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In Vitro Contractile Response of Rabbit Myometrium to BK_{Ca} and K_{ATP} Potassium Channel Openers

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Abstract

The aim of the study was to evaluate the participation of ligand-sensitive potassium large conductance calcium-activated channels (BK_{Ca}) and ATP-sensitive potassium channels in uterine smooth muscle reactivity during different stages of the experimentally induced proliferatory and secretory phase in the sexual cycle in ovariectomised rabbits *in vitro*.

The myometrial reactivity to oxytocin (10^{-6} mol·l^{-1}) was investigated by an *in vitro* method in female rabbits 14 days after ovariectomy treated with 17β-estradiol - 1 mg/kg/day i.m. for 7 days, or with a combination of progesterone 2 mg/kg/day s.c. for 7 days and 17β-estradiol - 0.2 mg/kg/day (day 3–7). The strips of myometrial smooth muscle were incubated with a specific opener (NS 1619) and an antagonist (TEA) of potassium large conductance calcium-activated channel, or with a specific opener (pinacidil) and an antagonist (glybenclamide) of ATP-sensitive potassium channels before the administration of oxytocin.

NS1619 produced more potent inhibition of the oxytocin-induced contraction during the gestagen dominance (experimental secretory phase) than the one observed during the oestrogen dominance (experimental proliferatory phase). TEA antagonized the NS1619 induced inhibition of the myometrial contraction.

In the matter of K_{ATP} potassium channels, after the administration of pinacidil we observed a similar situation in the changes of myometrial contractility. Pinacidil produced more pronounced inhibition of oxytocin-induced contraction during the secretory phase, and its effect was abolished by the selective inhibitor glybenclamide.

Our experimental results indicate that both potassium large conductance calcium-activated channels and ATP-sensitive potassium channels significantly participate in the regulation of myometrial oxytocin-induced contractions and the activity of these channels is probably influenced by the levels of oestrogens and gestagens.

Uterine contractility, pinacidil, NS1619, BK_{Ca} and K_{ATP} potassium channel, sex steroid regulation

The uterine smooth muscle, myometrium, undergoes large changes in contractility that are essential for the cyclic shedding of the endometrial lining, sperm transport, pre-implantation, positioning of embryos and also for the expulsion of a foetus during parturition. The contractility of the uterus is under the control of a variety of bioactive agents and cell membrane ion channel activity. However, the dominant factor participating in the modulation of uterine smooth muscle contraction is the steroid hormone activity during the reproductive cycle (Janíček et al. 2007). The action of oestrogen and progesterone on target cells are mediated through oestrogen (ERα, ERβ) and progesterone (PR) receptors. Both ER and PR are members of the steroid receptor superfamily that act though the steroid-modulated transcription factors (Kurita et al. 2000). The oestrogenic state of the pregnant as well as non-pregnant uterus (Richter et al. 2003) is linked to the elevation of myometrial smooth muscle reactivity: increased expression of oxytocin (OXY) receptors and a blockade of the potassium current (Knock et al. 2001). Progesterone is responsible for uterine quiescence, hence

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in relation to uterine contractility it produces the opposite activity to oestrogen by decreasing the number of OXY receptors and increasing the potassium current (Knock et al. 2001). Potassium channels are the most abundant class of ion channels. At least 16 types and many more subtypes have been identified. Activation of voltage-gated channels is regulated by the changes in the membrane potential (delayed K_r, inward K_{IR} and transient outward K_A rectifier). Ligand-sensitive channels are on the other hand activated by a number of ligands: calcium ions (large conductance BK_{Ca}, intermediate conductance IK_{Ca} and small conductance SK_{Ca} channels), ATP (K_{ATP}), neurotransmitters and G-protein (Ertel et al. 2000; Khan et al. 2001). Potassium (K^+) channels are the largest group of ion channels in a cell and represent the primary importance in the membrane potential regulation and the cell excitability in myometrium, and thus are functionally important in the regulation of the tone in the uterine smooth muscle. Pharmacological manipulation of this pathway could be helpful to prevent pre-term uterine contractions and to relieve dysmenorrhea.

The link connecting potassium large conductance calcium-activated channels and ATP-sensitive potassium channels to uterine contraction and sex hormones levels is not fully understood.

In view of this fact the aim of the study was to investigate the participation of potassium large conductance calcium-activated channels and ATP-sensitive potassium channels in uterine smooth muscle reactivity during the different stages of experimentally induced oestrous cycle after progesterone and 17-β estradiol administration in ovariectomised rabbits in the condition *in vitro*.

Materials and Methods

Drugs and chemicals

17ß-estradiol, progesterone, NS1619, tetraethylammonium (TEA), pinacidil, glybenclamide, oxytocin and other chemicals were purchased from Sigma Chemicals Co, Germany.

Experimentally induced secretory and proliferatory phase of the oestrous cycle

Mature females (approximately 9 months old) of the Hill rabbit were housed in standard temperature-controlled conditions, given food and water *ad libitum* and acclimatised for 3–4 weeks after shipment. Experimental animals were bilaterally ovariectomised 14 days before each experiment. Ovariectomy was performed in continual intravenous thiopental anaesthesia. Starting on day 14 after the surgery, in order to mimic the proliferatory and secretory phases of the sexual cycle (Fig. 1), the rabbits were treated with 17β-estradiol - 1 mg/kg/day intramuscularly in saline for 7 days (group 1; n = 12) or with a combination of progesterone 2 mg/kg/day s.c. in oil solution (7 days) and 17β-estradiol - 0.4 mg/kg/day (day 3 - 7) (group 2; n = 12). Group 3 (n = 12) represented the female rabbits after ovariectomy treated 14 days with saline.

In vitro contractile studies

Animals were killed by cervical dislocation, and the uterine tissue samples were mounted in a 20 ml tissue bath containing Krebs-Henseleit buffer of the following composition (μM): NaCl, 110.0; KCl, 4.8; $CaCl_2$, 2.35; $MgSO_4$, 1.20; $KHPO_4$, 1.20; $NaHCO_3$, 25.0; in glass-distilled water. Organ chambers were maintained at 36.5 ± 0.5 °C and were aerated continuously with a mixture of 95% O_2 and 5% CO_2 to maintain pH = 7.5 ± 0.1. Tissue strips were set to 2 g of tension for the period of 1 h, during which the tissue was washed at 15-min intervals. Those muscle strips that developed spontaneous contractions at this stage were considered not viable and were discarded.

The control represented the mean peak values of the contractile amplitudes induced by the application of oxytocin (10^{-6} mol·l^{1}) into the organ bath. The mean peak amplitude of contraction (mN) of the myometrial smooth muscle strips to oxytocin (10^{-6} mol·l^{-1}) after 10-min incubation with K_{ATP} opener pinacidil (10^{-5}mol·l^{-1}) and inhibitor of this channel glybenclamide (10^{-6}mol·l^{-1}), opener of potassium large conductance calcium-activated channels NS1619 (10^{-6}mol·l^{-1}) and its inhibitor TEA (10^{-4}mol·l^{-1}) was used as a indicator of myometrial reactivity. Contractile activity was measured isometrically using force transducer (TSR 10G, Vývoj Martin, Slovakia), amplifier (M 1101 SUPR, Mikrotechna Praha, Czech Republic).

The experimental study was approved by the Ethics Committee of Jessenius Faculty of Medicine in Martin.

Data analysis

Statistical analysis was performed using one-way ANOVA, followed by Tukey post hoc test. Observed differences were considered significant at $p < 0.05$. The results were expressed as mean ± SEM (n = 12).

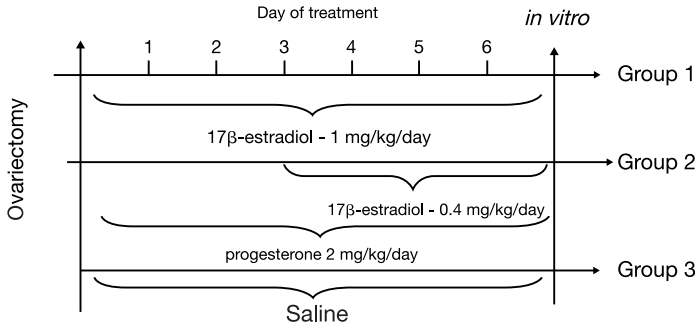

Fig. 1. Diagrammatic illustration of treatment regimens. Experimental animals bilaterally ovariectomised 14 days before each experiment. Starting on day 14 after surgery the rabbits treated with 17β-estradiol - 1 mg/kg/day intramuscularly in saline for 7 days (group 1; n = 12) or with a combination of progesterone 2 mg/kg/day s.c. in oil solution for 7 days and 17β-estradiol – 0.2 mg/kg/day (day 3–7) (group 2; n = 12). Group 3 (n = 12) female rabbits after ovariectomy treated 14 days with saline.

Results

The stripped myometrial smooth muscles from the ovariectomised rabbits treated with 17β-estradiol (group 1); or with a combination of progesterone +17β-estradiol (group 2) were incubated for 10 min with a specific opener (NS 1619) and antagonist (TEA) of potassium large conductance calcium-activated channels, or with a specific opener (pinacidil) and antagonist (glybenclamide) of ATP-sensitive potassium channels before the oxytocin-induced contraction. This approach indirectly allowed investigating the participation of the above mentioned potassium channels in the uterine smooth muscle reactivity during different stages of the experimentally-induced oestrous cycle.

NS1619 produced more potent inhibition of oxytocin-induced contraction during gestagen (experimental secretory phase) than during oestrogen dominance (experimental proliferatory phase). TEA antagonized the NS1619 induced inhibition of the myometrial contraction. In the case of female rabbits treated after ovariectomy with saline only, the reactivity of myometrial smooth muscle was not significantly affected (Fig. 2).

As for K_{ATP} potassium channels, after the administration of pinacidil and glybenclamide we observed a similar mode in the changes of myometrial contractility. However, the inhibition of oxytocin-induced contraction produced by pinacidil was more significant in the secretory phase and the effect was abolished by the selective inhibitor glybenclamide (Fig. 3).

Discussion

Nowadays, from the experimental and the therapeutic point of view, the role of potassium channels in the regulation of myometrial reactivity represents an actual problem. In our experiments we have investigated the participation of potassium large conductance calcium-activated channels and ATP-sensitive potassium channels in uterine smooth muscle reactivity during different phases of an experimentally-induced sexual cycle in ovariectomised rabbits. Our findings showed that both BK_{Ca} and K_{ATP} potassium channels play a role in the regulation of uterine smooth muscle contractility. However, the activity of the above-mentioned channels is influenced by the levels of oestrogens and gestagens during experimentally-induced proliferative and secretory phase of the sexual cycle.

One of the best characterized subclasses of calcium-activated potassium channels is the BK_{Ca} channel. The potassium large conductance calcium-activated channel is probably a predominant potassium-channel type expressed in non-pregnant (Pérez et al. 1993) and

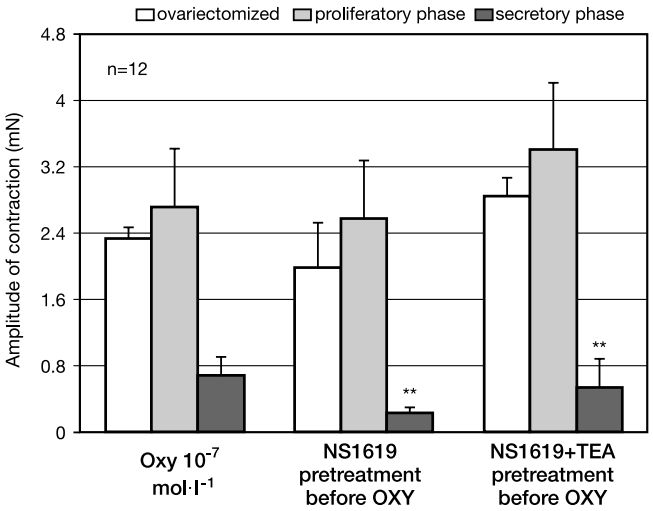

Fig. 2. Changes in the myometrial amplitude of contraction induced by oxytocin in ovariectomised rabbits treated with 17β-estradiol; with a combination of progesterone + 17β-estradiol; or with saline after 10-min incubation with a specific opener (NS 1619 - 10^{-6}mol·l^{-1}) and antagonist (TEA - 10^{-4}mol·l^{-1}) of ligand-sensitive large conductance BK_{Ca} potassium channels. Data are expressed as mean ± S.E.M.; n = 12 for each group; significant differences *$p < 0.05$ and **$p < 0.01$, ovariectomised vs. saline-treated (control) experimental groups.

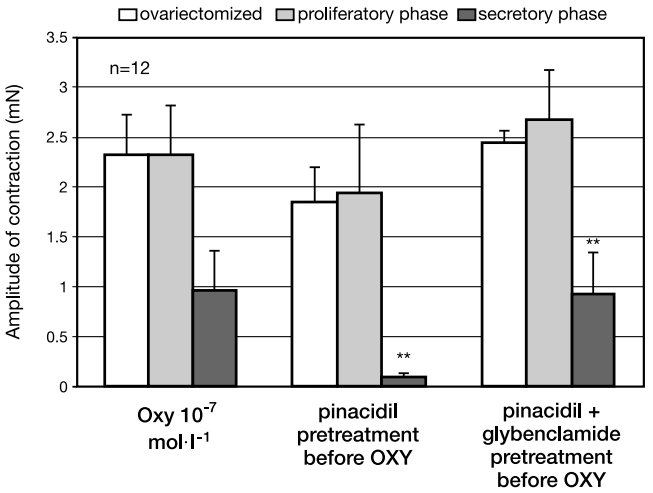

Fig. 3. Changes in the myometrial amplitude of contraction induced by oxytocin in ovariectomised rabbits treated with 17β-estradiol; with a combination of progesterone + 17β-estradiol; or with saline after 10-min incubation with a specific opener (pinacidil - 10^{-5}mol·l^{-1}) and antagonist (glybenclamide - 10^{-6}mol·l^{-1}) of K_{ATP} potassium channels. Data are expressed as mean ± S.E.M.; n = 12 for each group; significant difference *$p < 0.05$ and **$p < 0.01$, ovariectomised vs. saline-treated (control) experimental groups.

pregnant (Anwer et al. 1993) human myometrium. This channel is unique in the sense that it is activated by membrane depolarization and by an increase in the intracellular calcium levels ($[Ca^{2+}]_i$), thereby playing a pivotal role in modulating uterine activity (Matharoo-Ball et al. 2003). Our findings showed that the activity of this type of potassium channel is

strongly influenced by the levels of oestrogens and gestagens. The BK_{Ca} opener NS1619 did not have a significant effect on the amplitude of uterine contraction during the proliferative phase of the cycle. Gestagens probably increase the activity of BK_{Ca} channels during the secretory phase, hence a NS1619 pre-treatment significantly lowered the amplitude of contraction and this relaxant effect was completely abolished by TEA - the specific blocker of BK_{Ca} channels.

The role of K_{ATP} channels in the smooth muscle tissue is unclear and probably reflects the enormous diversity of functions of these channels ranging from the smooth muscle type and animal species. ATP-sensitive potassium channels are regulated by the intracellular level changes in ATP. According to some experimental results, K_{ATP} channels may be present in myometrium but at a very low density; however, possibly at present there are technical problems with the estimation of its expression and activity in the cell (Khan et al. 2001). Khan et al. (1998) presented that pinacidil-mediated relaxation of myometrial strips may be partially attributable to the opening of uterine potassium large conductance calcium-activated channels in addition to ATP-sensitive potassium channel activation. Mandi et al. (2005) showed the ability of pinacidil to inhibit spontaneous rhythmic contractions and to antagonize oxytocin-induced contraction in the pregnant goat myometrium collected at the mid-gestation. The study of Longo et al. (2003) also demonstrated that the increase in the expression and function of ATP-sensitive potassium channels may play an important role in the uterine preparation for pre-term or term labour.

Our findings confirmed the direct participation of K_{ATP} channels in the mechanism of uterine contraction induced by oxytocin. K_{ATP} opener pinacidil significantly lowered the amplitude of uterine contraction during the secretory phase of the experimental cycle, and the effect of pinacidil was abolished by the selective inhibitor of this channel glybenclamide. Gestagens probably increase the activity of K_{ATP} channels, whereas 17ß-estradiol probably inhibits the myometrial K^+ channel activity. These results are consistent with the respective contractile and proquiescence roles for 17ß-estradiol and progesterone in human uterus.

Abnormalities in uterine contractility are thought to contribute to several problems in human medicine. It is suggested that the dyscontractile phenomenon of the non-pregnant myometrium might also be mediated via 17-ß-estradiol, oxytocin and the oxytocin receptor.

The results of our experiment indicate that both potassium large conductance calcium-activated channels and ATP-sensitive potassium channels significantly participate in the regulation of non-pregnant myometrial, oxytocin induced contractility and that the activity of these channels is probably influenced by the levels of oestrogens and gestagens.

Kontrakčná odpoveď myometria králika na otvárače BK_{Ca} and K_{ATP} káliových kanálov v podmienkach *in vitro*

Cieľom práce bolo sledovanie participácie ligand-senzitívnych káliových kanálov aktivovaných kalciom typu BK_{Ca} a ATP senzitívnych káliových kanálov (K_{ATP}) v mechanizme kontrakcie hladkého svalu maternice počas experimentálne navodenej proliferačnej a sekrečnej fázy pohlavného cyklu králikov v podmienkach *in vitro*.

Reaktivita myometria k oxytocínu (10^{-6} mol·l^{-1}) bola sledovaná metódou *in vitro* u dospelých samičiek králika, ktorým bol 14 dní po ovariektómii podávaný 17β-estradiol (1 mg/kg/deň i.m.) po dobu 7 dní, alebo kombinácia progesterónu (2 mg/kg/deň s.c.) po dobu 7 dní s 17β-estradiolom (0,2 mg/kg/deň – podávaný v deň 3.-7.).

Stripy hladkej svaloviny maternice boli pred kontrakciou vyvolanou oxytocínom 10 minút inkubované so špecifickým otváračom (NS1619) a antagonistom (TEA) káliových kanálov typu BK_{Ca}, alebo špecifickým otváračom (pinacidilom) a antagonistom (glybenklamidom) K_{ATP} káliových kanálov.

Inkubácia s NS1619 vyvolala signifikantnejší pokles kontrakcie myometria indukovanej oxytocínom počas dominancie gestagénu (experimentálne navodená sekrečná fáza) ako počas dominancie estrogénu (experimentálne indukovaná proliferačná fáza). Pričom kontrakčná odpoveď myometria vyvolaná NS1619 bola antagonizovaná TEA.

V prípade sledovania aktivity K_{ATP} káliových kanálov po inkubácii s pinacidilom sme pozorovali rovnaký obraz zmien v kontrakčnej aktivite myometria. Pinacidil však signifikantnejšie inhiboval kontrakciu myometria vyvolanú oxytocínom počas sekrečnej fázy a účinok pinacidilu bol minimalizovaný selektívnym inhibítorom glybenklamidom.

Výsledky našich experimentov naznačujú, že obidva typy káliových kanálov BK_{Ca} aj K_{ATP} participujú v regulácii kontrakcie hladkej svaloviny maternice vyvolanej oxytocínom a aktivita uvedených kanálov je pravdepodobne ovplyvňovaná hladinami estrogénov a gestagénov.

Acknowledgement

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