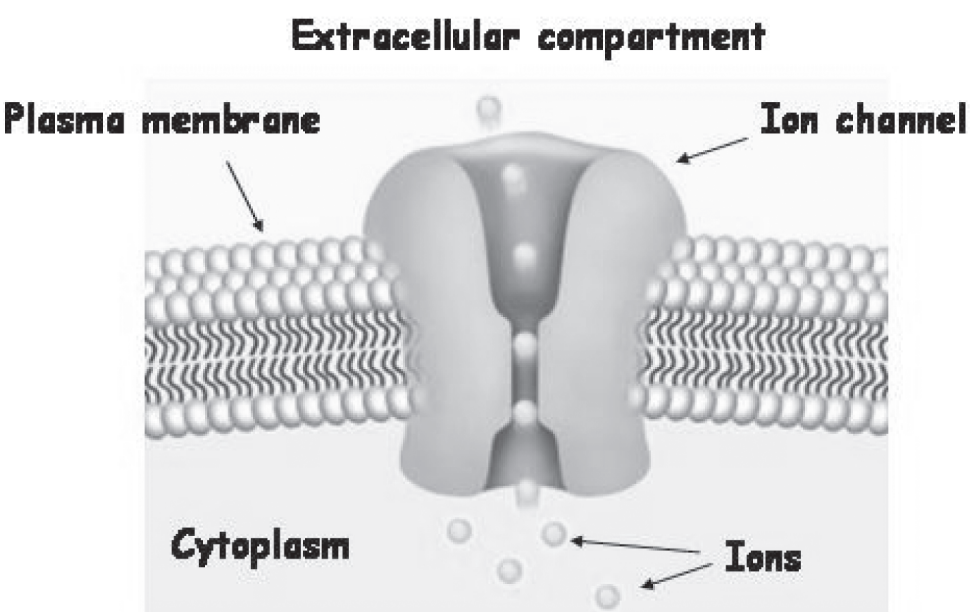

Fig. 1 Ion channel is a protein pore in the plasma membrane that allows specific ions to pass in and out the cell. (adapted from http://campus.lakeforest.edu/light/teaching.html)

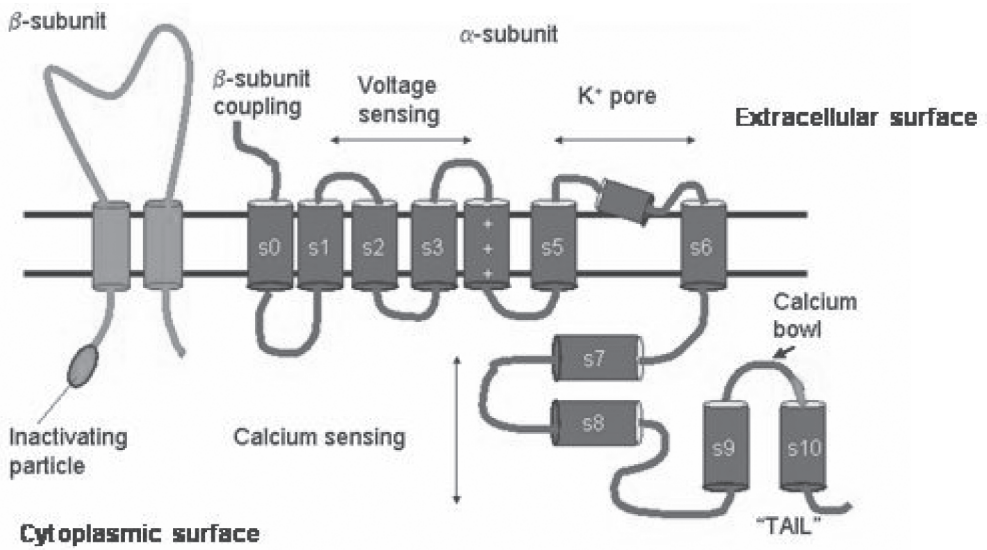

Fig. 2 Figure illustrates both α and β subunit of BK_{Ca} channel. S1 – S6 of the a subunit are homologous to voltage-dependent ion channels whereas S7 – S10 are the hydrophobic regions at the carboxy terminus. The β subunit is a modulatory protein which imparts Ca^{2+} sensitivity of BK_{Ca} channel (adapted from http://www.cf.ac.uk/biosi/staff/jacob/teaching/ionchan/Maxi-K%20channel%20(slo).gif)

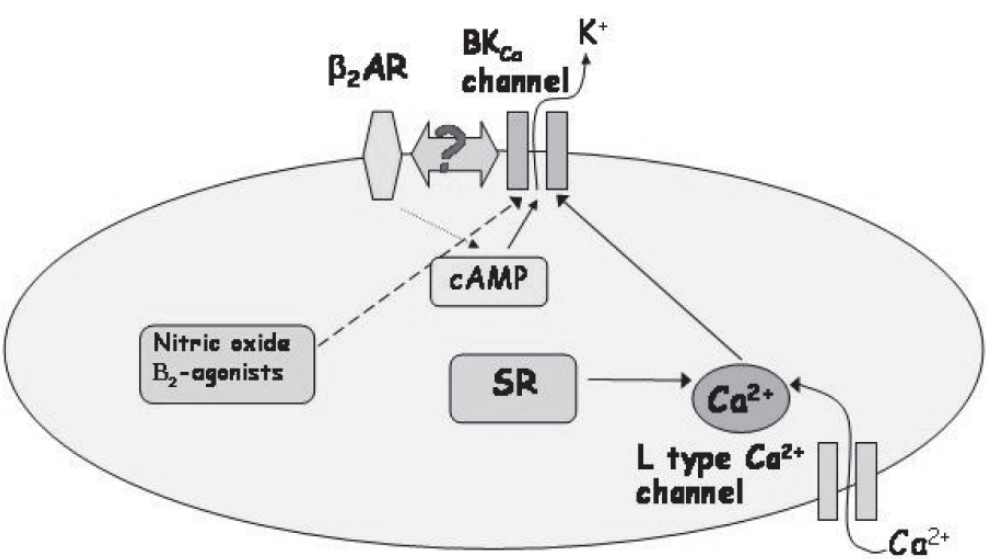

Fig. 3 Simplified scheme of the contraction/relaxation mechanisms of pregnant human myometrial cell. Ca^{2+} released from sarcoplasmic reticulum (SR) activates influx of Ca^{2+} through L type Ca^{2+} channel initiating myometrial contractions. Increased intracellular Ca^{2+} level is then activates BK_{Ca} channel opening leading to uterine relaxation

We have shown that BK_{Ca} channels are predominantly located on the plasma membrane of pregnant human myometrial cells both before and after the onset of labor[18,21]. Our results demonstrated that both α and β subunits of BK_{Ca} channel proteins are decreased in human after the onset of labor at term[18,21,24]. This may be resulted from the changing level of sex steroid hormones during pregnancy[30-32]. Downregulation of BK_{Ca} channel at term may allow contraction associated proteins (CAPs), such as oxytocin receptors, Ca^{2+} channels, to enhance myometrial contractility at the onset of labor.

β_2 adrenoceptor (AR) is the target of ritodrine, a β_2 AR agonist, which is the only drug that has been approved by the US Food and Drug Administration (FDA) for the management of preterm labor[33]. Interestingly, we found that the level of β_2 AR, which mediated uterine relaxation, is also reduced at the similar level, approximately 50%, after the onset of labor[21,34]. Our further study has shown that BK_{Ca} channel and β_2 AR are closely situated on the plasma membrane of human myometrium[22]. Immunoprecipitation study confirmed the protein-protein association between both of them whereas pharmacological and electrophysiological studies demonstrated their functional association[22,23].

This evidence suggested that BK_{Ca} channel and β_2 AR form a macromolecular complex and may be involved in the same signaling cascade which controls the uterine excitability and may have direct interaction between these two proteins (Fig. 3). Interestingly, our recent experiments suggested that L type Ca^{2+} channel, which is one of the contraction associated proteins (CAPs) is also a member of the same compartmentation with BK_{Ca} channel/ β_2 AR macromolecular complex (personal communication). Further studies are underway to examine the mechanisms that regulate BK_{Ca} channel/ β_2 AR/ L type Ca^{2+} channel macromolecular complex in pregnant human myometrial cells.

Conclusion

More effective and more selective tocolytic agents are urgently needed. The high rate of mortality, short and long term morbidity causing by preterm delivery have a significant impact not only on health care but also on education, social, and families. The consequences of preterm birth continue beyond neonatal period and have both direct and indirect impact on parents and society. Evidence has suggested that BK_{Ca} channel/ β_2 AR/ L type Ca^{2+} channel macromolecular complex in pregnant human myometrial cells may play a major role in regulating uterine (in) excitability. Further studies are needed to examine this compartmentation in more details. The component of this signaling complex including second messengers, anchoring proteins and effectors may be the potential target for the next generation of tocolytic agents. Multidiscipli-nary approach is essential for complete understanding of the mechanism of uterine contraction. Knowledge from basic sciences research needs to be translated to clinical trials in order to develop and improve clinical care for preterm labor.

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Ion channels: เป้าหมายใหม่ของยาระงับการเจ็บครรภ์ในอนาคต

บุญศรี จันทร์รัชชกูล

Ion channels เป็นโมเลกุลที่น่าสนใจเนื่องจากมันเกี่ยวข้องทั้งในกระบวนการหดรัดตัวและคลายตัวของมดลูก เราได้ศึกษาถึงปริมาณของ channels การทำงาน และความสัมพันธ์ระหว่าง large conductance calcium-activated potassium (BK_{Ca}) channel β_2 adrenoceptor (AR) และ long-lasting (L) type calcium (Ca^{2+}) channel ซึ่งเป็น channels และ receptor ที่สำคัญในกระบวนการหดรัดตัวและคลายตัวของมดลูก จากการศึกษาพบว่า BK_{Ca} channel β_2 AR อยู่บนตำแหน่งที่ใกล้เคียงกันบนเซลล์เมมเบรนของเซลล์กล้ามเนื้อมดลูกและปริมาณโปรตีนของทั้ง BK_{Ca} channel และ β_2 AR ของกล้ามเนื้อมดลูกลดลงประมาณครึ่งหนึ่งภายหลังการเจ็บครรภ์คลอด เป็นที่น่าสนใจว่า L type Ca^{2+} channel ซึ่งเป็น channel ที่อยู่ในกระบวนการหดรัดตัวของมดลูกกลับอยู่ในตำแหน่งที่ใกล้เคียงกับ BK_{Ca} channel/ β_2 AR complex ซึ่งอยู่ในกระบวนการคลายตัวของมดลูก ขณะนี้เรากำลังศึกษาถึงองค์ประกอบที่อยู่ใน signalling complex นี้ ซึ่งอาจจะเป็นเป้าหมายใหม่ของยาระงับการหดรัดตัวของมดลูกในอนาคต