Effects of the polyphenol resveratrol on contractility of human term pregnant myometrium


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ABSTRACT: The ideal agent for prevention and treatment of uterine abnormal contractility has not been found. The polyphenol resveratrol possesses a wide spectrum of pharmacologic properties, but its influence on the contractility of human myometrium is not defined. The present study evaluated the effect of resveratrol on the oxytocin-induced contractions of human term pregnant myometrium in vitro and the contribution of different K⁺ channels to resveratrol action. Resveratrol induced a concentration-dependent relaxation of myometrium contractions (pD₂ values and maximal responses were 4.52 and 82.25%, respectively). Glibenclamide, a selective blocker of ATP-sensitive (KATP), iberiotoxin, a selective blockers of big calcium-sensitive (BK Ca) and 4-aminopiridine, a non-selective blocker of voltage-sensitive (Kv) channels induced a significant shift to the right of the concentration–response curves of resveratrol. Inhibition achieved by 0.1 mM resveratrol was insensitive to all K⁺ channel blockers. A K⁺ channel opener, pinacidil, inhibited oxytocin-induced contractions of pregnant myometrium with comparable potency and efficacy to resveratrol (pD₂ values and maximal relaxation were 4.52 and 83.67%, respectively). Based on K⁺ channel opener/blocker affinities, it appears that the inhibitory response of resveratrol involves different myometrial K⁺ channels. When applied in high concentrations, resveratrol has an additional K⁺ channel-independent mechanism(s) of action. Furthermore, immunohistochemistry staining and western blot analyses detected the presence and distribution of KATP, BKCa and Kv channel proteins in pregnant myometrium.

Key words: contraction / K⁺- channels / myometrium / pregnancy / resveratrol

Introduction

Approximately 50% of all preterm deliveries are due to preterm labor which is the cause of death and morbidity of neonates (Di Renzo et al., 2011). It is clear that once initiated, preterm labor is difficult to arrest fully, even though transient suppression by pharmacologic means is possible. Furthermore, preterm labor is more common and serious problem in a specific group of women from in vitro fertilization (IVF) programs (Van Voorhis, 2007). Despite little evidence to support improvement in key perinatal outcomes with these drugs, the β2-adrenoceptor agonists remain the most popular option for the suppression of preterm myometrial contractions.

Previous data have shown that the K⁺ channel family affects cell function and plays a major role in regulation of myometrial contractility (Khan et al., 2001; Brainard et al., 2007). Potassium efflux from myometrial smooth muscle cells results in membrane repolarization and this is responsible for maintaining the resting membrane potential. Changes in the expression or activity of K⁺ channels can translate into an inadequate repolarization leading to aberrant uterine activity. Thus, K⁺ channels alterations may contribute to certain pathophysiological conditions such as preterm labor. A number of studies have shown that opening of different types of K⁺ channel leads to relaxation of non-pregnant as well as pregnant myometrium (Morrison et al., 1993; Khan et al., 1998; Longo et al., 2003; Novakovic et al., 2007). It seems that function and molecular expression of K⁺ channels are dependent on stage of pregnancy, the mother’s age and hormones influences (Song et al., 1999; Knock et al., 2001; Lovasz et al., 2011; Du et al., 2013). For instance, it has been shown that activity of ATP-sensitive K⁺ (KATP) and big Ca²⁺-sensitive K⁺ (BKCa) channels are diminished in late pregnancy (Matharoo-Ball et al., 2003; Lovasz et al., 2011). Holdiman et al. (2002)
suggest that alternative splicing of the BK<sub>Ca</sub> channel transcript is regulated by 17β-estradiol and may allow for differential expression of channel isoforms, leading to altered modulation without a change in channel density. Recently, it has been demonstrated that K<sub>ATP</sub> channels are up-regulated with increasing age of the human myometrium. The myometrium of parturient over 35 years old possess more K<sub>ATP</sub> channels than the myometrium of younger parturient (Du et al., 2013). It is well known that K<sup>+</sup> channel family includes more than 100 various protein subunits (Coetzee et al., 1999). The identification of subunits/proteins of K<sup>+</sup> channels in myometrial smooth muscle cell is important for understanding preterm labor.

Our knowledge regarding the mechanisms that underlie normal physiologic labor has advanced considerably, but the discovery of new drugs that lack the side effects of commonly used tocolytics is not satisfactory. Within the last 10 years, resveratrol is found under spotlight like ‘one molecule—many targets’ (Pirola and Frojdo, 2008). Several potential therapeutic applications of resveratrol have come to light (Pirola and Fröjdö, 2008; Li et al., 2012). It has been shown that resveratrol promotes smooth muscle relaxation in non-pregnant rat uterus (Hsia et al., 2011; Novakovic et al., 2013) as well blood vessels (Novakovic et al., 2006a, b; Gojkovic-Bukarica et al., 2008; Protic et al., 2013) and gubladder (Wang et al., 2008). Moreover, K<sup>+</sup> channels have been implicated directly or indirectly in the action of resveratrol (Novakovic et al., 2006a, b; Novakovic et al., 2013; Gojkovic-Bukarica et al., 2008; Protic et al., 2013). The relaxant action of resveratrol in non-pregnant rat uterus results in reduced intracellular Ca<sup>2+</sup> levels and inhibition of different types of contractions: spontaneous rhythmic contractions and contractions induced by oxytocin, prostaglandins, high K<sup>+</sup> concentration and Ca<sup>2+</sup> channel activator (Bay K 8644) (Hsia et al., 2011; Novakovic et al., 2013). Reservatol supplements widely present on the market have been proposed as a potential therapeutic to improve metabolic health (considering their possible cardioprotective, neuroprotective, anti-diabetic effects); however, little is known about effects on the contractility of pregnant myometrium.

Thus, the aims of the present study were to elucidate whether resveratrol is able to modulate contractility of pregnant human term myometrium and to investigate a role of K<sup>+</sup> channels in the action of resveratrol. The expression and distribution of the different K<sup>+</sup> channels subunits were examined by immunohistochemistry staining and by western blot.

**Materials and Methods**

**Tissue collection and preparation**

Patients were recruited in the Institute of Gynecology and Obstetrics, Clinical Center of Serbia. The study was approved by the Ethics Committee of Faculty of Medicine, University Belgrade (No 4211/2), and recruitment was carried out by the provision of information sheets and by obtaining written informed consent. The women were from IVF programs. In vitro fertilization program and care during pregnancy have been carried in accordance with the recommendations of the ‘Fertility: Assessment and Treatment for People with Fertility Problems’ (NICE clinical guideline 11, 2004).

Myometrial samples were obtained from 42 non-laboring women undergoing elective Cesarean section in the third trimester of pregnancy. The maternal demographic details were as follows: mean age 35.46 years (range 27–43); median period of gestation 39 weeks (range 37–40). No tocolytic agents have been administered to the mother 24 h prior to Caesarean delivery. The reasons for Cesarean section included breech presentation, previous Cesarean section and cephalopelvic disproportion. Biopsies were excised from the midline portion of the upper lip of the incision in the lower uterine segment. Tissue samples for isometric recordings were placed in fresh Krebs–Ringer solution and tissue was stored at 4°C and used within 12 h of collection. Additional tissue samples were rinsed with Krebs–Ringer solution and immediately frozen in liquid nitrogen at 80°C or placed in formalin.

**Tissue bath experiments**

Longitudinal myometrial strips were dissected, measuring 2 × 2 × 10 mm, and mounted under 2 g of tension in organ tissue baths (TSZ-04/1.2, Experimentia, Budapest, Hungary) for isometric recording as described previously (Longo et al., 2003). Data were recorded via computer using IsoLAB software (Elunet, Belgrade, Serbia). The tissue baths contained 10 ml of Krebs–Ringer solution, which was maintained at 37°C and at a pH of 7.4 and gassed continuously with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub> (Crankshaw, 2001). The strips were allowed to equilibrate in Krebs–Ringer solution for at least 1 h and solution was changed every 15 min during the equilibration period. Following equilibration, contractions were stimulated by the addition of the uterotonin agent oxytocin to achieve a tissue bath concentration of 20 nM for a period of 30 min.

Concentration–response curves were constructed by adding resveratrol (1–100 μM) or the K<sup>+</sup> channel opener (KCO) pinacidil (0.01–100 μM) directly to the bathing solution in a cumulative way, taking the amplitude of the response immediately before addition of a drug as the control contraction. The results are expressed as the percentage inhibition of the control contraction. Increasing concentrations of resveratrol or pinacidil were added only after the previous concentration had produced an equilibrium response or after 20 min if no response was obtained.

To test the involvement of K<sup>+</sup> channels in a mechanism of action of resveratrol, the different K<sup>+</sup> channel blockers were examined. In separate experiments, glibenclamide, ibehotoxin or 4-aminopyridine (4-AP) were added to the bathing solution ~20 min before exposure to resveratrol. The concentration–response curves to resveratrol were obtained in the presence of K<sup>+</sup> channel blockers. Vehicle-matched control experiments were conducted.

**Drugs and solutions**

All chemicals were obtained from Sigma-Aldrich Inc., St Louis, MO, USA. Resveratrol was dissolved in 70% v/v ethanol with further dilution in distilled water before use. Working concentrations of ethanol in the bath were <0.01% (v/v). Glibenclamide was dissolved in polyethylene glycol. Stock solution of pinacidil was dissolved in dilute acid solution (0.1 N HCl) with further dilution in water before use. Iberiotoxin, 4-AP and oxytocin were dissolved in distilled water. The Krebs–Ringer solution had the following composition (mmol/l): NaCl 120, KCl 5, CaCl<sub>2</sub> 2.5, MgSO<sub>4</sub> 1.2, NaHCO<sub>3</sub> 25, KH<sub>2</sub>PO<sub>4</sub> 1.2, glucose 11. All drugs were added directly to the bath in a volume of 50 μl and the concentrations given are the calculated final concentrations in the bath solution.

**Immunohistochemistry staining**

Thick paraffin sections (5 μm) from the formalin-fixed, paraffin-embedded tissue samples of human myometrium were deparaffinized and dehydrated. For antigen retrieval, citrate buffer (pH 6.0, 20 min in microwave) was used. Primary polyclonal rabbit antibodies Anti-Kir6.1, Anti-Kir6.2, Anti-Kv2.1, Anti-Kv4.2 and Anti-K<sub>Ca</sub>1.1 (all diluted 1:50, Alomone Labs, Jerusalem, Israel) were incubated for 1 h at room temperature. Sections were then treated with EnVision<sup>TM</sup> Detection System (Dako, Germany) using 3-amino-9-ethylcarbazole (AEC) as substrate and counterstained with hematoxalin. Negative controls were performed by omitting the primary.
Western blot analysis

The tissue samples of human myometrium were homogenized in RIPA (Radio-Immunoprecipitation Assay) buffer (1 M TRIS—HCl, pH 7.5, 0.5 M EDTA, 10% SDS, 10% sodium deoxycholate, 10% Triton X-100) with protease inhibitors (Roche Applied Science, Mannheim, Germany). Cell lysate was centrifuged for 20 min, 10 000g at 4°C and the supernatant was transferred to a new tube. Protein concentration was measured at 595 nm with spectrophotometer using the Bio-Rad Protein Assay, based on the method of Bradford. Prior to sodium dodecyl sulfate—polyacrylamide gel electrophoresis, LDS (lithium dodecyl sulfate, pH 8.4) Sample Buffer and Reducing Agent were added to the sample, denatured for 10 min at 70°C and loaded onto a precast 4–12% Bis-Tris gel (Life Technologies, Carlsbad, CA, USA).

After electrophoresis, separated proteins were transferred to a nitrocellulose membrane. A membrane was blocked with 5% non-fat dry milk and 0.1% Tween 20 for 1 h at room temperature and probed with primary antibody for Kir6.1 and Kir6.2 subunit of KATP channels, α-subunits Kv2.1 and Kv4.2 subtype of Kv channels and KCa1.1 subunit of BK Ca channels (Anti-Kv4.2 subtype of Kv channels and K Ca1.1 subunit of BK Ca channels (Anti-Kir6.1 and Anti-Kir6.2) subunit of KATP channels, α-subunits Kv2.1 and Kv4.2 subtype of Kv channels and KCa1.1 subunit of BK Ca channels (Anti-Kir6.1 and Anti-Kir6.2; Anti-Kv2.1; Anti-Kv4.2; Anti-KCa1.1; Alomone Labs, Jerusalem, Israel) overnight at 4°C. The membranes were incubated separately with different antibodies. After visualization, the membranes were stripped with 0.2 M NaOH, blocked and reprobed with another antibody. Membranes were probed with horse-radish peroxidase-conjugated antirabbit secondary antibody for 1.5 h at room temperature. Western blotting kit (Roche, Applied Science, Mannheim, Germany) was used for the chemiluminescent detections of proteins. After visualization, the membranes were stripped with 0.2 M NaOH, blocked and reprobed with anti-β-actin antibody (Abcam, Cambridge, UK) and visualized. Protein loading was normalized to β-actin (Beleslin-Cokic et al., 2011). ImageQuant software was used for analysis.

Treatment of data and statistics

The amplitude of the contractions was measured from the baseline to the top of the spike. The mean amplitude during the control period was taken as 100%.

EC50 value is defined as the concentration of resveratrol or pinacidil required to produce 50% of the maximum response of contractions, and it was determined for each curve by using a non-linear least square fitting procedure of the individual experimental data, and presented as pD2 (pD2 = −log EC50). The results were tested for normality and data are expressed as the mean ± SEM; n refers to the number of experiments. Statistically significant differences between means were determined by Student’s t-test; a value of P < 0.05 was considered statistically significant. Data analysis was performed in GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA).

Results

The effects of resveratrol and pinacidil on contractions provoked by oxytocin

Application of nanomolar concentration of oxytocin (20 nM final concentration in the bath solution) produced phasic contractions of constant amplitude (4.95 ± 0.08 g) and frequency (2.65 ± 0.35 contractions per 10 min), as shown in Fig. 1 (n = 12). Resveratrol (1–100 μM) significantly inhibited amplitude of oxytocin-induced contractions in a concentration-dependent manner with pD2 value of 4.52 ± 0.11, maximal responses: 82.25 ± 1.50%, n = 12 (Fig. 1A).

Glibenclamide (10 μM, circle) produced a significant rightward shift of the concentration–response curve of resveratrol (pD2 values: 4.52 ± 0.11 in the absence versus 4.12 ± 0.18 in the presence of glibenclamide, P < 0.05, n = 8) and suppression of the maximal response (82.25 ± 1.50% in the absence versus 50.79 ± 2.95% in the presence of glibenclamide, P < 0.05, n = 8, Figs 1B and 2A).

Iberiotoxin (100 nM) produced a significant rightward shift of the concentration–response curve of resveratrol (pD2 values: 4.52 ± 0.11 in the absence versus 4.05 ± 0.54 in the presence of iberiotoxin, P < 0.05, n = 8) and suppression of the maximal response (82.25 ± 1.50% in the absence versus 54.56 ± 3.87% in the presence of iberiotoxin P < 0.05, n = 8, Figs 1C and 2B).

Application of 4-AP (1 mM) produced a significant rightward shift of the concentration–response curve of resveratrol (pD2 values: 4.52 ± 0.11 in the absence versus 4.02 ± 0.38 in the presence of 4-AP, P < 0.05, n = 8) with a significant suppression of the maximal response (82.25 ± 1.50% in the absence versus 50.79 ± 2.95% in the presence of 4-AP, P < 0.05, n = 8, Figs 1D and 2C).
In order to compare the effect of resveratrol with the effect of KCO, pinacidil was used \((n = 8)\). Pinacidil \((10 \text{nM} - 100 \text{µM})\) inhibited contractions induced by oxytocin in the concentration-dependent manner with \(pD_2\) value of \(4.52 \pm 0.45\) and maximal responses of \(83.67 \pm 2.23\%\). There are no statistically significant differences between \(pD_2\) values and maximal responses of resveratrol and pinacidil \((4.52 \pm 0.11\) versus \(4.52 \pm 0.45; 82.25 \pm 1.50\) versus \(83.67 \pm 2.23\%\), respectively).

Detection of \(K_{\text{ATP}}, \text{Kv and BK}_{\text{Ca}}\) channels subunit by immunohistochemistry and western blot analyses

Immunohistochemical staining revealed expression of different \(K^+\) channels in human pregnant myometrium. In all analyzed tissues, Kir6.1 (Fig. 3A) and Kir6.2 (Fig. 3C) subunits of \(K_{\text{ATP}}\) channel, \(K_{\text{Ca}1.1}\) (Fig. 3E) subunit of \(\text{BK}_{\text{Ca}}\) channel and \(\alpha\)-subunit Kv4.2 (Fig. 3G) subtype of Kv channel were detected. Human pregnant myometrium clearly showed expression of Kir6.1, Kir6.2, \(\text{KCa}1.1\) and Kv4.2 subunits on smooth muscle cell (Fig. 3). The expression of all four channels on smooth muscle cells was mainly cytoplasmic but with a diffuse extension pattern. The distribution of the Kir6.2 was the most widespread since it was present on all smooth muscle cells (Fig. 3C). However, all other channels were detected on single smooth muscle cells such as Kir6.1 (Fig. 3A), or on groups of smooth muscle cells, such as \(\text{KCa}1.1\) and \(\alpha\)-subunit of Kv4.2 channels (Fig. 3E and G). In addition to diffuse cytoplasmic expression, channels Kir6.1, Kir6.2 and Kv4.2 revealed strong granular perinuclear localization.

Western blotting analysis detected Kir6.1 and Kir6.2 subunits of \(K_{\text{ATP}}\) channels, the \(K_{\text{Ca}1.1}\) subunit of \(\text{BK}_{\text{Ca}}\) channels and \(\alpha\)-subunit Kv4.2 subtype of Kv channels in human pregnant myometrium \((n = 5, each, Fig. 4)\). Results were normalized to \(\beta\)-actin expression. Immunohistochemical staining and western blotting analyses did not detect expression of Kv2.1 in all analyzed samples.

Discussion

This is the first report showing the inhibitory effects of resveratrol on contractility of human term pregnant myometrium. Resveratrol significantly decreased the amplitude of phasic contractions induced by oxytocin. This sensitivity was in line with sensitivity of oxytocin-induced contractions of non-pregnant rat uterus as well as uterine arteries (Naderali et al., 2000; Novakovic et al., 2013). There are differences between the \(pD_2\) value of resveratrol obtained here and the \(pD_2\) values in the investigations on the different types of human and rat blood vessels (Novakovic et al., 2006b; Nagaoka et al., 2007; Gojkovic-Bukarica et al., 2008) and the guinea pig gallbladder smooth muscles (Wang et al., 2008). This discrepancy in the results obtained in the various experimental models suggests tissue and species selectivity of resveratrol.

In order to analyze the contribution of \(K^+\) channels to the inhibition of myometrial contractions produced by resveratrol, we used agents that are known to possess a \(K^+\) channel-blocking/opening activity.

In the present study, glibenclamide, a highly specific \(K_{\text{ATP}}\) channel-blocker, partly antagonized the effect of resveratrol on the oxytocin-induced contractions, indicating the involvement of \(K_{\text{ATP}}\) channels in resveratrol mechanism of action. These data are in agreement with results obtained on the rat non-pregnant myometrium and heart, where resveratrol depressed contraction due to activation of \(K_{\text{ATP}}\) channels (Buluc et al., 2007; Novakovic et al., 2013). Structurally, \(K_{\text{ATP}}\) channels are composed of pore-forming inward rectifiers channels, Kir6.1 or Kir6.2, and regulatory subunits, SUR1, SUR2A or SUR2B that assemble to form an octameric complex, and the presence of all five subunits was confirmed in human pregnant myometrium (Du et al., 2013). Previous papers have shown two combinations of \(K_{\text{ATP}}\) channels subunits, namely Kir6.1/SUR2B and Kir6.2/SUR1, in the smooth muscle of non-pregnant rat and human uterus at the transcription level (Chien et al., 1999; Curley et al., 2002). Also, the immunohistochemical study presented here has confirmed existence of both Kir6.1 and Kir6.2 subunits in the pregnant human myometrium. These findings are in line with the research of Xu et al. (2011). The \(K_{\text{ATP}}\) channels composed of Kir6.1 and SUR2B subunits are expressed at higher levels in human non-pregnant myometrium compared with late gestation (Curley et al., 2002). It is possible that resveratrol may produce relaxation of pregnant myometrium, in part, by activation of Kir6.2/SUR1 channels.

According to the results with iberiotoxin, a highly specific \(\text{BK}_{\text{Ca}}\) channel blocker, and resveratrol, it is reasonable to conclude that \(\text{BK}_{\text{Ca}}\) channels are involved in the mechanism of action of resveratrol on human pregnant myometrium. Similarly, iberiotoxin antagonized the response to resveratrol on the spontaneous rhythmic contractions and phasic contractions induced by oxytocin, but did not antagonize...
the effect of resveratrol on the tonic contractions induced by oxytocin in non-pregnant rat myometrium (Novakovic et al., 2013). In the pancreatic β cells, Chen et al. (2007) have suggested that resveratrol could activate BKCa channels through an increase in intracellular Ca²⁺. Similarly, it has been shown in the portal vein that BKCa channels partly mediate the inhibitory effect of resveratrol by affecting the smooth muscle Ca²⁺.

Figure 3 Detection of four different K⁺ channels on the human term pregnant myometrium by immunohistochemistry staining. Expression of Kir6.1 (brown staining) on single smooth muscle cells (A); diffuse expression of Kir6.2 (brown staining) on smooth muscle cells (C); expression of KCa1.1 (brown staining) on smooth muscle cells (E); expression of Kv4.2 (brown staining) on smooth muscle cells (G). In all photos, nuclei were stained with hematoxylin (blue color). Negative controls (B, D, F and H). Original magnification 400× (A–H).
channels and/or Ca^{2+} mobilizing through cells (Protic et al., 2013). It is possible that resveratrol modulates intracellular Ca^{2+} homeostasis and in turn indirectly regulates potassium efflux. Our data suggest the involvement of BK_{Ca} channels in the relaxant effect of resveratrol, but according to relative resistance of this effect to iberiotoxin, it seems that resveratrol exerts inhibition of the pregnant myometrium by acting on additional sites. The results of experiments from immunohistochemistry and western blotting analysis showed the presence of BK_{Ca} channels. Despite being encoded by a single gene, BK_{Ca} channel diversity is high. The expression of α-subunits is highly tissue-specific. The presence of α-subunits was confirmed at transcript and protein level in non-pregnant rat as well as non-pregnant and pregnant human myometrium (Chanrachakul et al., 2003; Curley et al., 2004; Novakovic et al., 2013) and results present here correspond to the these findings.

The Kv subtype of K^{+} channels is a large group with a remarkably diversity of channels that are activated by voltage. The data presented here showed that 4-AP antagonized the effect of resveratrol on the oxytocin-induced contractions of pregnant myometrium. Previous studies have confirmed that Kv channels participate in the resveratrol effects in the different blood vessels and phasic contractions of non-pregnant rat myometrium (Novakovic et al., 2006a, b; Novakovic et al., 2013; Gojkovic-Bukarica et al., 2008). However, the relaxant effects of resveratrol on the tonic oxytocin-induced contractions of non-pregnant rat myometrium and tonic contraction of the retinal arteries were not blocked by 4-AP (Nagaoka et al., 2007; Novakovic et al., 2013). In contrast, it has been shown that resveratrol inhibits Kv channels in the pancreatic β cells (Chen et al., 2007). Based on electrophysiological studies, three separate Kv currents have been identified in myometrial cells: 4-AP insensitive, 4-AP sensitive rapidly inactivating current and 4-AP sensitive slowly inactivating current (Smith et al., 2007). These various Kv current components have different gating properties and sensitivities to drugs. Until recently, only evidence from animal models for the Kv4.x pore-forming subunit of Kv channels has been provided (Suzuki and Takimoto, 2005). The findings of molecular expression of Kv4.x channels in human myometrium were not established yet. A few studies on mouse and rat uterus have shown that Kv4.3 subtype expression is reduced during pregnancy (Suzuki and Takimoto, 2005; Smith et al., 2007). However, the fact that both immunohistochemistry staining and western blot confirmed the presence of α-subunit of Kv4.2 channels in human pregnant myometrium suggests that this type of channel was not down-regulated during pregnancy. This assumption is in line with the research on term-pregnant mice (Smith et al., 2007). Taken together, these findings suggest a possible role for Kv4.2 channels in the action of resveratrol.

In order to compare the pharmacologic profile of resveratrol to KCOs, we tested the effects of pinacidil in the same experimental model. Under the experimental conditions described here, pinacidil produced inhibition of oxytocin-induced contractions with the same pD2 value as resveratrol.

It can be concluded that resveratrol exerts a potent inhibitory effect on human pregnant myometrial contractility. Based on K^{+} channel blocker/opener affinity, it appears that resveratrol exerts its effect on induced contractions by modulating K^{+} channel, but it has an additional K^{+}-channels-independent mechanism(s) of action and further studies are needed to identify the intracellular targets of resveratrol in human myometrial cells. The present study demonstrated the presence of K_{ATP}, Kv and BK_{Ca} channel proteins in myometrial cells of the human term pregnant uterus.

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Authors’ roles

R.N. designed the research, carried out the pharmacologically experiments, analyzed data, wrote the paper, coordinated the study; N.R.: conducting Cesarean section, provided clinical data and the samples of myometrium; J.M.-L.: interpretation of IHH data; S.C.: carried out the WB experiments and analyzed data; B.I., V.Z. and H.H.: interpretation of data, contributed to discussion; L.G.-B. designed the research, analyzed the data, wrote the paper and coordinated the study.

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Conflict of interest

None declared.

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