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The steroid hormone dydrogesterone inhibits myometrial contraction independently of the progesterone/progesterone receptor pathway

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ABSTRACT

Aims: In this study, we aimed to investigate the direct effects of steroid hormones on pregnant myometrial contraction.

Main methods: The effect of steroids on oxytocin-induced contraction was examined in vitro using pregnant rat or human myometrium. Subsequently, we evaluated whether RU486, a potent progesterone antagonist, influenced the effects of progestin on myometrial contraction. Additionally, we evaluated the effects of progestin on high-concentration KCl-induced contraction caused by voltage-dependent calcium channels in order to investigate the mechanisms involved in this process.

Key findings: Of the natural steroids examined, 17β-estradiol, progesterone, testosterone, cortisol, and aldosterone did not influence oxytocin-induced contraction at concentrations $< 10^{-6}$ M. Of the tested progestins, medroxyprogesterone acetate, norethisterone, chlormadinone acetate, levonorgesterol, 17α-hydroxyprogesterone capronate, and dienogest had no effect on contraction at $< 10^{-6}$ M. However, dydrogesterone showed rapid and direct inhibition of contraction at 10^{-6} M, and this inhibitory effect was dependent on dose and time. RU486 did not block the inhibitory effects of dydrogesterone on contraction. High-concentration KCl-induced contraction was also inhibited by dydrogesterone, and the inhibitory effects of dydrogesterone were observed at concentrations as low as 10^{-7} M. Additionally, oxytocin-induced contraction in pregnant human myometrium was inhibited by 10^{-6} M dydrogesterone.

Significance: These results suggested that the rapid and direct effects of dydrogesterone on myometrial contraction were caused by a nongenomic pathway and that the progesterone receptor was not required for dydrogesterone action. Additionally, the mechanism of dydrogesterone action may involve voltage-dependent calcium channels.

1. Introduction

Steroid hormones play many important roles in living organisms and are involved in growth, development, anabolism, catabolism, reproduction, and other processes. In the reproductive system, steroid hormones are involved in reproductive organ growth and development, ovulation, fertilization, implantation, preservation of pregnancy, labor, and nursing.

Of the steroid hormones involved in the reproductive system, estrogen and progesterone are the two most important molecules and exhibit opposite effects in many processes. In the uterine myometrium, estrogen plays an important role in increasing the uterine contractile response, and progesterone counteracts this effect [1]. During parturition, many substances and factors, such as oxytocin, prostaglandin, cytokines, protease, ion channels, and contractile proteins, are involved in labor pain and participate in a common pathway to affect myometrial contraction. Thus, myometrial contraction, which causes labor pain, results in birth. Accordingly, elucidation of the substances and mechanisms that directly influence myometrial contraction is essential.

Some steroid hormones, including androgen and progestins, have been shown to have nongenomic effects on the inhibition of myometrial contraction in nonpregnant rats [2–4]. However, few reports have described the nongenomic effects of steroid hormones and their derivatives on the inhibition of pregnant myometrial contraction, although the genomic effects of steroid hormones on contraction have been studied extensively. Additionally, the steroid hormones used in the above studies were applied at extremely high concentrations of over 10^{-6} M. For testosterone and progesterone, myometrial contraction

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was inhibited at a concentration of $> 10^{-5}$ M, which would be about 10,000 and 100 times higher than the plasma concentrations in non-pregnant women, respectively.

Therefore, in the present study, we evaluated whether steroid hormones at the lower concentrations directly inhibited myometrial contraction through a nongenomic pathway using pregnant rats and women. We used oxytocin as a physiologic uterotonic to induce myometrial contraction because oxytocin is known to be involved in parturition.

2. Materials and methods

2.1. Chemicals

17β-Estradiol, progesterone, testosterone, cortisol, aldosterone, 17α-hydroxyprogesterone capronate, norethisterone, chlormadinone acetate, dimethylsulfoxide, and oxytocin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dienogest was obtained from Mochida (Tokyo, Japan). Dydrogesterone was purchased from Abcam Biochemicals (Cambridge, UK). Levonorgesterol was purchased from R &D Systems (Minneapolis, MN, USA). Medroxyprogesterone acetate was purchased from LKT Laboratories (St. Paul, MN, USA). RU486 was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Natural steroid hormones and synthetic steroid hormones were dissolved in dimethylsulfoxide.

2.2. Animals

Pregnant Wistar rats were obtained from Oriental Bioservice Corporation (Kyoto, Japan). The rats were housed under controlled conditions (12-h light/12-h dark cycle) and were provided water and rat chow ad libitum. All rats were euthanized by inhalation of CO_2 gas on day 20 or 21 of gestation, and the uterus was removed and used for experiments. This study was approved by the Animal Committee of Kansai Medical University and was conducted in accordance with the Guidelines for the Care and Use of Agricultural Animals in Agricultural Research and Teaching published by the Consortium for Developing a Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching.

2.3. Human tissues

The patients included in this study had undergone an elective cesarean section at 37–38 weeks of gestation due to a previous cesarean section, breech presentation, or pregnancy following myomectomy. Routine abdominal cesarean sections were performed at the lower uterine segment under spinal and epidural anesthesia. A myometrial sample was obtained after incising the upper edge of the lower uterine segment following delivery of the infant and placenta. The obtained tissue samples were immediately washed with normal saline solution and then stored in an ice-cold Via Span (Belzer UW; Bristol-Myers Squibb Company, Princeton, NJ, USA). All tissue samples were prepared and used within 24 h from dissection. This study was approved by the ethics committee of Kansai Medical University and performed according to the Declaration of Helsinki. Written informed consent was obtained from each patient who underwent cesarean section included in this study.

2.4. Preparation and contractile activity

Preparation of myometrial strips was performed as previously reported [5, 6]. After the rat uterus or human tissue samples were removed, myometrial strips were prepared (width: 2–3 mm, length: 10–15 mm). Each strip was attached to a holder under 1 g resting-tension. After equilibration for 60 min in physiological saline, each strip was repeatedly exposed to 72.7 mM KCl solution (high-KCl) until the response became stable. The physiological saline contained the following: 136.9 m NaCl, 5.4 mM KCl, 1.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, 23.8 mM $NaHCO_3$, 5.5 mM glucose, and 0.01 mM ethylenediaminetetraacetic acid. The high-KCl was prepared by replacing NaCl with an equimolar amount of KCl. These solutions were saturated with a 95% O_2/5% CO_2 mixture at 37 °C (pH 7.4). In this study, myometrial strips with spontaneous contraction after preloading of the high-KCl were excluded because we were not able to confirm whether the contractions were due to oxytocin.

2.5. Effects of steroid hormones on contraction

After preloading of the high-KCl, appropriate concentrations of oxytocin were added to induce contraction. After oxytocin-induced contraction was stable, vehicle or various steroid hormones were added to evaluate the direct effects of the steroid hormones on contraction. Dimethylsulfoxide was used as a vehicle in this study. Contractions were recorded isometrically with a force-displacement transducer (Model TB611T; Nihon Kohden, Tokyo, Japan) connected to a Model 3134 strain amplifier and Model 3056 ink-writing recorder (Yokogawa, Tokyo, Japan), and the data were simultaneously entered into a personal computer (Microsoft Windows 7) and were analyzed with a Unique Acquisition software package (Unique Medical Co. Ltd., Tokyo, Japan). To evaluate the effects of steroid hormones on oxytocin-induced contraction, we analyzed three peaks in the rhythmic contraction at each time after the administration of steroid hormones. We calculated the amplitude from the baseline to the top of each contraction. The average amplitude of three peaks was defined as the representative amplitude at each time. The amplitude of the rhythmic contraction was measured at 0, 5, 10, 15, and 20 min, and the effects of various steroid hormones were evaluated by comparison with the amplitude of the contraction of vehicle at the corresponding time.

2.6. Statistical analysis

The results were expressed as means ± standard deviations (SDs). Results were analyzed with Stat View version 5.0 (SAS Institute, Inc., Cary, NC, USA). Differences in the measured parameters across the different groups were statistically assessed using repeated measures analysis of variance followed by Fisher's protected least significant difference, multiple range tests. Differences with p values of < 0.05 were considered statistically significant.

3. Results

3.1. Effects of natural steroid hormones on contraction

First, we evaluated whether natural steroid hormones had direct effects on myometrial contraction. The serum concentration of natural steroids in women is known to be 10^{-9} to 10^{-6} M, even though estradiol and progesterone levels increase dramatically during pregnancy. Therefore, we evaluated the effects of natural steroids on myometrial contraction at this concentration range in the present study. The contractile response to oxytocin was 10–30 μU/mL in the myometrium obtained from rats at 21 days of gestation. Natural steroid hormones were added to the physiological solution at concentrations of 10^{-9} M to 10^{-6} M every 10 min after the oxytocin-induced contraction was stable. Estradiol neither stimulated nor inhibited oxytocin-induced contraction at concentrations of 10^{-9} to 10^{-6} M. Progesterone did not inhibit oxytocin-induced contraction at the same concentration as estrogen (Fig. 1A). Testosterone, cortisol, and aldosterone did not influence oxytocin-induced contraction at concentrations of 10^{-9} to 10^{-6} M (Fig. 1A and B).

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