Serbian Biochemical Society
Fifth Conference

Faculty of Chemistry, University of Belgrade,
13.11.2015. Belgrade, Serbia.

"Integrated research in life science"

Calcium-activated chloride channels and Na_2S-induced relaxation of non-pregnant rat uteri in estrus

Ana Mijušković, Nikola Tatalović, Aleksandra Nikolić-Kokić, Zorana Oreščanin-Dušić, Mihajlo B. Spasić i Duško Blagojević

Department of Physiology, Institute for Biological Research, University of Belgrade, Serbia,

e-mail: ana.m.mijuskovic@gmail.com

The uterus is a spontaneously active tissue, whose contractions have to be controlled and regulated for successful pregnancy and parturition. Spontaneous contractions might be initiated by spontaneous pacemaker activity, although pacemaker cells are not fully defined. Myometrial membrane potential changes are essential for uterine activity which is a function of ion movement across the membrane governed by ion channel activity [1]. Changes in membrane potential are achieved by coordinated actions of two classes of channels: those that exert hyperpolarizing current and those that exert depolarizing currents. Calcium-activated chloride channels (CaCCs) are important contributors to depolarizing currents [1,2]. However, the molecular identity of CaCCs is still not known. Several candidates have been proposed including bestrophins (BEST), CLCA and the anoctamin (ANO or TMEM) family of proteins [3].

Improper or irregular uterine activity may underlie the common pathological disorders such as infertility, improper implantation, dysmenorrhea, weak uterine contraction during labor and preterm labor. The contractile activity of the uterus is regulated by the complex electrophysiologic network which is highly sensitive to various pharmacological and signaling molecules.

Hydrogen sulphide (H_2S) appears to be an important signaling molecule in rat uterus. The production of H_2S and the presence of enzymes responsible for its endogenous production (cystathionine beta-synthase and cystathionine gamma-lyase) have been demonstrated in rat uterus [3,4]. Hydrogen sulphide reduces uterine contractility, and it is recognized as a promising treatment for uterine disorders. It decreases amplitude as well as frequency of uterine contractions. H_2S effect on the frequency of contractions appears to be mediated via pacemaker channels. CaCCs channel inhibitors were shown to reduce the frequency of spontaneous contractions in myometrial strips and were proposed to be the main pacemaker channels in smooth muscles [1]. Very little, however, is known about these channels in the myometrium.

The mechanism of the H_2S -induced relaxation in non-pregnant uteri has not been examined. Organ bath studies were employed to assess the pharmacological effects of sodium sulphide (Na_2S; hydrogen sulphide donor) in uterine strips by exposing them to Na_2S with or without Cl^- channel blockers (DIDS, NFA, T16Ainh-A01, TA). Relaxation was not affected by majority of CaCC modulators since T16Ainh-AO1, tannic acid and NFA failed to inhibit

Na_2S induced relaxation but is DIDS sensitive [3]. DIDS was recently found to be highly selective for bestrophin (BEST-1) channels [5].

BEST proteins were shown to recapitulate the properties of native CaCCs. Although both bestrophin and calcium-activated chloride channel families were proposed to be the candidate genes for smooth muscle contraction their exact function and regulation remain to be confirmed [6].

The aim of this study was to explore the expression of bestrophins channels (BEST-1 and BEST-2) in rat uterus in estrus. Expression studies of the BEST-1 and BEST-2 were performed by Western blotting, RT-PCR and immunohistochemistry. BEST-1 and BEST-2 are expresses at the mRNA level and at protein level in rat uterus in estrus, suggesting a role for BESTs in the control of uterine contractility. However, expression of BEST-1 is higher comparing to BEST-2. Moreover, BEST-1 seems to be major mediators of Na_2S induced uterine relaxation. Mechanistic insights of possible Na_2S-induced modulation od BEST-1 were performed by molecular docking studies.

Taken together, work undertaken strengthens the evidence of a physiologically important role for bestrophin channels in the normal physiology of uterine contractions. Moreover, is an important modulator of uterine contractions and bestrophins appear to be main modulator of its effects. This research add to our understanding of molecular mechanisms of H_2S effects and will be beneficial in designing future in vivo studies, and ultimately identifying new therapeutic targets to treat uterine disorders that are associated with disturbed contractility.

References

1. Jones K, Shmygol A, Kupittayanant S, Wray S (2004). Electrophysiological characterization and functional importance of calcium-activated chloride channel in rat uterine myocytes. Pflugers Arch 448: 36–43.
2. Bernstein K, Vink JY, Fu XW, Wakita H, Danielsson J, Wapner R et al. (2014). Calcium activated chloride channels anoctamin 1 and 2 promote murine uterine smooth muscle contractility. Am J Obstet Gynecol 211: 688.e1–688.e10.
3. Mijuskovic A, Orescanin-Dusic Z, Nikolic-Kokic A, Slavic M, Spasic MB, Blagojevic D (2015) Chloride channels mediate sodium sulphide-induced relaxation in rat uteri. Br J Pharmacol 172, 3671-3686.
4. Mijuskovic A, Orescanin-Dusic Z, Nikolic-Kokic A, Slavic M, Spasic MB, Spasojevic I, Blagojevic D (2014) Comparison of the effects of methanethiol and sodium sulphide on uterine contractile activity. Pharmacol Rep 66, 373-379.
5. Liu Y, Zhang H, Huang D, Qi J, Xu J, Gao H et al. (2014). Characterization of the effects of Cl-channel modulators on TMEM16A and bestrophin-1 Ca2+ activated Cl – channels. Pflugers Arch 467, 1417-1430.
6. Song J, Zhang X, Qi Z, Sun G, Chi S, Zhu Z et al. (2009). Cloning and characterization of a calcium-activated chloride channel in rat uterus. Biol Reprod 80, 788-794.

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

577.1(082)

SERBIAN Biochemical Society (Beograd). Conference(5th; 2015 ; Beograd)Integrated Research in Life Science : [proceedings] / Serbian Biochemical Society Fifth Conference, 13. 11. 2015. Belgrade, Serbia ;[edited by Mihajlo B. Spasić]. - Belgrade : Faculty of Chemistry :Serbian Biochemical Society, 2015 (Belgrade : Colorgrafx). -149 str. : ilustr. ; 23 cm

Tiraž 400. - Str. 7: Foreword / Mihajlo B. Spasić. - Bibliografija uz svaki rad.

ISBN 978-86-7220-069-0 (FOC)

а) Биохемија - Зборници

COBISS.SR-ID 218681100