Changes in Action Potential and $\beta$-Adrenergic Effects of the Circular Muscle of Postpartum Rat Uterus

Keiji MARUTA, Yumiko MIZOGUCHI, and Takuro OSA

Department of Physiology, Yamaguchi University
School of Medicine, Ube, 755 Japan

Abstract Spike potential dominated in the circular muscle of postpartum rat uterus during the period between 0 and 15 hr after the delivery of the first newborn. During the postpartum period ranging between 20 and 48 hr, the plateau potential was dominant. Application of $10^{-9} \text{M}$ isoprenaline strongly depressed the contraction during early postpartum period (0–15 hr), and the depressant effect was much smaller thereafter. In vivo treatment of postparturient rats with estradiol-17$\beta$ (50 $\mu$g) or progesterone (50 $\mu$g) for 2 days did not alter the postpartum change in action potential or the effect of isoprenaline. The postpartum changes in muscle properties mentioned above were prevented in the distended portion of uterus, when several fetuses and placentas were artificially kept inside the uterus for 2 days, while other fetuses were delivered out. The hormonal influences on the circular muscle of postpartum rat uterus were discussed in view of the above experimental findings.

Key words: postpartum uterus, circular muscle, isoprenaline, Mg, fetoplacental unit.

Parturition is the final event of pregnancy, and attention in the field of reproductive physiology has been often directed to the mechanism via which the onset of labor is brought about. The maturation of uterine muscle is one factor. For example, oxytocin receptors in the surface membrane increase towards parturition (SOLOFF et al., 1979), and gap junctions are formed between myometrial cells (GARFIELD et al., 1977). In the rat uterus, the circular muscle exhibiting plateau potential during midpregnancy is transformed to show spike potentials at the end of pregnancy (OSA and FUJINO, 1978; KISHIKAWA, 1981; KAWARABAYASHI and MARSHALL, 1981). The configuration change of action potential is likely a useful indicator of the hormonal influence upon the circular myometrium. The adrenergic responses and effects of external Mg ions are also changeable (CHow and MARSHALL, 1981; OSA and OGASAWARA, 1983, 1984).

Having observed in a preliminary experiment that the plateau potential was

Received for publication January 23, 1985
dominant in the rat circular muscle obtained 2 days after parturition, it seemed of interest to study the hormonal influence that causes change in action potential. Hence, in the present report, changes in membrane excitability and \( \beta \)-adrenergic effects were studied for the circular muscle of postpartum rat uteri. Hormonal status during postparturient stage was altered by keeping several fetuses and placentas inside the uteri, while other fetuses were delivered out. Effects of exogenous application of ovarian hormones on postparturient rats were also investigated.

METHODS

Wistar rats weighing 250–300 g were used. Proestrous rats were housed with males overnight, and day 1 of pregnancy was the day that sperm were found in the vaginal smear. Delivery usually occurred on day 22 or 23 of pregnancy. The present work was carried out on uteri taken from animals sacrificed between 0 and 48 hr after the delivery of the first newborn. Litters were housed with the maternal rat. In some experiments, ovarian hormones (estradiol-17\( \beta \), Sigma; progesterone benzoate, Sigma) dissolved in sesame oil (50 \( \mu \)g) were injected intraperitoneally about 2 hr after the onset of delivery of the first newborn and on the next day. In other experiments, the one uterine horn was ligated on day 20 of pregnancy, and 4 fetuses and placentas in the ovarian portion were kept inside for a few days after other fetuses were delivered. When successful, fetuses contained within the uterine portion were alive for at least 2 days after the parturition. By this procedure, the date of delivery was not significantly different. The uterus was cut open along the mesometrium, and the endometrium was removed carefully using jeweler’s forceps. Circular muscle strips were dissected with fine scissors from the antimesometrial part of placental attachment.

Intracellular recording of the membrane activity was made on muscle strips 1 mm wide and 5 mm long by conventional microelectrodes filled with 3 m KCl, in the partition chamber as described by Abe and Tomita (1968). For mechanical recording alone, muscle strips about 0.5 mm wide and 3 mm long were set up vertically in a recording chamber, and connected to a force displacement transducer. The solutions were continuously flowed at a rate of 8 ml/min, and the capacity of the chamber was 2 ml. Ag-AgCl electrodes were placed near ends of muscle strips, and electric pulse with 300–500 msec duration was applied when necessary. Stimulus intensity was about twice as strong as rheobase.

The control solution (Krebs solution) had the following composition (mm): NaCl 121.9, NaHCO\( _3 \) 15.5, KCl 4.7, KH\( _2 \)PO\( _4 \) 1.2, CaCl\( _2 \) 2.5, MgCl\( _2 \) 1.2, and glucose 11.5. It was equilibrated with a gas mixture of 95% O\( _2 \) + 5% CO\( _2 \) (pH of 7.3). The test solutions were the Krebs solution containing \( 10^{-8} \) m isoprenaline (Nikken), or the Mg-free Krebs solution. Another drug used was propranolol (Inderal, Sumitomo). The preparation was equilibrated with warm Krebs solution (37 ±
0.5°C) for 2–3 hr before the experiment began.

RESULTS

The circular muscle strips excised at various postpartum stages were incubated with normal Krebs solution for 2–3 hr, then effects of the removal of external Mg and the application of $10^{-9}$ M isoprenaline on contractions were investigated (Fig. 1). The muscle strips excised during delivery (0 hr) gave off spontaneous activity for about 1 hr of incubation, and the contractile activity after 2 hr of incubation was such as shown in record A, i.e. the spontaneous activity stopped, and contractions were evoked by electric stimulation. Spontaneous contractions were generated when the external Mg was omitted, and they disappeared when isoprenaline was applied. Contractions having very small amplitude were evoked when electric stimuli were applied. Spontaneous contractions were generated in the muscle strip excised 9.5 hr after parturition (B), and the frequency was increased when the
external Mg was omitted. Spontaneous activity was abolished, and the amplitude of evoked contractions was strongly depressed by the application of isoprenaline. Spontaneous activity was frequent, and the frequency was greater in the muscle strip excised 17.5 hr after the onset of parturition when the external Mg was omitted (C). Isoprenaline depressed the amplitude of contractions very slightly. In the case of record D (24 hr), the basal tension was elevated gradually when the external Mg was omitted, and the amplitude of phasic contractions was decreased. The muscle strip underwent a contracture, then phasic contractions resumed again. When isoprenaline was applied, the basal tension was lowered, and the amplitude of phasic contractions was enhanced. The duration of phasic contraction became shorter. Record E shows the response of the muscle strip excised 48 hr after parturition. The duration of phasic contraction was more protracted than before. The amplitude and frequency of phasic contractions increased when the external Mg was omitted. Application of isoprenaline caused a slight depression of phasic contractions.

The effects of $10^{-9}$ M isoprenaline were essentially the same in normal Krebs solution as in Mg-free solution, and the depression of contraction by isoprenaline did not occur in the presence of $10^{-8}$ M propranolol, a β-blocker.

Taken such records as shown in Fig. 1 together, effects of 1.2 mM Mg and $10^{-9}$ M isoprenaline which changed depending on the postparturient period are summarized in Fig. 2. Here, the amplitude measured between the peak of phasic contraction occurred in the Mg-free Krebs solution and the basal tension level in normal Krebs solution was taken as a unity. The most remarkable feature depicted in Fig. 2 is that contractions were strongly depressed by isoprenaline in muscle strips taken between 0 and 15 hr after parturition, and the effect became very weak thereafter. The postpartum change in terms of the β-effect was nearly the same, whenever the delivery occurred on day 22, 23, or 24 of pregnancy. Depressant effects of the external Mg on the amplitude of contractions were also changeable depending on the postparturient period. Contractions were strongly depressed by Mg in the preparations excised about 5 hr after the delivery. The depressant effects became smaller thereafter.

Figure 3 illustrates comparative aspects of the membrane activity of circular muscle cells of uteri excised during postpartum stage between 0 and 20 hr. When the preparations were taken during delivery, the train of spike potential was generated by the electric stimulation (A). In another case (B), the tissue was spontaneously active, and the action potential was composed of spike and plateau potentials. In the case of record C, where the preparation was taken 5 hr after the delivery, action potentials having a short duration were evoked by the electric stimulation. Records D and E show the spontaneous membrane activities recorded in the muscle strips taken 15 hr after the delivery. Action potentials were the train discharge of spikes (D), or plateau potential preceded by the initial spike potential (E). Membrane activities in the postpartum preparations taken
20 hr after the delivery are shown in F and G. The action potential was preceded by the spike potential, then a slow potential supermounted by spike potentials followed. Supermounting spike potentials were in some case abortive (G).

Figure 4 shows the membrane activities in the muscle cells of uteri excised 24 hr (A–D) and 48 hr (E–G) after the delivery. In the former cases (A–D), the plateau potentials were dominant, and were supermounted by many or a few spike potentials. Such spike potentials exhibited a slower time course than those shown in Fig. 3. In latter cases (E–G), the plateau potential was definitely a dominant membrane activity.

Effects of the removal of external Mg and application of $10^{-9}$ M isoprenaline on the membrane activity were studied on the circular muscle strip taken 24 hr after the delivery (Fig. 5). The action potential was composed of a plateau and
Fig. 3. Membrane activities recorded intracellularly from the circular muscles taken at different periods after the onset of delivery. The periods are indicated by hr in parentheses. Faster sweeps of electrical activities are shown at the right side. Records were obtained in normal Krebs solution 2–3 hr after the beginning of incubation. Dots indicate the electric stimulation (300 msec).

Fig. 4. Membrane activities recorded intracellularly from the circular muscles taken 24 hr (A–D) and 48 hr (E–G) after the onset of delivery. Faster sweeps of electrical activities are shown at the right side in records A–D. Records were obtained in normal Krebs solution 2–3 hr after the beginning of incubation.
spikes in the normal Krebs solution. Contractions occurred in a one-to-one manner with the generation of action potentials (A, left). The membrane potential was not changed, but the duration of action potential became longer and the generation of supermounting spike potentials more frequent, when the external Mg was removed (A, right). The membrane was depolarized when the tissue was exposed to Mg-free solution for a longer period (B, left). The phasic contractions were not well correlated to the generation of action potentials. The action potential was likely generated before the preceding one was not completely repolarized. The membrane was hyperpolarized, and the basal tension was lowered when 10^-9 M isoprenaline was applied (B, right). The generation of spike potentials was more frequent in the presence of isoprenaline in Mg-free solution than in normal Krebs solution.

The transformations of the β-effect from strong to weak depression of contraction, and of action potential from spike-dominant to plateau-dominant are characteristic of the circular muscle of postpartum rat uterus. The experiments shown in Figs. 6 and 7 were undertaken in order to observe whether or not the above mentioned transformations were affected by exogenous injection to postpartum rats with estradiol or progesterone. Animals were injected with ovarian hormones 2 and 24 hr after the onset of delivery, and were sacrificed at 48 hr postpartum. Record A in Fig. 6 shows the effects of omission of the external Mg and application of 10^-6 M isoprenaline on the contractions in the circular muscle strip of estradiol-treated uterus. 10^-6 M isoprenaline was found to cause a slight depression of the phasic contractions. Records B–E show the membrane activities in the circular muscles of the estrogen-treated postpartum uteri. The action potentials were preceded by an initial spike (B–D). Plateau potentials were either accompanied by the generation of spikes having large or small amplitude (B–D),
Fig. 6. Record A: effects of the omission of external Mg and application of $10^{-9}$ M isoprenaline on the contraction in the circular muscle strip of the estrogen-treated postpartum rat. The muscle strip was obtained 48 hr after the onset of delivery. The removal and readmission of 1.2 mM Mg are shown by the line below, and the application of isoprenaline by the bar above. Records B-E: spontaneous membrane activities recorded intracellularly from the circular muscles of different estrogen-treated rat uteri. Records were taken in normal Krebs solution 2-3 hr after the beginning of incubation.

Fig. 7. Record A: effects of the omission of external Mg and application of $10^{-9}$ M isoprenaline on the contraction in the circular muscle strip of the progesterone-treated postpartum rat. The muscle strip was obtained 48 hr after the onset of delivery. The removal and readmission of 1.2 mM Mg are shown by the line below, and the application of isoprenaline by the bar above. Records B-D: spontaneous membrane activities recorded intracellularly from the circular muscles of different progesterone-treated rat uteri. Records were taken in normal Krebs solution 2-3 hr after the beginning of incubation.

*Japanese Journal of Physiology*
or not (E). Similar observation was made in the circular muscles of progesterone-treated uteri (Fig. 7). Neither the effect of isoprenaline (A), nor the membrane activity (B–D) was remarkably different from the responses in the uteri of non-treated postpartum rats (Figs. 1E, 4E–G) or of estrogen-treated postpartum rats (Fig. 6).

Experiments were designed so that 4 fetuses remained in the ovarian portion of the one uterine horn, while the fetuses located in the vaginal portion of the same uterine horn and in the other horn were delivered. The remaining fetuses were kept alive for at least 2 days after the other fetuses were delivered. The circular muscle strips were dissected from the fetus-retaining portion and from the other emptied horn. A pair of muscle strips taken from the same animal were suspended in a chamber, and effects of the removal of external Mg and application of $10^{-9}$ M isoprenaline were compared (Fig. 8). Records Aa and Ab show the responses of muscle strips removed from emptied and fetus-retaining uteri, respectively, taken 24 hr after the parturition. Spontaneous contractions were more frequent in the emptied muscle strip. A slight depression of contraction was caused in the emptied muscle strip (Aa), whereas in the fetus-retaining muscle strip spontaneous activity stopped and evoked contractions were strongly depressed (Ab) when $10^{-9}$ M isoprenaline was applied. Differences in spontaneous activity and the effect of isoprenaline between emptied (Ba) and fetus-retaining (Bb) muscle strips

![Fig. 8. Comparative effects of the removal of external Mg and applying $10^{-9}$ M isoprenaline on circular muscle strips taken from the emptied and fetus-retaining postpartum uterine horns. Postpartum uterus was obtained 24 (A) and 48 hr (B) after the onset of delivery. Muscle strips (a, emptied; b, fetus-retaining) were from the same animals, and were suspended in the same chamber. The change in Mg concentration is indicated by lines below, and application of isoprenaline by bars above. Dots indicate the electric stimulation (500 msec).](image-url)
which were obtained 48 hr after parturition were essentially the same as between Aa and Ab.

The membrane activities in the circular muscle cells in the emptied and fetus-retaining uteri are illustrated in Fig. 9 with simultaneous records of contractions. Records Aa and Ab were from the muscle strips taken 24 hr after the onset of delivery. The plateau potential dominated in the muscle cell of emptied uterus (Aa), whereas spike potentials did in the fetus-retaining uterus (Ab). Contractions having a small amplitude occurred, which were not associated with the generation of action potential in the impaled muscle cell (Ab). The electrical features mentioned above were much the same in the circular muscle obtained 48 hr after the parturition (Ba, Bb). Plateau potential had a longer duration in the emptied circular muscle (Ba) than in the case of Aa. Spike potentials dominated in the fetus-retaining muscle (Bb). In this muscle cell, action potentials which were not associated with the contraction were generated. The amplitude was small, and this potential was probably the electrotonic one which failed to invade the impaled cell. On the other hand, contractions having small amplitude were generated, which were not associated with the generation of action potential in the impaled cell. Therefore, it is suggested that conduction of excitation between muscle cells or muscle bundles was impaired for some reason in the circular muscle strip of fetus-retaining uterus (cf. Tomita, 1967).

Fig. 9. Spontaneous membrane activities recorded intracellularly (lower traces) with simultaneous records of contractions (upper traces) from the circular muscles of the emptied (a) and fetus-retaining (b) uterine horns. Postpartum uterus was obtained 24 (A) and 48 hr (B) after the onset of delivery. Muscle strips (a, b) were from the same animals. Records were taken in normal Krebs solution 2–3 hr after the beginning of incubation.
DISCUSSION

Spike potentials are generated in the longitudinal muscle of the rat uterus at pre- and postpartum status (Kuriyama and Suzuki, 1976; Kishikawa, 1981), whereas the membrane activity in the circular muscle is either spike- or plateau-dominant depending on pregnant as well as postpartum stages (Osa and Fujino, 1978; Kishikawa, 1981; Kawarabayashi and Marshall, 1981; Figs. 3, 4). Thus, the excitability of circular muscle is more likely under hormonal influence. The main findings to be discussed in the present report are listed in Table 1. Here, postpartum uterus (A) implies one in which all fetuses of both uterine horns were normally delivered, emptied uterus (Ba) the uterine horn of the animal in which the other side of pregnant uterus was ligated, and fetus-retaining uterus (Bb) the uterine portion in which fetuses were kept contained due to the ligation (see METHODS).

Blood progesterone rapidly decreases after day 19 of pregnancy (Pepe and Rothchild, 1974), possibly due to the luteolytic action of prostaglandin F₂α (Fuchs et al., 1974). On the other hand, ovarian follicles of the rat are kept developed during late pregnancy owing to follicle stimulating hormone (FSH) and luteinizing hormone (LH) released from anterior hypophysis (Greenwald, 1966). FSH secretion is not suppressed by suckling whereas the suckling stimulus is a potent inhibitor of LH (Taya and Sasamoto, 1981). Provided the hormonal status is like the above, the plateau potential can be regarded as a manifestation of estrogen dominance in the circular muscle of postpartum uterus taken 48 hr after the parturition. In fact, the treatment of spayed rat with exogenous estradiol induces the generation of plateau potential in the circular muscle (Osa and Ogasawara, 1979). A contradictory event is the generation of plateau potential in the circular muscle of rat uterus during midpregnancy (Osa and Fujino, 1978; Kishikawa, 1981; Kawarabayashi and Marshall, 1981; Osa and Ogasawara, 1984) when blood progesterone predominates. Thus, the plateau potential occurs in the circular myometrium by the dominance of either estrogen or progesterone. Spike potentials are possibly generated in the circular muscle at term and during early postpartum period in response to the decline of blood progesterone.

Table 1. Changes in membrane activities and effects of $10^{-9}$ M isoprenaline on the circular muscles of postpartum rat uteri. For further descriptions, see text.

<table>
<thead>
<tr>
<th>Uterine status</th>
<th>Hours after parturition</th>
<th>Membrane activity</th>
<th>$\beta$-inhibition (contraction)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–15</td>
<td>Spike dominant</td>
<td>strong</td>
</tr>
<tr>
<td>A</td>
<td>24</td>
<td>Spike or plateau</td>
<td>weak</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>Plateau dominant</td>
<td>weak</td>
</tr>
<tr>
<td>Ba</td>
<td>48</td>
<td>Plateau dominant</td>
<td>weak</td>
</tr>
<tr>
<td>Bb</td>
<td>48</td>
<td>Spike dominant</td>
<td>strong</td>
</tr>
</tbody>
</table>

Vol. 35, No. 4, 1985
Trains of spike potentials could be generated when "spike channels" are newly developed, or merely when the amplitude of plateau potential is depressed. These alternatives remain to be distinguished. Chamley and Parkington (1984) have recently demonstrated that relaxin specifically inhibits the plateau component of the action potential in the circular myometrium of the rat.

The transformation of action potential from the spike-dominant to plateau-dominant potential in postpartum circular muscle was prevented when uterus was ligated and fetuses and placentas were kept inside while other fetuses were delivered (Fig. 9). The ovarian function is possibly maintained in the same way as at term or during delivery by pituitary gonadotropins and prolactin, because the maternal rat was feeding pups, and/or by placental gonadotropins. Provided that the circulating hormones which originated from ovarium, fetoplacental unit or elsewhere were responsible for the generation of spike potentials mentioned above, the circular muscle in the emptied uterine horn in the same animal should have behaved in the same way as in the fetus-retaining uterine portion. However, this was not the case. Because of this difference between the fetus-retaining and emptied horns, some local influence on the fetus-retaining portion of uterus has to be taken into consideration. The possibilities are: first, hormones produced by fetoplacental unit or endometrium acted locally on the uterine muscle; second, a persistent distension of the uterine wall by containing fetuses affected the membrane property.

It has been reported for the circular muscle of rat uterus that noradrenaline causes a potentiation of contraction during midpregnancy, and then a depression at term (Kishikawa, 1981; Chow and Marshall, 1981). The \( \beta \)-inhibitory effect appears mainly altered depending on pregnant stage, but not the \( \alpha \)-excitatory effect. The present results indicate that \( 10^{-9} \) M isoprenaline caused a strong depression of contraction during the early postpartum stage, but the effect became much smaller about 15 hr after parturition. The depression of contraction meets with shortening of burst discharge or plateau potential and vice versa (Kawarabayashi and Osa, 1976; Osa and Watanabe, 1978). The \( \beta \)-inhibition could be enhanced, when the population of \( \beta \)-adrenergic receptors is increased, a Ca-sequestering mechanism linked to the \( \beta \)-action is developed, or intracellular ionic content is changed so as to cause more hyperpolarization. Although it has not been determined which of the above possibilities fits, it seems likely that the potentiation of the \( \beta \)-inhibition at term was brought about in the circular muscle by the decline of blood progesterone and/or distension of the uterine wall caused by fetal growth, because the postpartum gravid horn continued to undergo a strong \( \beta \)-inhibition (Fig. 8). Effects of the distension on the membrane properties with regard to the excitability and \( \beta \)-adrenergic action of circular muscle might be appreciated, because the exogenous application of estradiol or proestrone did not cause as remarkable an alteration in the postpartum uterus 2 days after the parturition (Figs. 6, 7) as the distension did.
This work was supported by Grant-in-Aid (No. 59570038) for Scientific Research of the Ministry of Education, Science and Culture of Japan. We are indebted to Dr. H. Kato, Department of Gynecology and Obstetrics, Yamaguchi University School of Medicine, for endocrinological knowledge of rat puerperium.

REFERENCES


Vol. 35, No. 4, 1985