Potassium Currents in Freshly Dissociated Uterine Myocytes from Nonpregnant and Late-Pregnant Rats

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ABSTRACT In freshly dissociated uterine myocytes, the outward current is carried by K⁺ through channels highly selective for K⁺. Typically, nonpregnant myocytes have rather noisy K⁺ currents; half of them also have a fast-inactivating transient outward current (Iᵥₒ). In contrast, the current records are not noisy in late pregnant myocytes, and Iᵥₒ densities are low. The whole-cell Iᵥ of nonpregnant myocytes respond strongly to changes in [Ca²⁺]o, or changes in [Ca²⁺]i, caused by photolysis of caged Ca²⁺ compounds, nitr 5 or DM-nitrophene, but that of late-pregnant myocytes respond weakly or not at all. The Ca²⁺ insensitivity of the latter is present before any exposure to dissociating enzymes. By holding at −80, −40, or 0 mV and digital subtractions, the whole-cell Iᵥ of each type of myocyte can be separated into one noninactivating and two inactivating components with half-inactivation at approximately −61 and −22 mV. The noninactivating components, which consist mainly of iberiotoxin-susceptible large-conductance Ca²⁺-activated K⁺ currents, are half-activated at 39 mV in nonpregnant myocytes, but at 63 mV in late-pregnant myocytes. In detached membrane patches from the latter, identified 139 pS, Ca²⁺-sensitive K⁺ channels also have a half-open probability at 68 mV, and are less sensitive to Ca²⁺ than similar channels in taenia coli myocytes. Ca²⁺-activated K⁺ currents, susceptible to tetraethylammonium, charybdotoxin, and iberiotoxin contribute 30–35% of the total Iᵥ in nonpregnant myocytes, but <20% in late-pregnant myocytes. Dendrotoxin-insensitive, small-conductance delayed rectifier currents are not seen in nonpregnant myocytes, but contribute ~20% of total Iᵥ in late-pregnant myocytes. Thus, in late-pregnancy, myometrial excitability is increased by changes in K⁺ currents that include a suppression of the Iᵥₒ, a redistribution of Iᵥ expression from large-conductance Ca²⁺-activated channels to smaller-conductance delayed rectifier channels, a lowered Ca²⁺ sensitivity, and a positive shift of the activation of some large-conductance Ca²⁺-activated channels.

KEY WORDS: smooth muscle cells • uterine myocytes • K⁺ channels • pregnancy • ovarian hormones

INTRODUCTION

Under influences of ovarian hormones and during pregnancy, ionic currents of uterine myocytes undergo some profound changes, such as the emergence of a high-affinity tetrodotoxin-sensitive Na⁺ current, and its increasing density relative to a coexisting Ca²⁺ current as pregnancy progresses to term (Yoshino et al., 1997). Another striking change occurs in the outward current where a noisy Ca²⁺-sensitive K⁺ current, prominent in nonpregnant and early-pregnant myocytes, is largely replaced by a smooth Ca²⁺-insensitive current in late-pregnant myocytes (Kao et al., 1989; Wang et al., 1996). Such a transformation could be due to changes in the properties of some K⁺ channels, to changes in the relative roles of different types of K⁺ channels, or combinations of these possibilities.

Multiple types of K⁺ currents have been known for some time (see Hille, 1992), and more than a score of different K⁺ channels have been identified by recombinant DNA methods (Chandy and Gutman, 1995; Jan and Jan, 1997). A chief aim of this work is to determine the contributions of different K⁺ channels to the total outward current of uterine myocytes at different stages of pregnancy in the rat. To this end, we separated the whole-cell K⁺ currents of nonpregnant and late-pregnant myocytes into components containing fewer overlapping currents, and studied their kinetic and steady state gating properties, responsiveness to intra- and extracellular Ca²⁺, and susceptibility to selective blocking agents. We also examined single-channel properties of the large-conductance Ca²⁺-activated K⁺ channel and related them to whole-cell K⁺ currents. We find that during pregnancy the expression of the outward current shifts from these channels to other types of K⁺ channel, and that the shift together with other changes in K⁺ currents can increase myometrial excitability. Preliminary accounts of some of this work have been presented (Suput et al., 1989; Kao et al., 1989; Yoshino et al., 1989, 1997; Wang et al., 1996).

METHODS

Multicellular Preparations

Myometrial strips were taken from pregnant rats of known gestation. Small strands of the longitudinal myometrium were studied in a double sucrose-gap chamber, where the region (“node”) un-
nder current or voltage clamp averaged 65 μm, with total capacitance of ~100 pF (Kao and McCullough, 1975). The nodes, formed by interfaces of flowing sucrose and Krebs solution, are now known to contain ~1,000 myocytes (Yoshino et al., 1997). Aside from being dissected free from the uterus and subjected to two cuffs of high-resistance isotonic sucrose solution, these strands were not exposed to any enzymes or mechanical disruptions, nor were their cell interior exposed to any artificial Ca²⁺ buffers.

**Dissociated Myocytes and Single-Channels Studies**

Myocytes were obtained from nonpregnant (estrus phase) and late-pregnant (17–21 d) rat uteri (see details in Yoshino et al., 1997). The main differences for the present study lay in the use of some agents and solutions for specific projects to sort out different types of K⁺ channels. They are 4-aminoptyridine (Hach Chemical Co., Ames, IA), charybdotoxin (Calbiochem Corp., San Diego, CA), iberiotoxin (Peptides International, Louisville, KY), desacetylotoxin (Calbiochem Corp.), apamin (ICN Biochemicals Inc., Costa Mesa, CA), mast-cell degranulating peptide (Peninsula Laboratory, Belmont, CA), nitr-5 and DM-nitrophe (Calbiochem Corp.).

In experiments to identify charge carriers of the outward current, the bath solution contained (mM): 140 KCl, 0.6 EGTA, and 0.01 CaCl₂ at pH 7.3, with a maximum free [Ca²⁺] of 7 nM. To test the role of Cl⁻ in the outward current, the 140 mM KCl was replaced with 100 mM K₂SO₄, the two being equiosmolar as determined by osmometry.

**Photolysis of Caged Ca²⁺ Compounds**

These experiments aimed at increasing intracellular Ca²⁺ ([Ca²⁺]i) directly to see how the outward current might be affected. An inverted microscope with an epifluorescence attachment was used (Diaphot; Nikon Inc., Melville, NY). The photo-labile caged Ca²⁺ compound, nitr 5 (Gurney et al., 1987), was introduced into the cell by diffusion from the pipette, which contained 2 mM nitr 5, 1 mM Ca²⁺, and 140 mM KCl. Filtered light of 330–380 nm was focused onto the myocyte through a 40 × “Fluor” objective (Nikon Inc.) that had a numerical aperture of 0.85 and transmittance to 340 nm. Exposure was controlled by a shutter (Vincent Associates, Rochester, NY). The photoenergy was insufficient to produce “flash” photolysis, and exposures lasted 100–800 ms. Such long exposures did not interfere with our interest in steady state effects. DM-nitrophe, another caged Ca²⁺ compound (Kaplan, 1990) was used in a generally similar way.

The concentration of Ca²⁺ attained on photolysis of nitr 5·Ca was estimated under simulated conditions. Ca²⁺-selective microelectrodes were made by introducing a neutral Ca²⁺-selective ion exchange resin (ETH 1001; World Precision Instruments, New Haven, CT; Amman, 1986) into the first 200 μm of previously silanized microelectrodes with tip openings of 1–1.5 μm. In standard solutions of pCa 7 to 3, the response of the microelectrodes was linear from pCa 6.5 to 3, with a slope of 29 mV/pCa U. Between pCa 7 and 6.5, the slope was 20 mV. To estimate the [Ca²⁺] released by photolysis, a Ca²⁺ microelectrode and a reference electrode were placed in a 10-μl droplet of the pipette solution within the microscope field. The droplet was exposed to UV light for 10–800 ms. The response of the microelectrode stabilized within 26 to 40 s. The baseline [Ca²⁺] before UV exposure was 0.4–0.47 μM (six trials; see also Gurney et al., 1987). Upon irradiation, the increment of [Ca²⁺] was 0 μM for 10 ms, 1.8 μM for 100 ms, 8.2 μM for 400 ms, 14.6 μM for 800 ms, and 44 μM on continuous exposure. The true [Ca²⁺] attained must be less because of the presence of additional Ca²⁺-buffering system in the cell.

**Single-Channel Studies**

Detached inside-out patches were used because [Ca²⁺], could be confidently controlled and readily altered. Openings identified as K⁺ channels were surveyed, and large-conductance Ca²⁺-activated K⁺ channels were selected for study. The methods used were similar to those described for other smooth myocytes (taenia coli, Hu et al. 1989a,b; Fan et al., 1993; ureter, Sui and Kao, 1997). Separated or overlapped openings of different amplitudes were considered as different channels rather than subconductance levels of the same channel, because the larger (assumed full) and smaller (assumed sublevels) openings were random and unrelated. Overlapped openings of the same amplitude were assumed to be of the same channel type. In each condition, 1,000–10,000 channel events were collected. The records were examined for the highest overlap level in the more active recordings taken at highly positive voltages (80 mV), and in high [Ca²⁺] (pCa 6). Relative activities of different types of channels were determined by analyzing all channel openings during a recording period in 0.1 pA bins every 150–200 μs. The number of channels in a patch was derived from the highest overlapped opening level, and the averaged single channel activities were calculated for all channels. The average open-probability (Pₒ) for patches with multiple channels of the same amplitude was estimated when the number of channels in the patch could be reasonably determined. When the number of channels was uncertain, the open-probability was shown as nₒPₒ.

In results, averaged values are given as means ± SEM. Significance of differences were evaluated by Student’s t test in either the paired or unpaired form, as appropriate.

**R E S U L T S**

**CHARGE CARRIER OF THE OUTWARD CURRENT**

In the myometrium, at the usual resting potential of approximately −50 mV, E_Cl is approximately −20 mV (Kao and Siegman, 1963); in principle, Cl⁻ influx during depolarization could contribute to the whole-cell outward current (Parkington and Coleman, 1990). The charge carrier is identified as follows: when uterine myocytes were immersed in 140 mM KCl or 100 mM K₂SO₄ (pCa = 8.13), the resting potential was close to 0. When they were held to −80 mV, and then depolarized, the steady state current (at 0.5 s) was inward at negative voltages and outward at positive voltages. This phenomenon was confirmed in nine myocytes, regardless of whether Cl⁻ or SO₄⁻ was the anion. The 0-mV reversal potential observed under asymmetric chloride concentrations indicates that potassium is the dominant charge carrier.

**W H O L E - C E L L K⁺ CURRENTS OF UTERINE MYOCYTES AND THEIR RESPONSES TO CA²⁺**

The outward currents of freshly dissociated nonpregnant and late pregnant uterine myocytes are quite different with regard to time dependence, relative amplitudes, inherent noise, and calcium dependence. To delineate separate potassium channel contributions, it is necessary first to differentiate the general properties of the outward current in the nonpregnant and late-pregnant myocytes.
Nonpregnant Myocytes

In nonpregnant myocytes (Fig. 1, A–C), the outward currents first appeared at approximately −30 mV. At ~0 mV, they began to exhibit frequent large fluctuations (noisy) and distinct outward rectification. When elicited from a holding potential (HP) of −80 mV, about half of the myocytes had an initial surge that peaked at 3.8 ± 0.5 ms (10 myocytes), and then fell in another few milliseconds to merge into a current that rose and declined more slowly (Fig. 1 A). The initial surge is due to a transient outward current (ITO). In the other half of nonpregnant myocytes, no ITO was present and the current rose gradually to reach a maximum at 24.8 ± 2.6 ms (10 myocytes). In both types of myocytes, the outward current decayed appreciably. In myocytes with an ITO, the current was smaller than that at HP −80 mV by 235 ms and ~50% by 1.1 s (data not shown). In either case, the noisiness and the extensive decay distinguish the outward current of the nonpregnant myocyte from that of the late-pregnant myocyte.

When elicited from −50 mV, ITO was absent (Fig. 1 B). The slower current was about half that at HP −80 mV. This current declined to ~90% by 235 ms (Fig. 1 C), and to ~75% by 1.1 s. The lesser decay resembled that of the late-pregnant myocyte, but the noisiness remained.

Fig. 2, A and B, shows the typical responses of nonpregnant myocytes to a rise in [Ca²⁺]o. At HP −80 mV, when all types of K⁺ channels were expressed, raising [Ca²⁺]o to 30 mM had little effect on the average current (see small difference current in Fig. 2 A, A3). At HP −50 mV, at which ITO was absent, raising [Ca²⁺]o markedly increased the total IK (Fig. 2 B, B1). The initial surge peaked at 2.8 ms and had all the kinetic features of the ITO (Fig. 2 B, B2). At −70 mV, the ITO was 3.7X larger, and the steady state IK at (245 ms) was 1.9X larger than the isochronal currents in 1 mM Ca²⁺ (Fig. 2 B, B3). Similar changes were seen in five other nonpregnant myocytes.

Late-Pregnant Myocytes

In late-pregnant myocytes (Fig. 1, D–F), the outward current first appeared at approximately −30 mV. Up to −10 mV, some outward rectification was evident, but, more positive than −10 mV, rectification was slight. The currents at all voltages had few fluctuations (smooth). Typically, they rose gradually to reach a maximum at 32.5 ± 2.1 ms (51 myocytes). Although an early rapid phase was apparent at small depolarizations from HP −80 mV (Fig. 1 D), no ITO similar to those in nonpregnant myocytes were seen in any late-pregnant myocyte. For ~300 ms, the currents were well sustained (at ~90% by 235 ms; Fig. 1 F), but at >1–2 s, some decline occurred (at ~60% by 2.1 s, not shown). From HP −50 mV, the current was smaller than that from HP −80 mV, and showed similar little decay, remaining at ~90% at 235 ms, and ~80% at 2.1 s.

Fig. 2, C and D, show the typical responses of changing [Ca²⁺]o on the IK of two late-pregnant myocytes. Although reducing [Ca²⁺]o to 0 mM (Fig. 2 C), or raising it to 30 mM (Fig. 2 D) led to a disappearance or an increase of the inward ICa, respectively, IK remained virtually unchanged (see also difference currents in Fig. 2, C, G, and D, D3). A similar stability of IK in different [Ca²⁺]o was observed in 11 other late-pregnant myocytes. In five of these, ICa had first been blocked with Co²⁺ (5 mM), and the stability of IK was the same as those in myocytes with ICa.

Ca²⁺-Insensitive IK as an Intrinsic Property of Late-Pregnant Uterine Myocytes

To exclude a possible artifactual nature of the unexpected Ca²⁺-insensitive IK of late-pregnant myocytes, we turned to evidence gathered on small multicellular preparations in which the myocytes were neither exposed to proteolytic enzymes nor their interior to EGTA. Fig. 3 shows that, in a double sucrose-gap method, such preparations produced action potentials under current-clamp conditions and ionic currents under voltage-clamp conditions. In these preparations, effects of procedures on IK can be gauged by comparing the current at 500 ms, when the inward current had inactivated. Mn²⁺ (5 mM), which blocked the inward Ca²⁺ current, had no effect on the IK (Fig. 3 A). A similar outcome was observed with Co²⁺ (3 mM; not shown). Conversely, when [Ca²⁺]o was raised, the inward current increased, but the steady state outward current was not appreciably different (Fig. 3 B). These results show that Ca²⁺-insensitive IK is present before cell dissociation, and represents an intrinsic physiological property of late-pregnant myocytes.

Effects of Photolysis-Released Ca²⁺ on IK of Different Types of Myocytes

To avoid altering surface negative charges that can occur when manipulating [Ca²⁺]o, the effects of [Ca²⁺]o on IK can be tested by use of caged calcium compounds, nitr 5, and DM-nitrophene.

Nitr 5–Ca complex was diffused from the pipette solution into myocytes to which it imparted a brownish fluorescence. Unirradiated, nitr 5 had no effect on the depolarization-induced IK, which was identical in density and kinetics to that in myocytes without nitr 5. In other control myocytes, irradiation, in the absence of...
nitr 5, produced no effect on the depolarization-induced $I_K$. The effects of irradiating cells containing nitr 5–Ca complex were tested on 15 nonpregnant and 36 late-pregnant uterine myocytes, and 29 guinea pig *taenia coli* myocytes (for comparative control). Fig. 4 shows the responses in the different types of cells.

All 15 nonpregnant myocytes loaded with the nitr 5–Ca complex responded to irradiation with an increase in the $I_K$ (Fig. 4, A and B), which averaged 4.8 ± 1.5-fold over the control (nonirradiated) $I_K$. The current noise was larger (Fig. 4 B), the holding current became slightly inward, and the tail current was bigger (Fig. 4 A). All these changes are consistent with an activation of a large-conductance $K^+$ channel.

In late-pregnant myocytes, the responses were varied. 16 myocytes (44%) showed no response (Fig. 4 C) and 20 myocytes (56%) showed an $I_K$ increased by 2.0 ± 0.3-fold (Fig. 4, D and E). In all responding myocytes, the current noise increased, but an inward holding current was seen in only 13 myocytes (Fig. 4 D). Pooling the responding and nonresponding myocytes, the average irradiation-induced increase in $I_K$ was 1.5 ± 0.2-fold over the control current. Thus, Ca$^{2+}$-activated $K^+$ channels, while present in late-pregnant myocytes, are expressed at a lower level.

By contrast, in guinea pig *taenia coli* myocytes in which whole-cell $I_K$ is mostly due to large-conductance Ca$^{2+}$-activated $K^+$ channels (Yamamoto et al., 1989; Hu et al., 1989; Fan et al., 1993), 28 myocytes (97%) responded to irradiation with a 5.3 ± 0.9-fold increase in $I_K$ (Fig. 4 F).
To address possible species differences, in three myocytes from the analogous rat cecum, irradiation increased the average \( I_K \) by 21.9 ± 3.3-fold above the control level. 

DM-nitrophene (Kaplan, 1990) was tested on 24 late-pregnant myocytes. The qualitative changes observed with DM-nitrophene were similar in every respect to those seen using nitr 5-Ca: the irradiation-induced increases in \( [\text{Ca}^{2+}]_o \) always caused much smaller increases in \( I_K \) in late-pregnant uterine myocytes than in \( taenia coli \) myocytes. The evidence presented above, the whole-cell \( I_K \) of nonpregnant and late-pregnant uterine myocytes are complex and substantially different from each other. In the following, we will attempt to sort and apportion the components of \( I_K \) in each type of myocyte.

**Basis of Paradigm: Steady State Availability of \( K^+ \) Currents**

Fig. 5 shows the voltage–steady state inactivation (V-\( h \)) relation of the outward current, obtained on eight nonpregnant and seven late-pregnant myocytes. Each myocyte was held at \(-80 \text{ mV}\), first subjected to a 10-s conditioning voltage step, and then to a 180-ms test step of up to \(+70 \text{ mV}\) to elicit outward currents. The data are complex, and can only be fitted by assuming the presence of three populations of currents with distinct Boltzmann
distribution functions. Two of the components inactivate at depolarized potentials, whereas a third does not. For nonpregnant myocytes (Fig. 5 A), the inactivating components represent 59% (C1) and 30% (C2) of the total current, with half-inactivating voltages at −59.5 and −22.9 mV, respectively. The noninactivating component (C3) represents 11% of the current. For late-pregnant myocytes (Fig. 5 B), the inactivating components are 67% for C1 and 23% for C2, with half-inactivation voltages, respectively, at −62.7 and −21.2 mV. The noninac-
activating component (C₃) represents 10% of the total. Thus, in pregnancy, the C₁ component enlarged at the expense of the C₂ component.

These results suggest that a paradigm using holding potentials, −80, −40 (or −50), and 0 mV, can sort the whole-cell Iₖ into smaller components. Holding at 0 mV gives the nonactivating component (C₃). Holding at −40 mV gives the C₂ and C₃ components, whereas the difference between these currents yields the C₃ component. Holding at −80 mV gives the total Iₖ, and the difference between currents from HP −80 and −40 mV yields the C₁ component. Thus, currents in the C₃ component are excluded from the C₂ component, as are currents in the C₁ and C₃ components from the C₂

Figure 4. Effects of photolysis-induced increase of [Ca²⁺], on nonpregnant, late-pregnant uterine myocytes and on taenia coli myocyte. Caged nitř 5–Ca complex was introduced intracellularly by diffusion from pipette (see text for details). In each panel, five consecutive traces, recurring at 3s intervals, are shown. Traces marked C represent three superimposed traces of depolarization-induced whole-cell Iₖ in cells that have been loaded with nitř 5–Ca complex, but not irradiated. Traces marked F represent the fourth trace, during which myocyte was exposed to 360 nm light at time indicated by bar beneath traces. Traces marked F+1 represent the fifth trace in series. (A and B) Nonpregnant myocytes, 10.6 and 8.4 pF, respectively. In A, average Iₖ showed an increase upon irradiation. The increase in this cell is unusually large. Other changes also evident include more prominent current noise, downward shift of baseline, indicating holding current became more inward, and larger tail current. In B, increase in current noise is especially evident. (C–E) Late-pregnant myocytes; from 18-d pregnant uterus, 78 (C) and 60 (D) pF, and 19-d pregnant uterus, 120 pF (E). These examples show representative responses in late-pregnant myocytes. (C) Typical of 44% of test samples (36 myocytes), this cell showed no response. (D) In this myocyte, in addition to an increase in average Iₖ, there was an inward shift of holding current, an increase in tail current, and an increase of current noise. (E) In this myocyte, response consisted of an increase in average Iₖ and in current noise. 56% of all samples responded as in D and E.
Currents in Nonpregnant and Pregnant Rat Uterine Myocytes

Component. A residue of $C_1$ currents remains in the combined $C_2$, $C_3$ components, but its relative size can be estimated from the $V$-current curves. This paradigm can be assessed by evaluating the average current densities (Table I) observed on a larger sample of myocytes used in other experiments. From a group of nonpregnant myocytes, separate from those used in the $V$-current study, the total current density at $H = 80$ mV was $41.4 \mu A/\mu F$ (Table I). On the basis of the $V$-current relation (Fig. 5 A), this total might be apportioned as: $C_1$, $24.4 \mu A/\mu F$ (59%); $C_2$, $12.4 \mu A/\mu F$ (30%); and $C_3$, $4.6 \mu A/\mu F$ (11%). Outward currents elicited from $H = 40$ mV contain components $C_2$ and $C_3$, which are the same as above, and a residue of $C_1$, which is $10.7$% (Fig. 5 A) or $4.4 \mu A/\mu F$. So, the deduced total current for $H = 40$ mV is $21.4 \mu A/\mu F$, which can be compared with the observed value of $21.6 \mu A/\mu F$ (Table I).

For late-pregnant myocytes, the total outward current elicited from $H = 80$ mV was $40.1 \mu A/\mu F$ (Table I), which can be apportioned as: $C_1$, $26.9 \mu A/\mu F$ (67%); $C_2$, $9.2 \mu A/\mu F$ (23%); and $C_3$, $4 \mu A/\mu F$ (10%). At $H = 50$ mV, the $C_2$ and $C_3$ components are the same as above, and the residual $C_1$ (8.3%; Fig. 5 B) is $3.3 \mu A/\mu F$. Therefore, the total deduced current for $H = 50$ mV is $16.5 \mu A/\mu F$, which is close to the observed current of $17.1 \mu A/\mu F$ (Table I).

The paradigm was further tested by gauging the sizes of the various components on six late-pregnant myocytes. Each of these cells was held successively at $-80$, $-40$, and $0$ mV, and $I_{K}$ at $+70$ mV and $200$ ms were compared. The fractional sizes were: $C_1$, $0.67 \pm 0.07$ (six myocytes); $C_2$, $0.23 \pm 0.04$; and $C_3$, $0.09 \pm 0.03$, comparable with those derived from the $V$-current relations (Fig. 5 B).

Such close agreements support a general usefulness of the paradigm. Although each component still contains multiple currents, there are fewer and some overlap can be estimated. For clarity of later presentation, we will refer to the various components by their pregnancy status and designation as used in Fig. 5. Thus, $I_{LP1}$ refers to the $C_1$ component of late-pregnant myocytes, and $I_{NP2}$ refers to the $C_2$ component of nonpregnant myocytes, etc. When two components are not separated, they are designated as the sum of the two, $I_{LP2,3}$, etc.
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组件的全细胞K⁺电流

因为这些组件含有较少的重叠电流，除了全细胞IK，详细观察它们的动态和稳态激活和失活性质（见图6），它们的Ca²⁺敏感性（见图7），以及它们对阻断剂的敏感性（见图8–12），可能有助于更好地理解非妊娠与晚期妊娠子宫肌肉之间的差异。

表1

几种非妊娠子宫肌肉IK的性质

<table>
<thead>
<tr>
<th>HP (mV)</th>
<th>Nonpregnant</th>
<th>Late-pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>£80*</td>
<td>-80 -40</td>
<td>-80 -50</td>
</tr>
<tr>
<td>tₘₐₓ (ms)</td>
<td>3.8 ±0.5 (10)</td>
<td>24.8 ±2.6 (12)</td>
</tr>
<tr>
<td>Iₘₐₓ (µA/pF)</td>
<td>68.0 ±11.3 (10)</td>
<td>41.4 ±7.5 (12)</td>
</tr>
<tr>
<td>g (µS/cm²)</td>
<td>493 ±68 (7)</td>
<td>15 ±3 (7)</td>
</tr>
<tr>
<td>V₀.5,act (mV)</td>
<td>7.2 (7)</td>
<td>39.1 (7)</td>
</tr>
<tr>
<td>V₀.5,inact (mV)</td>
<td>-59.5 (8)</td>
<td>-22.9 (8)</td>
</tr>
<tr>
<td>g (µS/cm²)</td>
<td>254 ±20 (22)</td>
<td>64 ±14 (11)</td>
</tr>
<tr>
<td>V₀.5,act (mV)</td>
<td>7.7 (22)</td>
<td>4.2 (11)</td>
</tr>
<tr>
<td>V₀.5,inact (mV)</td>
<td>-62.7 (7)</td>
<td>-21.2 (7)</td>
</tr>
</tbody>
</table>

所有值均为平均±SEM，括号中的数为肌肉数。

在两步命令协议中，半失活位于−76.5 mV，斜率为6.9 mV（图6 A）。−40 mV时，仅有0.1%的INO可用。

其他K⁺电流。所有K⁺电流的非妊娠子宫肌肉分析使用myocytes，而没有INO。INO的发育和衰减在HP −80和−40 mV之间和INO（HP −80和0 mV）之间是指数的。这种激活是电压依赖的，且τ为INO (10 ±2 ms +20 mV, 6 ±1 ms +70 mV; 11 myocytes) 比τ为INO (19 ±3 ms +20 mV, 9 ±2 ms +70 mV; 4 myocytes) 快30%。INO的激活 (HP 0 mV) 瞬间。INO的失活和INO没有过期1.2 s。

在图6中，稳态激活和失活性质INO (图6 B), INP (图6 C), 和INP (图6 D) 在图1中给出；它们的Boltzmann分布用虚线表示。半激活电压和相关的斜率在图1中给出，作为最大导通性。V-h的关系在图6中，B和C，被从图5 A, 和半激活电压和相关的斜率在图1中给出。在两种情况下，有连续与激活曲线，包围12%的范围在−40 mV INP1，和30%的范围在−20 mV INP2。

当[Ca²⁺]₀从1到30 mM增加时，激活曲线的INO和INP2,3在移向正向时，与V₀.5,act移动14和16 mV，分别（图7, A和B）。

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Component Currents of Late-Pregnant Myocytes

The development and decay of $I_{LP1}$ and $I_{LP2}$ were also exponential. Activation of both currents were voltage dependent; $\tau$ for $I_{LP1}$ was faster ($10 \pm 1$ ms at $+20$ mV; $4 \pm 0.4$ ms at $+70$ mV; 20 myocytes) than $\tau$ for $I_{LP2}$ ($18 \pm 1$ ms at $+20$ ms; $9 \pm 1$ ms at $70$ mV; 20 myocytes), but neither rate was significantly different from the corresponding rate of nonpregnant myocytes. The activation of $I_{LP2}$ was instantaneous. The decay of $I_{LP1}$ could be described by two exponential terms; the faster term was...
voltage dependent and stabilized at \(\sim 200\) ms, whereas the slower term was voltage independent at \(\sim 2.5\) s. \(I_{\text{LP2}}\) and \(I_{\text{LP3}}\) showed little decay over 2.1 s.

The steady state activation and inactivation properties of \(I_{\text{LP1}}\), (Fig. 6 B), \(I_{\text{LP2}}\) (Fig. 6 C), and \(I_{\text{LP3}}\) (Fig. 6 D) are shown in Fig. 6 as filled symbols, and their Boltzmann distributions in broken lines, for comparison with those of nonpregnant myocytes. Their half-activation voltages and the associated slopes as well as their maximum conductances are given in Table I. The \(V-h\) relations in Fig. 6, B and C, were rescaled from Fig. 5 B, and the half-inactivation voltages and associated slopes are given in Table I. Regions of overlap with the activation curves are similar to those seen in nonpregnant myocytes.

Unlike nonpregnant myocytes, increasing \([\text{Ca}^{2+}]_o\) to 30 mM caused no significant shifts in the activation curve of any of the component currents of late-pregnant myocytes (Fig. 7, C and D).

Among many similarities in the component currents of nonpregnant and late-pregnant myocytes, significant

<table>
<thead>
<tr>
<th>Blocking agent</th>
<th>Nonpregnant</th>
<th>Late-pregnant</th>
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<tbody>
<tr>
<td>TEA, 0.5 mM</td>
<td>35</td>
<td>19</td>
</tr>
<tr>
<td>Charybdotoxin, 100 nM</td>
<td>21</td>
<td>13</td>
</tr>
<tr>
<td>Iberiotoxin, 1 nM</td>
<td>30</td>
<td>18</td>
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<tr>
<td>Apamin, 100 nM</td>
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<td>5</td>
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<tr>
<td>4-aminopyridine, 5 mM</td>
<td>56</td>
<td>50</td>
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<tr>
<td>(\alpha)-dendrotoxin, 290 nM</td>
<td>0</td>
<td>19</td>
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<tr>
<td>Mast cell degranulating peptide, 100 nM</td>
<td>0</td>
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differences were found in three areas: maximum conductances of their C1 components (493 mS/cm² for INP1 vs. 254 mS/cm² for ILP1; \(P\), 0.001 by t test); the steady state half-activation voltages of their C3 components (39.1 mV for INP3 vs. 63.4 mV for ILP3; \(P\), 5x0.004); and their responses to raised Ca\(^{2+}\) concentrations in the bath. These differences underlie important characteristics of the whole-cell K\(^{+}\) currents (see discussion).

**Pharmacological responses of myometrial K\(^{+}\) channels**

As the total I\(_{K}\) is separated into smaller units by different holding potentials, additional use of selective blocking agents may identify some individual channel types and reveal their contributions to the total current (Table II).

**Tetraethylammonium Ion**

Fig. 8 shows the typical actions of tetraethylammonium (TEA) on I\(_{LP1}\) and I\(_{LP2,3}\) of late-pregnant myocytes. At 0.5 mM, TEA appreciably reduced the average current (Fig. 8, A and B) as well as the current noise at all voltages (Fig. 8 B). Similar effects were seen in nonpregnant myocytes. The noisiness of the affected component and its stability over 2.1 s (see difference currents, I\(_{control}\) - I\(_{TEA}\), Fig. 8 C) suggest that only a large-conductance channel was blocked. In 2 mM or higher concentrations, the blocked current also contained an early decaying phase (Fig. 8 D), possibly attributable to additional channel types. Therefore, for differentiating channel types, we will focus on the effects of 0.5 mM TEA.

On average, the TEA-sensitive component in I\(_{LP1}\) amounted to 17% (I\(_{TEA}\)/I\(_{control}\) = 0.83 ± 0.09, five myocytes), which contributed 11% of the total I\(_{K}\) (0.17 ± 0.67; see Fig. 5 B). On I\(_{LP2,3}\), the TEA-sensitive component represented 26% (I\(_{TEA}\)/I\(_{control}\) = 0.74 ± 0.11, five myocytes). As it contained a residue of 8.3% of ILP1, the blocked fraction in the C2,3 components was 24%, which contributed 8% (0.24 × 0.33) of the total I\(_{K}\). Thus, the susceptible current(s) represented 19% of the total I\(_{K}\) of late-pregnant myocytes (Table II).

In nonpregnant myocytes, the blocked fraction in I\(_{NP1}\) was 36%, which contributed 21% (0.36 × 0.59) of the total I\(_{K}\). In I\(_{NP2,3}\), the blocked fraction after correction for residual I\(_{NP1}\) was 33%, contributing 14% (0.33 × 0.41) of the total current. In sum, the TEA-sensitive component constituted 35% of the total I\(_{K}\) of nonpregnant myocytes (Table II).

**Charybdotoxin**

This peptidyl toxin from the scorpion, *Leiurus quinquesstriatus*, blocks several Ca\(^{2+}\)-activated K\(^{+}\) channels and also voltage-gated potassium channels (Miller et al., 1985; Garcia et al., 1995). It was tested on three nonpregnant and seven late-pregnant myocytes at 100 nM (IC\(_{50}\), 100 pM, Vasquez et al., 1989). On nonpregnant...
myocytes, charybdotoxin (ChTX) reduced the $I_{TO}$ (Fig. 9 A), the average current, and the current noise. The susceptible current(s) (as $I_{control} - I_{ChTX}$, Fig. 9, C and D) had three components: an $I_{TO}$ that peaked at $\sim$3 ms and was already present at $-30$ mV; a noisy current in $I_{NP1}$ (at 30 and 50 mV, Fig. 9 A) that was inactivated at HP $-50$ mV (for eliciting $I_{NP2,3}$, Fig. 9 B); and another that appeared at voltages positive to 50 mV (Fig. 9 D). The blocked fraction in $I_{NP1}$ represented 24%, contributing 14% of the total current. In $I_{NP2,3}$, the blocked fraction less the residual $I_{NP1}$ was 18%, contributing 7% of the total. In sum, ChTX blocked 21% of the whole-cell $I_K$ of nonpregnant myocytes (Table II).

On late-pregnant myocytes (Fig. 9, E–H), the main effect of ChTX was a reduction of the average current (Fig. 9, E and F). Although outward currents were already evident at $-30$ to 0 mV, the susceptible current(s) did not appear till 10 mV, and increased with more positive voltages (Fig. 9 E). The blocked current had two components: an early part that peaked at $\sim$10 ms, and a late part that had a noisiness and activation similar to those in nonpregnant myocytes (Fig. 9 G). In $I_{LP2,3}$ (Fig. 9 H), the susceptible current rose gradually over $\sim$25 ms, and did not decay over 230 ms (Fig. 9 H), but it differed from its counterpart in nonpregnant myocytes in emerging at a much less positive voltage of 10 mV. In $I_{LP1}$, the blocked fraction averaged 9%, contributing 6% of the total $I_K$. In $I_{LP2,3}$, the blocked fraction after correction for residual $I_{LP1}$ averaged 21%, contributing 7% of the total current. In sum, 13% of the whole-cell $I_K$ of late-pregnant myocytes were susceptible to ChTX (Table II).

Figure 9. Effects of charybdotoxin (100 nM) on $I_K$ of uterine myocytes. Conventions are similar to those in Fig. 8, except that A and E represent directly recorded total currents; $I_{ChTX}$ (light traces) overlaid on $I_{control}$ (heavy traces). (C, D, G, and H) are different currents, or currents blocked by ChTX. (A–D) Nonpregnant myocyte. 18.4 pF. ChTX reduced peak-to-peak current fluctuations. In A, $I_{TO}$ is distinct in traces of $-10$, 10, and 30 mV in $I_{control}$, and is blocked by ChTX. In B, at HP $-50$ mV, only $I_{NP2,3}$ is elicited. Effects of ChTX are rather small, and are not manifested until more positive than 50 mV. (G) Difference currents, $I_{control} - I_{ChTX}$ at HP $-80$ mV. For clarity, only two traces at fast time scale are shown. Note the particularly prominent block on the $I_{TO}$, manifested here as an initial surge, peaking at 3 ms. The subsequent current seen in the 70-mV trace is clearly of a different and noisy type. In the full trace (not shown), the blocked current shows no decay. (D) Difference currents, $I_{control} - I_{ChTX}$ at HP $-50$ mV confirm that ChTX had no effect until beyond 50 mV. (E–H) Myocyte from 20-d pregnant uterus; 117.6 pF. At HP $-80$ (E) and $-50$ (F) mV, $I_{ChTX}$ for the $-10$-mV trace is superimposed on $I_{control}$. (G) Difference currents, $I_{control} - I_{ChTX}$ for HP $-80$ mV, on a fast time scale. The blocked currents show an initial hump, contrast with the blocked $I_{TO}$ in C, followed by another sustained current. (H) Difference currents, $I_{control} - I_{ChTX}$ at HP $-50$ mV.

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Iberiotoxin

This peptidyl toxin from the scorpion, *Buthus tamulus*, is more potent (IC$_{50}$ ≈ 25 pM) and more specific than ChTX for the large-conductance Ca$_{2+}$-activated K$^+$ channel (Galvez et al., 1990). It was tested at 1 nM concentration on four nonpregnant and four late-pregnant myocytes. Fig. 10 shows the typical effects on two nonpregnant myocytes (Fig. 10, A–G) and two late-pregnant myocytes (Fig. 10, H–K). The effects were qualitatively similar: it reduced the average current and the current noise (Fig. 10 G). The predominant susceptible current was nondecaying, but sometimes an early decaying component was seen (Fig. 10 C). The effects on I$_{TO}$ differed from those of ChTX: the I$_{TO}$ at small depolarizations were minimally affected, but I$_{TO}$ at more positive voltages were blocked, indicating that myometrial I$_{TO}$ originated from more than a single channel type. On I$_{NP1}$, the blocked fraction averaged 17% (four myocytes), contributing 10% of the total I$_K$. On I$_{NP2,3}$, the blocked fraction after correction for residual I$_{NP1}$ averaged 48%, contributing 20% of the total I$_K$. In sum, 30% of the whole-cell I$_K$ of nonpregnant myocytes were susceptible to iberiotoxin (IbTX; Table II).

On some late-pregnant myocytes, IbTX had no effect (Fig. 10 H). On average, the blocked fraction of I$_{NP1}$ av-
averaged 8%, comprising 5% of the total current. On $I_{LP_{2,3}}$, the blocked fraction after correction for residual $I_{LP_1}$ averaged 39%, contributing 13% of the total current. In sum, 18% of the whole-cell $I_K$ of late-pregnant myocytes were susceptible to IbTX (Table II).

**Apamin**

This toxin from the venom of honey bees blocks a small-conductance K⁺ channel that is sensitive to Ca²⁺, but not to voltage (Romey et al., 1984; Blatz and Magleby, 1986). It (100 nM) was tested on one nonpregnant and five late-pregnant myocytes. On the former, it had no detectable effects. On the latter, it had no effect on $I_{LP_1}$ ($I_{apamin}/I_{control} = 1.00 ± 0.02$, five myocytes), but blocked 15% of $I_{LP_{2,3}}$ ($I_{apamin}/I_{control} = 0.85 ± 0.02$), which should affect 5% of the total $I_K$.

**4-Aminopyridine**

Three concentrations of 4-aminopyridine (4-AP), 0.4, 1, and 5 mM, were tested on two nonpregnant and six late-pregnant myocytes (Fig. 11). Their actions were similar in both types of myocytes, and they differed from those of TEA, ChTX, or IbTX: (a) the noisy current fluctuations were unaffected (Fig. 11, B and F); (b) it slowed the activation of $I_{LP_{2,3}}$ (Fig. 11, F and G), resulting in a seemingly greater effect at 150 ms ($I_{4-AP}/I_{control} = 0.38 ± 0.03$, six myocytes) than at 2.1 s ($I_{4-AP}/I_{control} = 0.78 ± 0.03$); and (c) it hastened the decay of the TEA-insensitive component in $I_{LP_1}$. These effects occurred with all three concentrations, being most marked in 5 mM. On $I_{LP_3}$, 5 mM 4-AP had no effect. In $I_{LP_1}$, the blocked fraction averaged 48%, comprising 32% of the total $I_K$. After correction for residual $I_{LP_1}$,
the blocked fraction in $I_{\text{LP2,3}}$ averaged 56%, comprising 18% of the total current. In sum, 50% of the whole-cell $I_K$ of late-pregnant myocytes were susceptible to 4-AP (Table II).

Significantly, in nonpregnant myocytes, the $I_{\text{TO}}$, peaking at $\sim$3 ms, was not preferentially blocked (Fig. 11 A; also dose–response relations in Fig. 11 H). The blocked fraction of $I_{\text{NP1}}$ averaged 73%, comprising 43% of the total outward current. After correction for residual $I_{\text{NP1}}$, the blocked fraction of $I_{\text{NP2,3}}$ averaged 32%, comprising 13% of the total current. In sum, 56% of the whole-cell $I_K$ of nonpregnant myocytes were susceptible to blockade by 4-AP (Table II).

$\alpha$-Dendrotoxin

This member of a group of peptidyl toxins from the venom of mamba snakes ($Dendroaspis$ $a$ugusticeps) blocks a gradually activating and slowly decaying voltage-gated channel of small conductance that shows little outward rectification (see Dreyer, 1990). It was tested on five nonpregnant and four late-pregnant myocytes at 200 and 400 nM. On the former, $\alpha$-dendrotoxin (DTX) had no effect (Fig. 12, A and B). On late-pregnant myocytes, DTX did not reduce current fluctuations and was more effective in blocking $I_{\text{LP2,3}}$ ($I_{\text{DTX}}/I_{\text{control}} = 0.60 \pm 0.10$, four myocytes) than $I_{\text{LP1}}$ ($I_{\text{DTX}}/I_{\text{control}} = 0.90 \pm 0.10$; Fig. 12, C and D). Thus, the fractions blocked were 37% (after correction for residual $I_{\text{LP1}}$) and 10%, respectively, contributing 12 and 7% of the total $I_K$, for a sum of 19% (Table II; observed $I_{\text{DTX}}/I_{\text{control}}$ for whole-cell $I_K = 0.82 \pm 0.02$; four myocytes).

Mast-Cell Degranulating Peptide

Mast-cell degranulating peptide (MCDP), a peptidyl toxin from honey bee venom, blocks the same class of delayed rectifier as DTX (Stansfeld et al., 1987; Brau et al., 1990; Dreyer, 1990). It was applied to four late-pregnant myocytes at 100 nM. There was little effect on $I_{\text{LP1}}$. Its effects were confined to the $I_{\text{LP2,3}}$, reducing the average current ($I_{\text{MCDP}}/I_{\text{control}} = 0.89 \pm 0.03$) without affecting current fluctuations. The deduced effect on the whole-cell $I_K$ is 3.6% (Table II; observed $I_{\text{MCDP}}/I_{\text{control}}$ for whole-cell $I_K = 0.96 \pm 0.02$; four myocytes).

Table II summarizes the effects of the various agents. Allowing for some overlapping actions, a combination of ChTX, IbTX, and 4AP on nonpregnant myocytes, and additionally of apamin and DTX on late-pregnant myocytes, blocked all outward currents. The data show (a) $K_{\text{Ca}}$ currents constitute a smaller fraction of the total outward current in late-pregnant than in nonpregnant myocytes, and (b) DTX-susceptible $K_v$ currents are present in late-pregnant but not in nonpregnant myocytes.

**Single-channel observations**

To resolve an apparent contradiction between the presence of $K_{\text{Ca}}$ channels in late-pregnant uterine myocytes...
In patches from *taenia coli* myocytes, single channel activities were rarer; 8 of 51 (15.7%) randomly made patches showed no openings of any type, and in many patches only one channel was present, yielding an average of 1.8 channels per patch. In them, single-channel activities were also more complex. Of 92 single channels, the frequency of occurrence of various types (by their unitary conductance and charge-carrier) were: 140-pS K channels, 15.2%; 20-pS K channels, 60.8%; 50-pS K channels, 7.6%; 400-pS Cl channels, 16.3%; 300-pS Cl channels, 15.2%. However, when by chance a patch contained both small- and large-conductance channels, the small-conductance channels were usually much more active than the large-conductance channels, as evident in Fig. 13, A and B.

The myometrial maxi-K channels exhibited readily detectable activities at approximately −30 mV, and the current–voltage (i-V) relation in asymmetric K+ distribution (K/Ko = 5.4/135) showed significant outward rectification. They had a unitary conductance of 139 ± 3 pS (at 0 mV; n = 24), and, by extrapolation of the −30 to 0 mV segment of the i-V curve, a zero-current voltage at −83 mV (expected Nernst potential, −82 mV).

**Influence of Voltage and [Ca\textsuperscript{2+}], on Po of Maxi-K+ Channels**

Comparing *taenia* and myometrical patches, there are differences in the open probability of the maxi-K channels, voltage–Po, relations, and the sensitivity of Po, to internal Ca\textsuperscript{2+} concentrations. Fig. 14, A and B, shows the V–Po relations of two representative channels, one from a *taenia* myocyte and the other from a uterine myocyte, at pCa’s 7 and 8. Fig. 14 C summarizes such data from six *taenia* channels and nine myometrical channels. Several features are readily apparent. (a) The slopes of the curves (b), representing that the logarithmic voltage dependence of Po, is shallower for the myometrial channel (10.5 ± 0.9 mV at pCa 8; 12.2 ± 1.6 mV at pCa 7) than for the *taenia* channel (7.6 ± 0.6 mV at pCa 8; 8.6 ± 0.7 mV at pCa 7). By t-test, the difference in pCa 8 is significant (P = 0.05), whereas the difference in pCa 7 is not (P = 0.12). (b) The voltage at which Po = 0.5 (V1/2; i.e., when a channel is equally likely to be open as closed) is more positive for the myometrial channel (86.8 ± 9.1 mV at pCa 8; 68.3 ± 9.1 mV at pCa 7) than for the *taenia* channel (49.7 ± 5.4 mV at pCa 8; 24.1 ± 5.2 mV at pCa 7). The difference for either pCa is significant (P = 0.004 for pCa 8, and 0.012 for pCa 7). (c) The negative shift of V1/2 when pCa is changed from 8 to 7 is less in the myometrial channel (18 mV) than in the *taenia* channel (26 mV).

From Fig. 14 C, it is readily apparent that within the physiological range of voltages (−40 to +30 mV), the open probability at a fixed pCa in the myometrical maxi-K channel is only ~0.05–0.1 that of the *taenia* channel.
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Figure 14. Voltage-open probability relations of maxi-K channels from taenia coli myocyte and late-pregnant uterine myocyte, and effects of [Ca$^{2+}$], on them. (A and B) Data from representative individual patch for illustration. (C) Summary of data. In A and B, solid curves are Boltzmann distributions: $P_o = [1 + \exp(V_o - V)/k]^{-1}$, where $V_o$ is voltage at which $P_o = 0.5$, and $k$ is logarithmic voltage sensitivity. Filled symbols for pCa 8; hollow symbols for pCa 7. (A) For taenia coli channel, $V_o$ and $k$ are, respectively, 63.2 and 7.9 mV for pCa 8, and 35.4 and 8.9 mV for pCa 7. (B) For late-pregnant myometrial channels, they are, respectively, 76.3 and 9.1 mV for pCa 8, and 60.3 and 10.4 mV for pCa 7. Differences: in myometrial channel, $V_o$ is more positive, $k$ is shallower, and negative shift of $V_o$ on increasing [Ca$^{2+}$] is less. (C) Average P_o-N relations of late-pregnant maxi-K channels and their related Ca$^{2+}$ channels. Each curve is identified by average $V_o$ value used; triangles for myometrial channels, circles for taenia coli channels. Filled symbols for pCa 8, hollow symbols for pCa 7. See text for data.

Discussion

Confusion abounds in our knowledge of myometrial K$^+$ currents, possibly because of concurrent expressions of multiple types of channels and labile combinations of channel types engendered by hormonal influences. Previous studies centered on single states of the myometrium (Mironneau and Savineau, 1980; Miyoshi et al., 1991; Piedras-Renteria et al., 1991; Inoue et al., 1993), or identified tissue-cultured material with freshly dissociated myocytes (Toro et al., 1990; Erulkar et al., 1994). As whole-cell K$^+$ currents were generally treated in their entirety, variance could be expected between extant claims and the present results. Thus, the prominence of a Ca$^{2+}$-activated K$^+$ current in multicellular preparations of late-pregnant myometrium (Mironneau and Savineau, 1989) is inconsistent with the Ca$^{2+}$-insensitivity of myocytes from such preparations (Figs. 2 and 3; also Kao et al., 1989; Miyoshi et al., 1991; Inoue et al., 1993). A transient outward current in late-pregnant myocytes surmised solely on the basis of 4-AP action (Inoue et al., 1993) may have resulted from an unexpected slowing of activation of I$_K$ by 4-AP (see Fig. 11, F and G), because such a current was not seen in late-pregnant myocytes. The difficulties of equating tissue-culture material with freshly dissociated myocytes is exemplified by the fact that three K$^+$ currents in freshly dissociated nonpregnant myocytes (Piedras-Renteria et al., 1991) were very different from those seen by the same investigators in tissue-cultured material (Toro et al., 1990). They also lack counterparts in the present study, not least because they could not be recorded with pipette solutions containing Ca$^{2+}$ buffers (contrast also Miyoshi et al., 1991). The I$_{TO}$ of the present study, half inactivated at $-77$ mV and half activated at 5 mV (Fig. 14 A) is clearly different from an incompletely characterized transient K$^+$ current (K$_t$) that was half activated at 22 mV (Piedras-Renteria et al., 1991), and another seen in tissue-cultured material that was half inactivated at $-48$ mV (Erulkar et al., 1994).

Although the whole-cell approach used in this study cannot identify native K$^+$ channels with cloned K$^+$ channels (because of accessory unit influence or heteromultimeric assembly), the paradigm used has sorted out more concurrent K$^+$ currents in smooth myocytes than had been accomplished before. The combined sifting with holding potentials and blocking agents also recognized the appropriate roles of some channels that would have been masked in a whole-cell current approach. In uterine myocytes, the K$^+$ currents are due to voltage-gated (K$_v$) currents and their related Ca$^{2+}$-activated K$^+$ (K$_{Ca}$) currents. No inwardly rectifying K$^+$ currents were detected.

By their noninactivating nature, noisiness and susceptibility to IbTX (Fig. 10 G), 85–90% of the C$_g$ currents are attributed to large-conductance K$_{Ca}$ channels, a surmise consistent with their nonresponsiveness to 4-AP. The C$_g$ currents contained several types of K$_{Ca}$ currents and K$_v$ currents: K$_{Ca}$ currents of the small- or intermediate-conductance varieties were recognized by their susceptibility to ChTX and apamin, and K$_v$ currents by their suscepti-
bility to 4-AP, DTX, and MCDP. The \( C_1 \) currents contained the most diverse constituents of both \( K_\text{v} \) and \( K_\text{Ca} \) types. Of the \( K_\text{v} \) currents, because of vast differences in their steady state gating properties (Fig. 6), the \( I_{\text{TO}} \) and the delayed-rectifier currents probably originated in different channels rather than in a single channel type with different accessory-unit modification of their inactivation kinetics. That 4-AP had no preferential effect on the \( I_{\text{TO}} \) suggested that the native \( I_{\text{TO}} \) channel(s) might be closer to \( rK_\text{v} 1.4 \) than to \( rK_\text{v} 3.3 \) or \( rK_\text{v} 3.4 \) (see Chandy and Gutman, 1995). In late-pregnant myocytes, the gating and pharmacological properties of the native \( K_\text{v} \) channels resembled those of cloned \( rK_\text{v} \) 1.1, 1.2, 1.6 channels (see Chandy and Gutman, 1995).

**Changes in Myometrial \( K^+ \) Currents During Pregnancy**

Among many similarities in the \( K^+ \) currents of non-pregnant and late-pregnant myocytes, three differences are particularly notable: (a) \( I_{\text{TO}} \), often present in non-pregnant myocytes, is absent in late-pregnant myocytes; (b) \( K^+ \) currents of late-pregnant myocytes are insensitive or much less sensitive than those of nonpregnant myocytes to changes in intracellular or extracellular \( Ca^{2+} \); and (c) some delayed-rectifier currents are seen only in late-pregnant myocytes.

As \( I_{\text{TO}} \) regulates the membrane potential during burst spike discharges (Conner and Stevens, 1971), its absence in late pregnancy removes a constraint on repetitive action potentials that occur with greater frequencies as term approaches. Several factors underlie the relative \( Ca^{2+} \) insensitivity of late-pregnant myocytes. Firstly, there are differences in screenable surface negative charges (Frankenhauser and Hodgkin, 1957), whereas voltage-activation relations of nonpregnant myocytes were shifted 15 mV to the positive by elevated \( [Ca^{2+}]_o \), those of late-pregnant myocytes were unaffected (Fig. 7). Such a charge-screening effect must also influence the voltage-inactivation relations. Thus, for nonpregnant myocytes held at ~50 mV, a 15-mV shift would increase the available fraction of \( I_{\text{TO}} \) from ~1% in 1 mM \( Ca^{2+} \) (Fig. 6 A) to ~20% in 30 mM \( Ca^{2+} \), enough to largely account for a revival of an \( I_{\text{TO}} \) that had been inactivated (Fig. 2). Other factors involve more direct changes in \( K^+ \) channel types. Pharmacological responses indicate that as pregnancy progressed towards term, maxi-K (\( K_{\text{Ca}} \)) channels are replaced by smaller-conductance delayed rectifier (\( K_\text{v} \)) channels to express whole-cell \( K^+ \) currents. This change accounts for the difference between the noisy and outwardly rectifying current of nonpregnant myocytes and the rather smooth current with little rectification of the late-pregnant myocytes.

The lowered expression of maxi-K channels can result from a reduced density and/or altered conditions for their expression. A reduced density is suggested by the different responses of the \( I_{\text{p}} \) of nonpregnant and of late-pregnant myocytes to photolysis-induced increase of \( [Ca^{2+}]_i \) (Fig. 4). The possibility of altered conditions of expression is shown in the \( V_g \) relations of the \( C_3 \) currents (Fig. 6 D; 39 mV for nonpregnant myocytes and 63 mV for late-pregnant myocytes), which are mostly due to IbTX-sensitive large-conductance \( K_{\text{Ca}} \) channel(s) (Fig. 10 G). Single maxi-K channels from late-pregnant myocytes have a half-open probability in \( pCa \) 7 of 68 mV (Fig. 14). They are also less sensitive to \( Ca^{2+} \) than similar channels in *taenia coli* myocytes, which express them abundantly. Limiting the expression of maxi-K channels could increase myometrial excitability by setting the resting potential positive to the potassium equilibrium potential, and by decreasing the resting membrane conductance and thereby lowering the current needed to trigger action potentials. Fig. 6 D shows that, in the physiological range of voltages, differences in the fractional activation of these currents are substantial. For instance, at ~20 mV (near the spike threshold), the fractional activation is 0.03 for nonpregnant myocytes and 0.005 for late-pregnant myocytes; at 20 mV (near the peak of action potentials), these fractions are 0.26 and 0.08, respectively.

In conclusion, as pregnancy progresses towards term, myometrial maxi-K channels lose functional importance through a combination of factors that include a change in surface negative charges, a reduction in density, a positive shift of voltage-activation relation, and a lowered sensitivity to \( Ca^{2+} \). In concert with a suppression of \( I_{\text{TO}} \) and an increased expression of a fast \( Na^+ \) channel (Yoshino et al., 1997), these changes facilitate repetitive spike discharges for the needs of parturition.

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