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Progesterone suppressed vasoconstriction in human umbilical vein via reducing calcium entry

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ABSTRACT

The aim of this study was to evaluate the actions of progesterone on human umbilical vein (HUV) from normal pregnancies and the possible underlying mechanisms involved. HUV rings were suspended in organ baths and exposed to progesterone followed by phenylephrine (PE) or serotonin (5-HT). Progesterone suppressed PE- or 5-HT-induced vasoconstriction in HUV rings. The inhibitory effect induced by progesterone was not influenced by nitric oxide syntheses inhibitor, prostaglandins syntheses blocker, the integrity of endothelium, selective progesterone receptor or potassium channel antagonists. Further testing showed that progesterone and nifedipine (a blocker for L-type calcium channels) produced similar inhibitory effects on PE-, 5-HT-, Bay-k8644-, KCl-induced vasoconstriction in Krebs solution as well as $CaCl_2$-induced vasoconstriction in Ca^{2+}-free Krebs solution. But the inhibitory effect of mibefradil (mibe, a blocker for L-type ($CaV_{1.2}$) and T-type calcium channels ($CaV_{3.2}$)) on PE-, 5-HT-induced vasoconstriction was significantly greater than progesterone or nifedipine in Krebs solution. Furthermore, progesterone did not affect the vasoconstriction caused by PE, 5-HT, or caffeine in Ca^{2+}-free Krebs solution. In addition, incubation HUV with progesterone did not change $CaV_{1.2}$ and progesterone receptor (PR) expressions. The results gained demonstrated that progesterone could suppress multiple agonist-induced vasoconstrictions in HUV, mainly due to a reduction of calcium entry through L-type calcium channels, not endothelium-dependent vascular relaxation pathways, potassium channels, or Ca^{2+} release from intracellular stores, providing new information important to further understanding the contribution of progesterone in the regulation of the placental-fetal circulation.

1. Introduction

Progesterone, a natural steroid hormone, produced by the corpus luteum in ovaries, increases by as much as 100-fold during normal pregnancy in Human mainly due to placental secretion [1]. In recent years, a number of studies demonstrated vasorelaxation effects of progesterone in vasculatures [2–5]. To date, although the mechanisms on vasorelaxation effects of progesterone were not fully understood, it has been suggested that progesterone-induced vasorelaxation in vasculatures were associated with endothelium-dependent vascular relaxation, activated potassium channels, and blocked calcium channels [3,6,7].

Progesterone receptors (PR) have been identified in vasculatures [8]. Progesterone receptors, especially B isoform, showed a direct role in the regulation of gene transcription and proliferation for vascular smooth muscle cells [9]. However, whether PR participates in progesterone-induced endothelium-dependent vascular relaxation is still unclear. In adult models, progesterone induced endothelium-dependent vascular relaxation, mainly mediated by the production of endothelial prostacyclin (PGI_2) and nitric oxide (NO) [2,5,10,11]. The vasorelaxation actions of progesterone were found to be independent on the synthesis of PGI_2 and NO, and removal of endothelium did not prevent relaxation in other studies on adult models [4,12]. These different effects of progesterone on PGI_2 and NO production and endothelium may be caused by animal species, dose of progesterone, sizes of the vessels used, and experimental settings for investigation. Notably, there has been very limited information regarding effects of progesterone on umbilical cord blood vessels, while the umbilical cord is only pathway between the placenta and fetus for blood flow, oxygen, and nutrients. Any factor that can influence diameters of blood vessels in the umbilical cord would affect fetal development *in utero*. Therefore, the present study focused on the effects and possible mechanisms of progesterone on human umbilical vein (HUV).

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Ion channels such as potassium and calcium channels play important roles in vascular contraction. Progesterone was believed to suppress the contraction of the portal vein in rats by activation of potassium channels, and this response could be antagonized by potassium channel blockers [7,13]. Vascular smooth muscle contraction has largely been explained by increased intracellular Ca^{2+} via voltage-gated calcium channels, and both L-type calcium channels ($CaV_{1.2}$) and T-type calcium channels ($CaV_{3.2}$) are two important voltage-gated calcium channels [14,15]. Progesterone decreased Ca^{2+} influx and intracellular Ca^{2+} in vascular smooth muscle in adult blood vessels had been reported [3,12,16]. Because of the importance of the potassium and calcium channels in the control of vascular smooth muscle contraction, the possible link between those channels and progesterone in HUV was investigated in this study.

Although many potent vasoconstrictors are existed in the placental-fetal circulation, human umbilical blood vessels still keep a low resistance system in providing nutrition and oxygen for the growing fetus [17–19]. In general, the tension of human umbilical blood vessels is mainly controlled by autacoids substance and hormones, as the cord is deficient in autonomic nerve [20]. Progesterone, as one of important hormones during pregnancy, its effects and underlying mechanisms on human umbilical-placental blood vessels are still unclear, although numerous studies demonstrated direct relaxation effects of progesterone in other vasculatures [5,21,22]. Therefore, it is necessary to determine actions of progesterone on human umbilical vein as well as the possible underlying mechanisms involved.

2. Materials and methods

2.1. Preparation of HUV rings

All procedures used in this study were approved by the Institute's Ethics Committee of First Hospital of Soochow University. All participants in this study were given written informed consent. Human umbilical cords were obtained from healthy single pregnancy women ($N = 106$, 37–41 weeks) within 30 min after vaginal delivery in local hospitals. The middle parts of umbilical cords (10–20 cm in length) kept at 4 °C in Krebs solution containing: NaCl (119 mmol/L), $NaHCO_3$ (25 mmol/L), glucose (11 mmol/L), KCl (4.7 mmol/L), KH_2PO_4 (1.2 mmol/L), $MgSO_4$ (1.0 mmol/L), and $CaCl_2$ (2.5 mmol/L). The HUV were carefully isolated and cut into 4–5 mm long. And then the rings ($n = 737$) were suspended in an organ bath with 5 ml Krebs solution. Tissue baths were maintained at 37 °C, pH 7.35–7.45, and gassed continuously with a mixture of 95% O_2 and 5% CO_2. In some HUV rings, the endothelium was removed by gentle rubbing by a wooden probe with cotton. In the Ca^{2+}-free Krebs solution, $CaCl_2$ was omitted and EGTA (2 mmol/L) was added.

2.2. Isometric tension testing

HUV rings were suspended horizontally between two parallel stainless steelwires and fixed them to the bottom of the chamber for recording isometric tension. The rings were given 2 g of initial tension and allowed to equilibrate for 2 h. KCl (100 mmol/L) was used to achieve maximal tension (100%). And the contraction induced by the drugs was normalized by comparing to maximal tension elicited by KCl. PE (10^{-10}–10^{-4}mol/L) or 5-HT (10^{-10}–$10^{-4.5}$mol/L) was cumulatively added into the organ bath to obtain dose-response curves after adding progesterone (0.1 μmol/L, 1 μmol/L, 10 μmol/L, 100 μmol/L) or equivalent vehicles separately to different tissues for 60 min. There was at least a 3-min interval between successive doses of PE or 5-HT, during which time the plateau phase of the response from the preceding concentration was reached. In subsequent experiments, HUV rings were pretreated with indomethacin (10 μmol/L), N_ω-nitro-L-arginine (L-NMMA, 10 μmol/L), RU486 (10 μmol/L), tetraethylammonium (TEA, 10 μmol/L) for 30 min or removal of endothelium, then adding progesterone (10 μmol/L) or equivalent vehicles for 60 min, then the concentration-response curves to PE or 5-HT were recorded. To evaluate effects of progesterone on Ca^{2+} entry, the concentration-response curves to PE, 5-HT, Bay-K8644 (10^{-9}–10^{-5}mol/L), KCl (20–140 mmol/L) in Krebs solution, or $CaCl_2$ (10^{-5}–$3 * 10^{-2}$mol/L) in Ca^{2+}-free Krebs solution were obtained in the presence or absence of progesterone or (and) nifedipine (10 μmol/L), or mibefradil (1 μmol/L). At last, to test the effect of progesterone on Ca^{2+} release from intracellular stores, HUV rings were incubated with progesterone or equivalent vehicles in Ca^{2+}-free solution before added PE, 5-HT, or caffeine (25 mmol/L).

2.3. Real-time PCR

We isolated total RNA from HUV tissues from healthy single pregnancy mothers ($N = 20$) according to the manufacturer's instructions. First-strand cDNA was synthesized in a 20 μL reaction volume using reverse transcription kits (Takara). Real-time PCR was performed with SYBR Green SupermixTaq Kit (Takara) and analyzed on iCycler, MyiQ two Color Real-Time PCR Detection System (Bio-Rad). The Real-time PCR primers sequences were listed in the following: $CaV_{1.2}$-F: 5′catccggcaatctccgaaga3′, $CaV_{1.2}$-R: 5′tgcaagggcaggactgtctt3′; PR-F: 5′cagcttcgagtcattacctc3′, PR-R: 5′ccagcacataagtagttgtgc3′.

2.4. Western blot analysis

The protein abundance of $CaV_{1.2}$ and PR in HUV ($N = 10$) were assessed by western blotting normalized to β-actin. The primary antibodies were the rabbit polyclonal antibody (Santa Cruz Biotechnology) against $CaV_{1.2}$ (1:200) and PR (1:200). The secondary antibody goat anti-rabbit antibody (1:1000; Beyotime Biotechnology, Jiangsu, China) was used. Immunosignals were visualized using chemiluminescence detection (Amersham Biosciences) and the UVP imaging system (EC3-Imaging-System, Upland, CA, USA).

2.5. Drugs and chemicals

The following drugs were used: progesterone, 5-HT, L-NMMA, indomethacin, RU486, TEA, nifedipine, mibefradil, Bay-K8644, Caffeine (Sigma Chemical Co.); PE (shanghai, HeFeng, China); $CaCl_2$ (shanghai, MeiXing, China); KCl (Chinese medicine group).

2.6. Statistical analysis

All data were expressed as mean ± SEM. Significance ($P < 0.05$) was determined by two-way ANOVA or *t*-test. Concentration-dependent response curves were analyzed by computer assisted nonlinear regression (Graph Pad Prism software; Graph Pad, La Jolla, CA, USA).

3. Results

3.1. Progesterone suppressed dose-dependent vasoconstriction induced by PE or 5-HT in HUV

Incubated HUV rings with progesterone (0.1 μmol/L, 1 μmol/L, 10 μmol/L, 100 μmol/L) or equivalent vehicles for 60 min [22] before PE or 5-HT was cumulatively added in the organ bath. PE