Ca²⁺ sensitivity of skinned uterine muscle fibers in unilaterally pregnant rats

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Abstract

We prepared chemically skinned uterine muscle fibers from both horns of unilaterally pregnant rats and investigated the effect of gestation on the Ca²⁺ sensitivity of the Ca²⁺-induced contraction. The Ca²⁺ sensitivity was defined as the pCa (minus logarithm of free calcium concentration) required for half maximum activation of force production. Gravid horns did not show any statistical difference in the Ca²⁺ sensitivity between normal pregnant and unilaterally pregnant rats. Non-gravid horns of unilaterally pregnant rats before Day 14 of gestation showed the same behavior in the Ca²⁺ sensitivity as horns of normal pregnant rats. After Day 15 of gestation the Ca²⁺ sensitivity of non-gravid horn also increased but significantly less than that of gravid horn.

Taking into account the hormonal situation, the present results suggest that some local factors, such as an increased size of conceptus (fetus and placenta) and/or intercellular binding conditions of myometrium modulated by the gap junction formation and the appearance of connexin molecules, may also be requisite for the regulation of the contractile activity associated with parturition and delivery of the fetus and placenta.

Key words: unilaterally pregnant rats, gravid and non-gravid horn, chemically skinned fibers, Ca²⁺ sensitivity

Introduction

The uterine smooth muscle cell is known to undergo great changes in the contractility during gestation and delivery. From the view point of reproductive physiology, these changes are thought to play an important role in the maintenance of pregnancy and delivery, spending a major part of its time in a relatively quiescent state for implantation and development of the fetus and developing brief terms of the contractile activity associated with parturition and delivery of the fetus and placenta (Thorburn and Challis, 1979; Huszar and Roberts, 1982). The change in the uterine muscle contractility is mainly due to qualitative and quantitative changes in contractile and regulatory proteins in uterine smooth muscle cells, and pregnancy-associated endocrinological changes have been implicated as the principal cause of those changes (Cavaille and Leger, 1983).

The skinned muscle fibers method have been introduced, first by Natori (1954), as a good

muscle model to study the muscle contraction without complicated participation of the excitable membranes. The saponin method for the preparation of skinned cells was first introduced by Ohtsuki *et al.* (1978), and this method was successfully applied for the preparation of skinned smooth muscle fibers by Endo *et al.* (1977) and Saida and Nonomura (1978). Ochiai *et al.* (1981), Maruyama *et al.* (1986) and Ochiai *et al.* (1986) applied this method to the uterine smooth muscles.

In the present study using, we prepared saponin-treated (chemically skinned) uterine muscle fibers of gravid and non-gravid horns from unilaterally pregnant rats, keeping the endocrine milieu constant, and investigated, using the Ca^{2+} sensitivity as an indicator, whether there is the difference on the contractility of skinned muscle fibers obtained from the gravid and non-gravid horns during gestation.

Materials and Methods

1. Chemicals.

3-(N-morpholino) propane sulfonic acid (MOPS) and ethyleneglycol-bis (β -aminoethyl ether)-N, N, N', N'-tetraacetic acid (EGTA) were purchased from Dojindo Lab. (Kumamoto, Japan), and saponin from Sigma Chemicals (St. Louis, U.S.A.). Other reagents (analytical grade) were from Wako Pure Chemicals (Tokyo, Japan).

2. Solutions.

In the present experiments, we used the following solutions containing (in mM, at 20°C): (1) Locke's solution, 154 NaCl, 5.6 KCl, 1.8 NaHCO_3 , 2.2 CaCl_2 and 5.6 glucose (pH 7.4): (2) High- K^+ solution, 159.6 KCl, 1.8 NaHCO_3 , 2.2 CaCl_2 and 5.6 glucose (pH 7.4): (3) Relaxing solution, 160 KCl, 4 MgCl_2 , 4 EGTA, 4 ATP and 10 MOPS (pH 7.0): (4) Normal contraction solution, 160 KCl, 4 CaCl_2 , 4 MgCl_2 , 4 EGTA, 4 ATP and 10 MOPS (pH 7.0): (5) Saponin-relaxing solution, relaxing solution (3) containing 50 $\mu\text{g}/\text{ml}$ of saponin. Ca^{2+} concentration of the contraction solution was determined by mixing adequate volumes of solution (3) and (4) with the binding constant of Ca^{2+} and EGTA at 10^6 M^{-1} (Harafuji and Ogawa, 1980).

3. Experimental animals.

Female rats (Wister-Imamichi strain, 4 weeks old) were used in the present study. The oviduct of rats were ligated unilaterally through a dorsal back incision in the usual manner. Upon confirmation of the sexual cycle by daily examination of vaginal smear after the operation, the animals were mated with males. The Day 0 of gestation was defined as the day when spermatozoa were found in the vaginal plug after mating, and the night of Day 21 of gestation corresponds to the expected day of delivery. All operated animals have conceived at the non-ligated horn and there were no significant differences in gravid horn from the control pregnant rats as to the number of fetuses and the weight of placenta.

4. Preparation of muscle fibers, skinned muscle fibers and experimental procedures.

Rats on Day 7, 11, 14, 17, 19 and 21 of gestation were laparotomized, and the uterus was excised as quickly as possible. A small bundle of longitudinal muscle fiber, 150–200 μm in

width and several mm (approximately 8 mm) in length, was cut out of both gravid horn and non-gravid horn in the relaxing solution. The muscle fibers were mounted horizontally in a trough ($6 \times 20 \times 5$ mm) with cotton thread between a fixed position in the trough and a short rod extending to the transducer mounted on a micromanipulator (W-77, Narishige Co., Ltd. Tokyo). The viability of muscle preparations was observed by the stable spontaneous contraction in Locke's solution and the contractions in high- K^+ solution. Preparation of skinned muscle fibers and the experimental procedures were the same as described previously (Maruyama *et al.*, 1986). The muscle preparations were immersed in the saponin relaxation solution containing $50 \mu\text{g/ml}$ saponin to prepare chemically skinned fibers for 25 min at room temperature. Then the specimen was washed with the normal relaxing solution and left until tension became constant. As the criterion for the preparation of skinned muscle fibers, we adopted conventionally the following condition; the muscle fibers were skinned enough when they could not respond to the high- K^+ solution and oxytocin (10 mU/ml). Then a 5 mg resting tension was routinely applied to skinned fibers throughout the present experiments. Ca^{2+} -induced contraction of skinned muscle fibers were measured by applying the contraction solutions with a variety of Ca^{2+} concentrations.

5. Statistical Analysis

Statistical analysis was done in Student's *t*-test, and the significance of the data was accepted at the condition of $P < 0.05$.

Fig. 1. An example record of the relation between the relative tension and pCa of a chemically skinned uterine muscle fiber from unilateral pregnant rat at Day 7 (a) and at Day 21 (b) of gestation. Relative tension was normalized at the value in pCa 4.45. □, gravid horn; ●, non-gravid horn. Dashed line represents the mean value of the Ca^{2+} sensitivity from the normal pregnant rats (Ochiai *et al.*, 1986).

Results

There was no significant difference on the contractility, shown by the Ca^{2+} sensitivity, of skinned muscle preparations from both gravid horn and non-gravid horn on Day 7 of gestation, $\text{pCa } 5.41 \pm 0.14$ ($n=3$) from gravid horn and $\text{pCa } 5.36 \pm 0.21$ ($n=3$) from non-gravid horn (Fig. 1a). These values were less than that of the uterine skinned muscle fibers from non-pregnant rat ($\text{pCa } 6.2 \pm 0.2$) and almost the same as that from normal pregnant rats at Day 7 of gestation ($\text{pCa } 5.4 \pm 0.3$) (Maruyama *et al.*, 1986). On Day 14 of gestation, we could not yet observe the significant difference between the tension-pCa curves of skinned muscle fibers from gravid horn and non-gravid horn (data not shown). After then, the difference between the values of the Ca^{2+} sensitivity of the skinned muscle preparations from gravid horn and non-gravid horn became gradually obvious, and more remarkable over the period from Day 17 of gestation to Day 21 of gestation just before delivery. The values of pCa on Day 17 of gestation were $\text{pCa } 6.41 \pm 0.18$ from the preparations of non-gravid horn and $\text{pCa } 6.68 \pm 0.36$ from that of normal pregnant rats (Maruyama *et al.*, 1986). Fig. 1b. is an example of the the tension-pCa curves of the muscle fibers from Day 21 of gestation, in which we can observe the significant difference between the shinned muscle fibers from gravid horn and non-gravid horn. Fig. 2 is the final summary of the relation between the Ca^{2+} sensitivity and the stages of gestation. The skinned fibers from gravid horn showed a similar progressive increase in the Ca^{2+} sensitivity to that from the normal pregnant rats, and the Ca^{2+} sensitivity after Day 15 of gestation that from gravid horn and non-gravid horn exhibited little or no changes in each group, the values for gravid horn vs.non-gravid horn being $\text{pCa } 6.94 \pm 0.20$ vs. 6.52 ± 0.21 on Day 19, and $\text{pCa } 6.95 \pm 0.17$ vs. 6.47 ± 0.14 on Day 21 ($P < 0.05$) (Fig. 2).

Discussion

In the present study, we prepared saponin-treated (chemically skinned) uterine muscle fibers of gravid and non-gravid horns from unilaterally pregnant rats, and investigated changes

Fig. 2. Relationship between the Ca^{2+} sensitivity and the days of gestation. The data present mean $\pm$ S.D. from 3-5 different fibers, the number of which were represented in parenthesis. $\square$, gravid horn; $\bullet$, non-gravid horn. Dashed line represents the mean value of the Ca^{2+} sensitivity from the normal pregnant rats (Maruyama *et al.*, 1986). Asterisks represent $P < 0.05$.

in the Ca^{2+} -induced contraction using the Ca^{2+} sensitivity as an indicator. The Ca^{2+} sensitivity was defined as the pCa (minus logarithm of free calcium concentration) required for half maximum activation of force production. We observed that the overall behaviors in the time course of changes in the Ca^{2+} sensitivity in gravid horn during gestation was quite similar to that in the normal pregnant group (Fig. 2), indicating that the contractile system in pregnant uterine smooth muscle cells will already be complete at Day 17 of gestation enough to produce the big contractile force required at labor. These observations suggested that the change of endocrinological conditions in gravid horn during experiments were almost the same as that in the uterus of normal pregnant rats. On the other hand, of great interest is that muscle fibers from non-gravid horn, wherein neither fetus nor placenta was present, exhibited the noticeable dissimilar change in the Ca^{2+} sensitivity after Day 15 of gestation, after which, in the normal pregnant rats, the rate of increases in size of conceptus will become remarkably high.

Kawarabayashi and Marshall (1981) and Kawarabayashi (1983) represented electrophysiologically using the uterine muscle cells from unilaterally pregnant rats that the action potentials generated at myometrium were different between gravid horn and non-gravid horn, and also gravid horn showed the electrophysiological properties similar to those of myometrium in normal pregnant rats. These reports also support our present observations.

There are many reports providing experimental evidences that placental steroid hormones can regulate Ca^{2+} intrusion into sarcoplasm through the change in the membrane potential (Carsten, 1974), and also demonstrating that long-term loading of myocytes with steroid hormones gives rise to change in the amount of the intracellular contractile and regulatory proteins (Csapo, 1950). These will cause to modulate the ATPase activity (Micheal and Schofield, 1969) and the phosphorylation state of myosin molecules (Matsui *et al.*, 1983). Steroid hormones also modify the electrical properties (Kuriyama *et al.*, 1976) and enzymatic activities (Miyazaki *et al.*, 1980) of myometrium. Shimizu (1986) have shown possible pregnancy-associated alternations in contractile and regulatory proteins in uterine smooth muscle cells using polyacrylamide gel electrophoresis performed in the presence of sodium dodecyl sulfate (SDS-PAGE) and the fluorescence microscopy.

Present results indicate that gravid horn and non-gravid horn are in essentially the similar contractile properties during the progesterone dominant early stage of pregnancy, providing the evidence of the involvement of hormonal milieu in the relaxation mechanism of uterine muscles. In contrast, striking differences in the contractility were observed between gravid horn and non-gravid horn during the second half of gestation, suggesting the possibility that the increasing size of conceptus (fetus and placenta) contributes to the forceful contraction of uterus at the terminal stage of gestation. Maruyama *et al.* (1986) reported the change in the amount of calmodulin in the smooth muscle contractile system, and the changes in the effect of exogenously applied calmodulin and in the Ca^{2+} sensitivity on the Ca^{2+} -induced contraction during gestation using the skinned fibers. Ochiai *et al.* (1986) also showed that hormone load alone failed to give rise to such remarkable changes in uterine muscle contraction at the terminal stage of pregnancy. Moss (1983) reported that the mechanical stretching of skeletal muscle fibers causes to increase in their Ca^{2+} sensitivities, suggesting that there may be similar stretching-like changes in uterine smooth muscle cells during the terminal stages of gestation

due to the development of fetus.

Generally mentions should also be made on the contribution of local hormones associated with the progress of pregnancy in intact uterine fibers, modulating, directly and indirectly, the formation of gap junctions and the expression of connexins, possible gap junction proteins. Garfield and Kannan (1980) and Garfield and Hayashi (1981) reported that the number of gap junctions on uterine muscle cells were markedly increased in a pregnancy associated manner, suggesting the intimate relationship between the formation of gap junction and the labor contraction. We also obtained the evidence that the amount of connexin molecules increased on the progress of pregnancy by the immunofluorescent microscopy (Ohura *et al.*, unpublished data).

From these results, we cannot adequately succeed to explain the powerful contraction of uterine smooth muscle at labor by only the terms of endocrinological backgrounds. As mentioned above, local factors other than the humoral regulation are likely to play a role in the causation of powerful contractions of uterine smooth muscle at delivery. We contemplate conducting further studies to elucidate the mechanism in the initiation of delivery.

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