PACEMAKER CURRENT (HYPERPOLARIZATION-ACTIVATED INWARD CURRENT) IN ISOLATED SINGLE PREGNANT RAT UTERINE MYOCYTES

HIROYASU SATOH and TOSHIKATSU NAKASHIMA
Department of Pharmacology, Nara Medical University
Received March 8, 1993

Summary: Whole-cell patch clamp experiments were performed to examine the underlying currents to generate spontaneous activity in freshly isolated single longitudinal muscle cells of pregnant rat uterus (18-day gestation). Isolated single cells were spindle- or round-shaped (50-700 μm in length and 2-30 μm in diameter). The holding potential was −30 mV. Long-duration (3 sec) hyperpolarizing pulses were applied to −40 to −120 mV, in increments of 10 mV. Experiments were performed at room temperature (22 ℃). A hyperpolarization-activated inward current (I_f) was produced. Current density at −120 mV was −1.03±0.31 pA/pF (n=5). The average capacitance was 64.3±2.3 pF (n=8). The threshold potential for activation of I_f was about −50 to −60 mV. The reversal potential was −18.6±2.1 mV (n=4). In the presence of Cs^+ (3 mM), the I_f current at −120 mV was decreased by 76.5±2.1 % ($P<0.01$, n=5). These results indicate that the Cs^+-sensitive hyperpolarization-activated inward current is present in the longitudinal muscle cells of pregnant rat uterus. This I_f current may contribute somewhat to the electrogenesis of the spontaneous activity.

Index Terms

hyperpolarization-activated inward current, pregnant rat uterus, single smooth muscle cell, whole-cell voltage-clamp, electrophysiology

INTRODUCTION

Changes in the membrane potential of uterine smooth muscle play an important role in regulation of the contraction and relaxation of the uterus. The functions are modulated by neurotransmitters and hormones pharmacomechanically and electrophysiologically[1)2)]. Rat uterus possesses spontaneous contraction and automaticity. In cells possessing the automaticity, there might generally be an inward pacemaker current flowing during the pacemaker potential (or phase 4 depolarization). The hyperpolarization-activated inward current (I_f) is one of the inward currents contributing to depolarize the membrane to the threshold potential for action potential during diastole.

Recently, many informations about the ionic currents underlying the myometrial activity of isolated uterine single cells have been obtained by applying the patch-clamp technique[3)-8)]. However, little is known yet about the ionic currents underlying the spontaneous activity of uterine smooth muscle. In the present experiments, thus, we sought to examine the existence of the hyperpolarization-activated inward current (the pacemaker current), I_f, in 18-day

gestation cells using whole-cell patch clamp experiments.

MATERIALS AND METHODS

Single smooth muscle cells were obtained from the longitudinal layer of pregnant rat uterus (18-day gestation), according to the methods reported previously[6)7)8)]. The tissues were incubated in nominally Ca^{2+} -free solution with 0.2% protease (Type XIV, Sigma Chemical, St. Louis, MO, USA) for 20 min after 60-min incubation with 0.2% collagenase (Worthington Biochemical). Spindle- and/or round-shaped cells (50-700 μm in length, 2 -30 μm in diameter) were isolated.

Whole-cell voltage-clamp recording was carried out with patch electrodes (3 - 5 MΩ) using usually round-shaped cells and with an Axopatch patch-clamp amplifier (Axon Instruments, Burlingame, CA, USA)[9)-12)]. The patch electrodes were made from borosilicate glass capillary tubing. The series resistance error was less than 3 - 7 mV ; compensation was not used. All experiments were performed at room temperature (22℃). The data were stored and analyzed on a IBM-AT microcomputer, using the PCLAMP analysis program (Axon Instruments). Current traces were filtered, using a cutoff frequency of 1 KHz, for plotting. The values were expressed as mean ±SEM. The differences of the mean values were analyzed with Student's *t*-test for paired data. P value less than 0.05 was considered significant.

The composition of the modified Tyrode solution was (in mM) : NaCl 137, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.0, NaH_2PO_4 0.3, glucose 5.0, and HEPES 5 . To avoid the interferences of other currents, 10 μM TTX, 3 mM $CdCl_2$, and 3 mM $BaCl_2$ were added to external solution to block the fast Na^+ current, the Ca^{2+} current and the inward rectifier K^+ current, respectively. The pipette solution contained (in mM) : KOH 110, KCl 20, $MgCl_2$ 2 , EGTA 10, MgATP 5 , creatine phosphate 5 , aspartic acid 100, and HEPES 5 (pH 7.2). The concentration of Ca^{2+} in pipette solution was adjusted to pCa 7 , according to the calculations of Fabiato and Fabiato[13)] and Tsien and Rink[14)].

RESULTS

Long-duration (3 sec) hyperpolarizing pulses were applied to −40 to −120 mV from holding potential of −30 mV (Fig. 1A). Small I_f current was produced, as shown in Figs. 1B and C. The current was activated by hyperpolarizing pulses, and it had a very slow activation. Its amplitude was so small, and at −120 mV, the I_f current density was −1.03±0.31 pA/pF (n= 5) (Fig. 2). The average capacitance was 64.3±2.3 pF (n=8). The threshold potential was −60 to −70 mV. In the presence of Cs^+ (3 mM), the I_f current was inhibited to −0.25±0.07 pA/pF at −120 mV (Figs. 1D and 2). The percentage decrease was 76.5±2.1% (n=5, $P<0.01$).

To examine the reversal potential for I_f, test pulses were applied to −100 to +10 mV, following conditioning potential of −120 mV, as shown in Fig. 3A. The current traces elicited by the test pulses are represented in Fig. 3B. Figure 4 shows the current-voltage relationship for the currents elicited by the test pulses. The reversal potential was −18.6±2.1 mV (n=3).

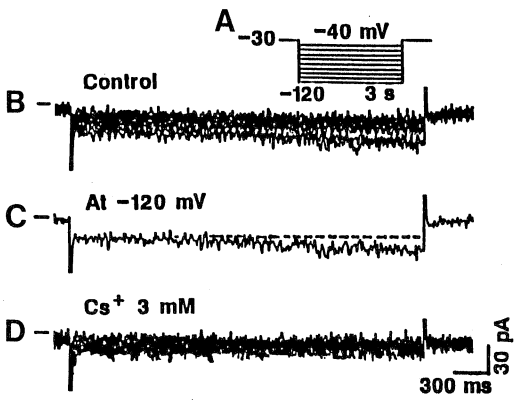

Fig. 1. Existence of hyperpolarization-activated inward current (I_f) in single rat uterine smooth muscle cells during pregnancy (18-day). A: The voltage-clamp protocol. Test pulses were applied from-40 mV to−120 mV, in 10 mV increments, from a holding potential of −30 mV. B: Presence of I_f in pregnant rat uterus. C: Activation of I_f at −120 mV from panel B. Dashed line indicates initial time-independent current level. D: Blockade of I_f by Cs^+ (3 mM).

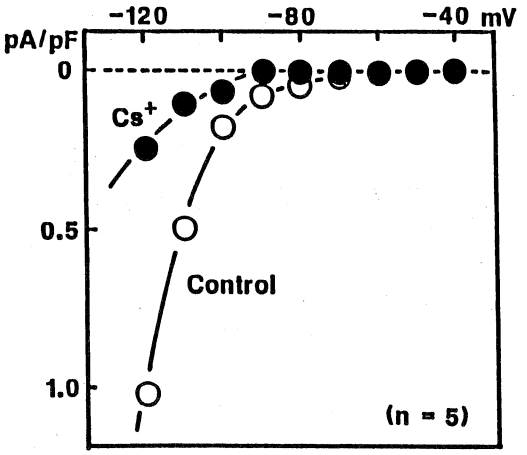

Fig. 2. Current-voltage relationship for I_f current density in the absence (open circles) and presence (filled circles) of Cs^+ (3 mM). Points plotted are the mean ±SEM (n=5); the SEM bars are less than the stickness of the symbols. External solution included 3 mM $BaCl_2$, 1 μM TTX, and 3 mM $CdCl_2$. Experiments were performed at room temperature (22°C). Dashed line presents the zero current level.

4-Aminopyridine (3 mM) was added further to the external solution to block the transient outward current.

DISCUSSION

In the present experiments, the longitudinal muscle cell from pregnant rat uterus (18-day gestation) exhibited the I_f current (but small) by hyperpolarizing pulses. The I_f was activated in a time- and voltage-dependent manner. The I_f required long hyperpolarizing steps for 3 sec or more to reach maximum. The results were as follows: (1) The I_f current density was approximately 1.0 pA/pF. The average capacitance was about 64 pF. (2) The threshold potential was around −60 to −70 mV. (3) The reversal potential was about −20 mV. (4) Cs^+ (3 mM) inhibited the I_f. The characteristics for the I_f current in this study are consistent with those from cardiac muscle cells[15)16)17)], and also with those from the circular muscle cells of pregnant rat uterus[18)]. Therefore, these results indicate that the Cs^+ -sensitive hyperpolarization-activated inward current in the longitudinal muscle of pregnant rat uterus is similar to the I_f current in cardiac muscles as well as in the circular muscle cells of pregnant rat uterus.

The membrane hyperpolarizes during gestation, whereas it depolarizes during parturition[19)]. Smooth muscle cells of isolated rat myometrial strips generate bursts of electrical activity, regardless of pregnant or non-pregnant status. Although the mechanisms generating this spontaneous activity remain unclear, the I_f current would be one of many factors involved. In cardiac muscles, I_f plays an important role in the pacemaker potential of Purkinje fibers[15)] and a relatively minor role in sino-atrial node cells[16)20)21)]. In pregnant rat uterus. the I_f current has

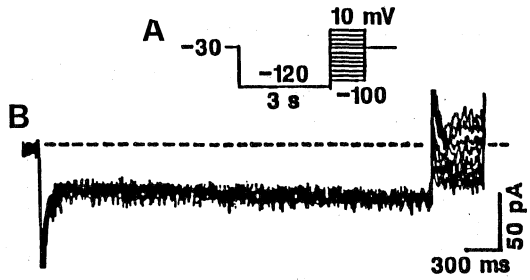

Fig. 3. Reversal potential for I_f current in rat uterine myocyte during 18-day pregnancy. A : Voltage-clamp protocol. Current recordings at test pulses (−100 to +10 mV) from conditioning pulse (at −120mV) of 3 sec. The holding potential was −30mV. B : The current traces by the conditioning pulse and test pulses (−100 to +10 mV). The dashed line indicates zero current level. External solution induced 3 mM $BaCl_2$, 1 μM TTX, 3 mM $CdCl_2$, and 3 mM 4-aminopyridine (4-AP).

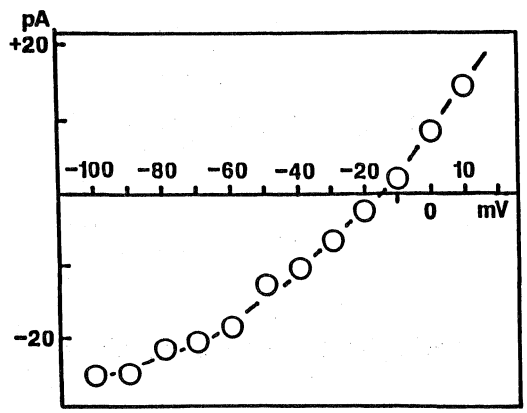

Fig. 4. Current-voltage curves showing to reversal potential. The curve crossed between −20 to −10 mV. The data were plotted against voltage axis from the recordings in a single pregnant uterine smooth muscle cell (in Fig. 3B).

been reported to be present in only circular smooth muscle cells, but not in longitudinal muscle cells[18]. If so, the spontaneous activity to be generated by the I_f current would be produced from only the circular muscle cells. However, the I_f current also actually existed in the longitudinal muscle cells, which has so small current density. Unfortunately, it is unlikely that the I_f current from the longitudinal muscle makes a major contribution to the spontaneous activity in pregnant rat uterus smooth muscles. Because the maximum diastolic potential of spontaneous action potential is not so negative (−50 to −60 mV), and the I_f current required long (3 sec) and high negative voltages (−60 to −120 mV) of hyperpolarizing pulses. In addition, because the I_f current amplitude was so much smaller, as compared to that of cardiac muscles.

Inoue and Sperelakis[8] have reported that the fast Na^+ current increased in the single longitudinal muscle cells of rat pregnant uterus (18-day gestation), as term approaches. The ratio of Ca^{2+}/Na^+ current decreases from 0.57 to 0.31 as term approaches in pregnant rat myometrium[22], suggesting that the Na^+ current as a major factor may be mainly involved in the spontaneous activity during gestation and near parturition. The Na^+ current may play an important role for parturition (i. e. spread of excitation throughout the uterine smooth muscle and its contraction).

In conclusion, the pregnant rat uterus smooth muscle cells exhibit a lot of ionic currents, as term approaches. Presumably the existence of these ionic currents seems to be preparation for parturition. Therefore, the I_f current in the pregnant rat uterus, as one factor, also must play an important role during gestation and near parturition.

REFERENCES

1) **Mironneau, J.** : Excitation-contraction coupling in voltage clamped uterine muscle. J. Physiol. (Lond.) **233** : 127-141, 1973.

2) **Honoré, E., Martin, C., Mironneau, C.** and **Mironneau, J.** : An ATP-sensitive conductance in cultured smooth muscle cells from pregnant rat myometrium. Am. J. Physiol. **257** : C297-C305, 1989.

3) **Coleman, H. A.** and **Parkington, H. C.** : Single channel Cl^- and K^+ currents from cells of uterus not treated with enzymes. Pflüger Arch. **410** : 560-562, 1987.

4) **Jmari, K., Mironneau, C.** and **Mironneau, J.** : Inactivation of calcium channel current in rat uterine smooth muscle : evidence for calcium and voltage-mediated mechanism. J. Physiol. (Lond.) **380** : 111-126, 1986.

5) **Kao, C. Y.** and **McCullough, J. R.** : Ionic currents in the uterine smooth muscle. J. Physiol. (Lond.) **246** : 1-36, 1975.

6) **Ohya, Y.** and **Sperelakis, N.** : Fast Na^+ and slow Ca^{2+} channels in single uterine muscle cells from pregnant rat. Am. J. Physiol. **257** : C408-C412, 1989.

7) **Ohya, Y.** and **Sperelakis, N.** : Tocolytic agents act on calcium channel current in single smooth muscle cells of pregnant rat uterus. J. Pharmacol. Exp. Ther. **253** : 580-585, 1990.

8) **Inoue, Y.** and **Sperelakis, N.** : Gestational change in Na^+ and Ca^{2+} channel current densities in rat myometrial smooth muscle cells. Am. J. Physiol. **260** : C658-C663, 1991.

9) **Satoh, H.** : Inhibition of L-type Ca^{2+} channel by stimulation of protein kinase C in isolated guinea-pig ventricular cardiomyocytes. Gen. Pharmacol. **23** : 1097-1102, 1992.

10) **Satoh, H.** and **Sperelakis, N.** : Identification of the hyperpolarization-activated inward current in young embryonic chick heart myocytes. J. Devel. Physiol. **15** : 247-252, 1991.

11) **Satoh, H.** and **Sperelakis, N.** : Taurine inhibition of Na^+current in embryonic chick ventricular myocytes. Eur J. Pharmacol. **218** : 83-89, 1992.

12) **Satoh, H.** and **Sperelakis, N.** : Taurine effects on Ca^{2+} currents in young embryonic chick cardiomyocytes. Eur. J. Pharmacol. **231** : 443-499, 1993.

13) **Fabiato, A.** and **Fabiato, F.** : Calculator programs for computing the composition of the solutions containing multiple metals and ligands used for experimemts in skinned muscle cells. J. Physiol. (Lond.) **75** : 463-505, 1979.

14) **Tsien, R. Y.** and **Rink, T. J.** : Neutral carrier ion-selective microelectrodes for measurements of intracellular free calcium. Biochim. Biophys. Acta **559** : 623-638, 1980.

15) **DiFrancesco, D.** and **Ojeda, C.** : Properties of the pacemaker current i_f in the sinoatrial node of the rabbit : a comparison with the current i_{k2} in Purkinje fiber. J. Physiol. (Lond.) **308** : 331-351, 1980.

16) **Hagiwara, N.** and **Irisawa, H.** : Modulation by intracellular Ca^{2+} of the hyperpolarization-activated inward current in rabbit single sino-atrial node cells. J. Physiol. (Lond.) **419** : 121-141, 1989.

17) **Satoh, H.** and **Sperelakis, N.** : Modulation of hyperpolarization-activated inward current in embryonic chick cardiac myocytes. Eur. J. Pharmacol. 1993 (in press).

18) **Okabe, K., Inoue, H.** and **Osa, T.** : Hyperpolarization-activated inward currents (I_f) in single smooth muscle cells of pregnant rat uterus. Jpn. J. Physiol. **42** (**supplement**) : S259, 1992.

19) **Kuriyama, T.** and **Suzuki, H.** : Changes in electrical properties of rat myometrium during gestation and following hormonal treatments. J. Physiol. (Lond.) **260** : 315-333, 1976.

20) **Nobel, D.** : The surprising heart : A review of recent progress in cardiac electrophysiology. J. Physiol. (Lond.) **353** : 1-50, 1984.

21) **Satoh, H.** : Pharmacology and therapeutic effects of mepirodipine. Cardiovasc. Drug Rev. **9** : 340-356, 1991.

22) **Nakai, Y.** and **Kao, C. Y.** : Changing properties of Na^+ and Ca^{2+} components of early inward current in the rat myometrium during pregnancy. Federation Proc. **42** : 313, 1983.