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Effects of Conditioning Polarization on the Membrane Ionic Currents in Rat Myometrium

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Summary. Membrane ionic currents were measured in pregnant rat uterine smooth muscle under voltage clamp conditions by utilizing the double sucrose gap method, and the effects of conditioning pre-pulses on these currents were investigated. With depolarizing pulses, the early inward current was followed by a late outward current. Cobalt (1 mM) abolished the inward current and did not affect the late outward current *per se*, but produced changes in the current pattern, suggesting that the inward current overlaps with the initial part of the late outward current. After correction for this overlap, the inward current reached its maximum at about +10 mV and its reversal potential was estimated to be +62 mV. Tetraethylammonium (TEA) suppressed the outward currents and increased the apparent inward current. The increase in the inward current by TEA thus could be due to a suppression of the outward current. The reversal potential for the outward current was estimated to be −87 mV. Conditioning depolarization and hyperpolarization both produced a decrease in the inward current. Complete depolarization block occurred at a membrane potential of −20 mV. Conditioning hyperpolarization experiments in the presence of cobalt and/or TEA revealed that the decrease in the inward current caused by conditioning hyperpolarization was a result of an increase in the outward current overlapping with the inward current. It appears that a part of the potassium channel population is inactivated at the resting membrane potential and that this inactivation is removed by hyperpolarization.

The electrical activity of excitable membranes is modified by a polarizing current. In axons (Hodgkin & Huxley, 1952; Cole & Moore, 1960), skeletal muscles (Adrian, Chandler & Hodgkin, 1970; Ildefonse & Rougier, 1972) and cardiac muscles (Weidmann, 1955; McAllister, Nobel & Tsien, 1975), the amplitudes of the action potentials are usually decreased when the membrane is conditioned by a depolarizing pre-pulse and increased following hyperpolarization. These effects on the membrane potential are the result of changes in the availability of Na-channels (Hodgkin & Huxley, 1952). In the smooth muscle of the cat intestine, however, the amplitude of the spike potential is reduced by either depolarization or hyperpolarization (Nagai & Prosser, 1963). In the guinea

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pig *taenia coli*, Kuriyama and Tomita (1965) showed an increase in spike amplitude produced by hyperpolarization analogous to that seen in nerve and striated muscle, but Tomita (1966, 1967) in subsequent papers found that the spike amplitude was reduced by either depolarization or hyperpolarization as seen in the cat intestine.

Voltage-clamp experiments on smooth muscle have shown that an outward current is activated early, so that the early part of the outward current overlaps with the early inward current (Kumamoto & Horn, 1970; Vassort, 1975; Muramatsu, Fujiwara & Kumamoto, 1976). Thus, the amplitude of the spike potential of smooth muscles is an algebraic sum of the inward (Ca^{++} and/or Na^{+}) and the outward (K^{+}) currents, and the effects of membrane polarization on the spike amplitude cannot simply be determined by conductance changes for the inward current carrying ions.

The present experiments were designed to determine the effects of conditioning hyperpolarization and depolarization on ionic conductances in the rat uterine smooth muscle under conditions in which either inward or outward current is attenuated. The results obtained indicate that an apparent decrease in the inward current following conditioning hyperpolarization is the result of an increase in the outward current overlapping with the inward current and that the actual current does not change after conditioning hyperpolarization. A preliminary report was presented at the 61st Annual Meeting of the Federation of American Societies for Experimental Biology (Kumamoto *et al.*, 1977*a*).

Materials and Methods

Wistar rats, 200–250 g and 18 to 21 days pregnant, were sacrificed by a blow on the head and bled from the carotid arteries. The uteri were excised immediately; an incision was made along the mesometrial borders, and fetuses and placentas were removed. From the short segments of the opened uteri the myometrium was cut along the muscle bundle, and longitudinal muscle strips, 150 to 250 μm wide, about 100 μm thick and about 10 mm long, were prepared. The strips were mounted so as to maintain an appropriate tension and constant length in the double sucrose-gap chamber and incubated at 37 °C for at least 30 min before the experiment was started.

Details of the apparatus and procedures were the same as described by Kumamoto and Horn (1970). Briefly, the double sucrose-gap chamber was composed of three compartments separated from one another by two cuffs (2.5 mm long) of high-resistance isotonic sucrose solution. The central compartment, less than 100 μm wide, served as the test compartment, isolated by the two insulating sucrose streams. This central nodal area of the preparation was superfused with Krebs solution or a test solution. Two lateral compartments at each end of the preparation were *I*-pool for current injection and *V*-pool for potential

recording, and were filled with isotonic KCl solution. Low-resistance, Ag-AgCl electrodes were connected (through Krebs-agar bridges) to the lateral *I*- and *V*-pools and the upstream end of the central compartment. Membrane potentials were measured with a high input impedance operational amplifier, which is a unity gain voltage follower, connected between the electrode in the *V*-pool and the ground. The electrode in the central compartment was connected directly to the current recording operational amplifier (*I-V* converter), whose summing point was maintained at the virtual ground. To clamp voltage, another operational amplifier was used as a feedback amplifier (high gain, inverting). To clamp current, stimulating pulses were injected through a high resistance to the *I*-pool from an electronic stimulator. The resistance of the sucrose-gap without the preparation was 50 to 100 MΩ, and it fell to about 1 MΩ when preparations were mounted in the sucrose cuffs. Therefore, the short-circuiting factor (Stämpfli, 1954) was of the order of less than 5% in this series of experiments. The space constant of the rat myometrium is 1.8 mm at the resting state (Abe, 1971), and Bolton (1975) suggested that it is reduced to approximately one third of the resting value during the active state. Therefore, the width of less than 100 μm of the central nodal area should be sufficiently narrow for space clamping of the membrane. There was a series resistance of 10 to 15 KΩ in our system which might cause a voltage drop with large inward currents (Johnson & Lieberman, 1971; Tarr & Trank, 1974). In most of our experiments, however, the maximal inward currents were too small (less than 0.5 μA) to significantly affect the voltage control.

The composition of the Krebs solution used was as follows (mM): Na^+ 137.4; K^+ 5.9; Mg^{++} 1.2; Ca^{++} 2.5; Cl^- 134; HCO_3^- 15.5; $H_2PO_4^-$ 1.2; glucose 11.5. This solution was aerated by 95% O_2+5% CO_2 (pH 7.4). For experiments with cobalt (Co^{++}) and TEA, the Krebs solution was buffered by 10mM trishydroxymethylaminomethane-HCl (Tris) from which carbonate and phosphate were omitted, and was aerated with 100% O_2 (pH 7.4–7.5). Osmolarity was adjusted by replacement of an appropriate amount of NaCl. Isotonic sucrose solution (10% w/v) was deionized to reduce the conductivity to less than 2 μmho/cm. All solutions were maintained at 37 °C.

Voltage clamp experiments were carried out with a holding potential equal to the resting membrane potential. In the experiments using double command pulses, the duration of the conditioning pre-pulse was usually 400 msec and did not significantly change the ionic composition in the cells (Kao & McCullough, 1975). Ionic currents produced by depolarizing pulses were corrected for leakage currents by using hyperpolarizing pulses of equal magnitude and assuming that the leak conductance remained constant. In double command pulse experiments, direct analysis from the total membrane currents was also performed. The inward current is downward in the figures.

Results

Membrane Activities under Normal Conditions

Current clamp experiments. In myometrial bundles isolated from 18- to 21-day pregnant rats, the resting membrane potential was -47.2 ± 0.5 mV (mean $\pm$SE, $n=134$). This value is slightly less negative than that obtained by microelectrode impalements of individual myometrial cells (-54.5 ± 0.5 mV; Casteels & Kuriyama, 1965).

Small depolarizing currents produced graded responses. Full-sized spikes of about 50 mV were usually elicited by currents of high intensity (200 nA) (Fig. 1). The spike was followed by an undershoot.

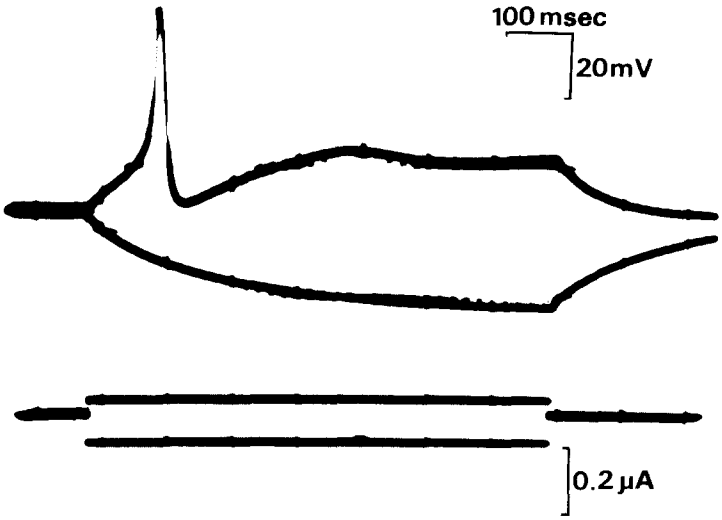

Fig. 1. Electrical activity of pregnant rat uterus under current clamp conditions. Upper trace is voltage recording and lower trace is the applied current. The resting membrane potential here is −55 mV

Hyperpolarizing current produced an exponential potential change with a time constant of 100 to 150 msec (Fig. 1). Since the amplitude of the voltage change was proportional to the intensity of the applied current at potentials ranging from the resting level to −100 mV, the membrane resistance is constant (about 300 kΩ) in this potential range.

Voltage clamp experiments. Membrane ionic currents of rat myometrium are composed of an early inward current and a late outward current (Anderson, 1969; Anderson & Ramon, 1976; Mironneau, 1974; Kao & McCullough, 1975). However, we found that the pattern of the outward current was rather complex. A small depolarizing step (< +30 mV) caused an even steady-state outward current following the early inward current, but with larger depolarizations, the outward current rapidly reached a maximal value and then declined slowly to a steady state (Fig. 2*A*). A tail current also appeared clearly at large depolarizations, indicated by arrows in the figure. To elucidate the relationship of the inward and outward currents, we measured the early inward current and the steady-state outward current at the three points in time shown in the inset of Fig. 2*B*, i.e., at 10–15, 30–50, and 300 msec, respectively. The three current-voltage relationship are plotted in Fig. 2*B*. On the one hand, the leakage currents measured at the end of the hyperpolarizing

pulses increased in a linear fashion with stepwise hyperpolarizations from −45 to −100 mV. On the other hand, when the membrane currents associated with the hyperpolarizing pulses were measured at the point corresponding to the peak of downward deflection by depolarization, a nonlinear curve was obtained (open circles), possibly related to remaining capacitive current. After correction for these leakage and capacitive currents, the early inward current is seen to reach a peak amplitude at −10 to 0 mV and reverses its polarity at +10 to +20 mV (filled circles). It is clear that the outward currents measured at both points, 30 to 50 msec and 300 msec, are almost the same, as shown in this figure.

Effects of conditioning voltage pulses. The ionic currents of pregnant rat uterus were influenced in various ways by different conditioning of the membrane. Figure 3*A* shows the effects of conditioning hyperpolarization or depolarization on the ionic currents of the tissue. The numbers in the figure represent the amplitude of the conditioning prepulse from the resting membrane potential. The level of the depolarizing test pulse was always set at 0 mV, at which maximal conductance of the early transient inward current was expected. When the membrane was conditioned by a depolarizing pulse (e.g., +30 mV step in Fig. 3*A*) from the resting level, the early inward current decreased while the outward current was little affected. When the membrane was conditioned with a hyperpolarizing pre-pulse (e.g., −20 to −40 mV steps in Fig. 3*A*), the early inward current was also reduced. The outward current increased, however, even though the depolarizing test pulse was at the same level as control. These changes increased with the magnitude of the conditioning hyperpolarization.

As shown in Fig. 3*B*, the inward current decreased while the outward current increased when the duration of hyperpolarizing pre-pulse was prolonged, and the maximal change was obtained at about 1000 msec duration. These results show that changes in inward and outward currents are dependent on the voltage and the duration of the conditioning prepulse. The decrease in the inward current (filled circles) by conditioning hyperpolarization follows the same time course as the increase in the initial part of the outward current (filled triangles). This finding suggests the possibility that the reduction of the inward current is due to the increase in the outward current. In order to elucidate this problem, we carried out voltage clamp experiments under conditions attenuating the early inward and/or the outward currents.

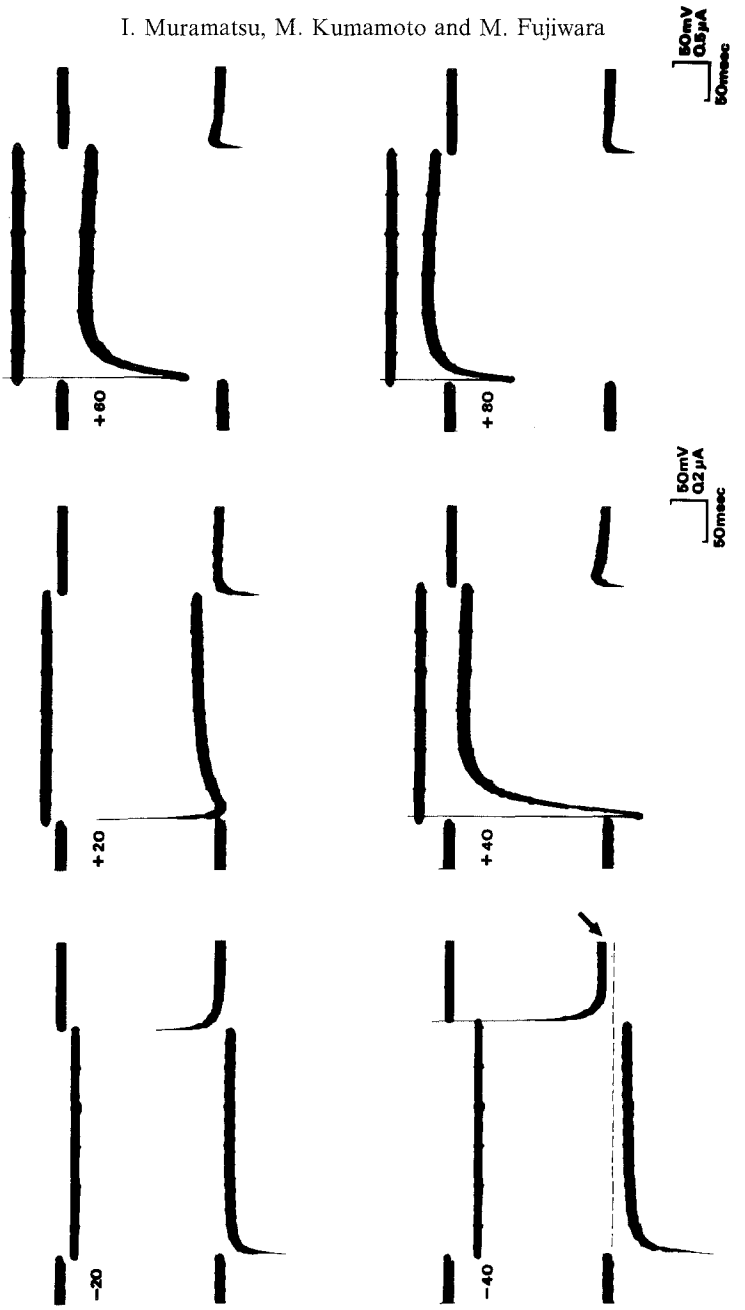

Fig. 2. (*A*) Representative currents of pregnant rat uterus under voltage clamp conditions. The resting membrane potential was, in this case, −45 mV and the holding potential was kept at this resting level. Numbers at the left of the voltage traces represent the amplitude of depolarization(+) or hyperpolarization(−) measured from the resting membrane potential. Note the outward tail current after cessation of a large hyperpolarization (at the arrow). (*B*) Current-voltage relationship obtained from records in *A*. Ionic currents were measured at three points as shown in the inset. Directly measured currents (open symbols) have here been corrected only for capacitive and leakage currents, and the values after this correction are represented by filled symbols

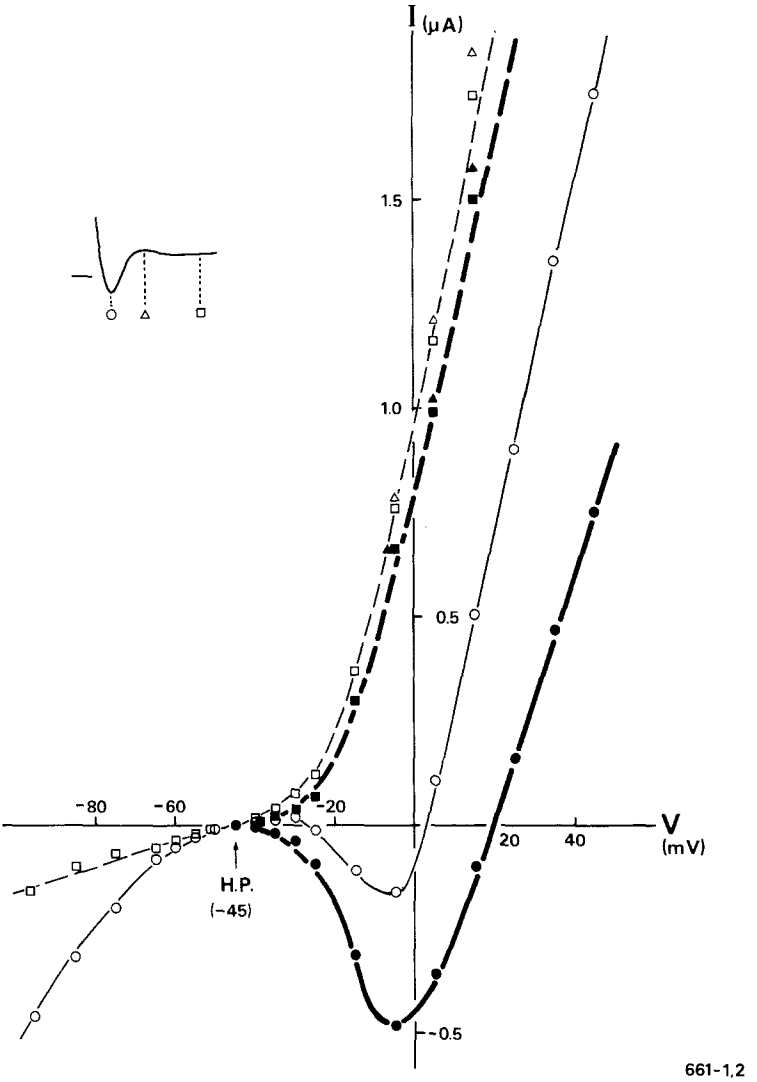

Fig. 2*B*

Membrane Activities under Conditions in which the Inward and/or Outward Currents are Suppressed

Effects of cobalt ion. Transition metal ions such as cobalt (Co^{++}) and manganese (Mn^{++}) suppress the spike (Abe, 1971; Nonomura, Hotta & Ohashi, 1966) and the early inward current (Kumamoto & Horn, 1970; Mironneau, 1974; Bury & Shuba, 1976; Inomata & Kao, 1976) of uterine and other smooth muscles. Recently, Muramatsu *et al.* (1976) found that 1 mM Co^{++} suppressed the early inward current completely

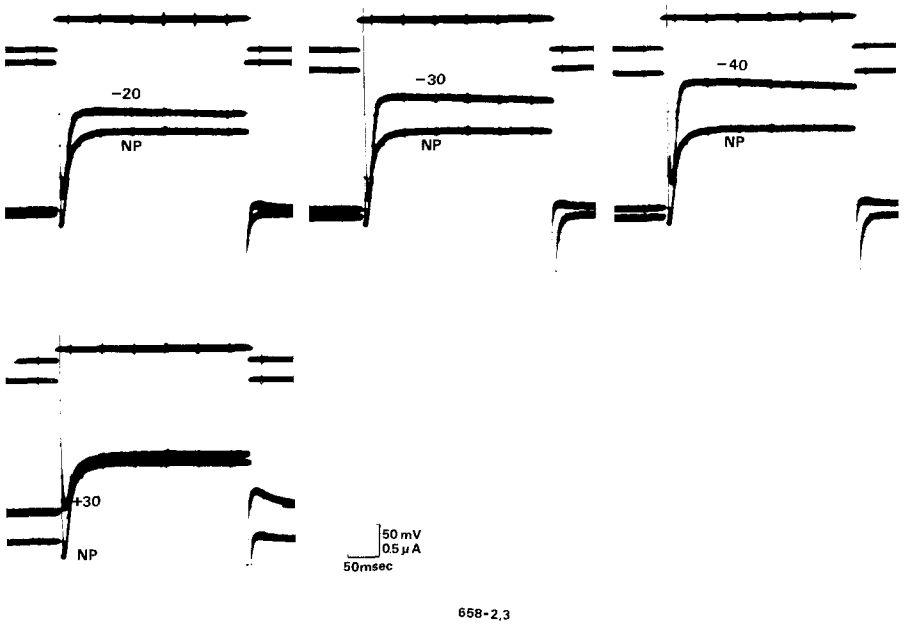

Fig. 3. Effects of conditioning polarizations on the ionic currents of pregnant rat uterus. (*A*): Effects of different amplitudes of conditioning pre-pulse. The holding potential was the same as the resting membrane potential (−50 mV). After a conditioning pulse of 400 msec, the membrane was depolarized to 0 mV for 300 msec. Numbers on each of the current traces represent the amplitudes of pre-pulses measured from the resting membrane potential. *NP* shows effects of the test pulse without pre-pulse. Note that the current trace is shifted in the upward direction by conditioning hyperpolarization. (*B*): The conditioning pre-pulses were fixed at 20 mV more negative (here at −67 mV) than the resting membrane potential of −47 mV and had durations varying from 10 to 1,000 msec (*T* in the inset). Membrane currents resulting from the subsequent test pulses were measured at the peak downward deflection (●), 50 (▲), 100 (■) and 300 (□) msec after the onset of the test pulse (*see* inset). Changes in the membrane currents from the control with no pre-pulse (ordinate) are plotted against various duration of pre-pulse (abscissa). All currents are shifted towards more positive, and thus the inward current (●) decreased and the outward currents (▲, ■ and □) increased with increasing duration of the pre-pulse

whereas the outward current was unaffected in pregnant rat myometrial muscle. Similar results were reported by Hagiwara, Hayashi and Takahashi (1969), who found that even several times that concentration (2–5 mM) had no effect on the steady-state current of barnacle muscle fibers. Therefore, we used the Co ion as a selective blocker of the early inward current. Cobalt at a concentration of 1 mM abolished the spike completely, but did not affect the resting potential and the leakage current.

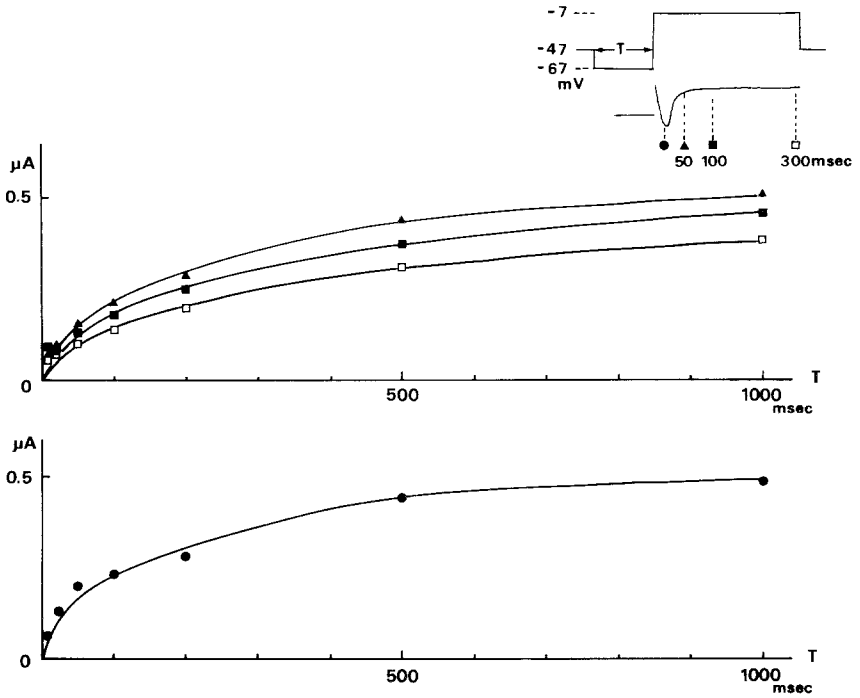

Fig. 3*B*

Figure 4*A* shows the effects of Co^{++} on the ionic currents elicited by a depolarizing pulse under voltage-clamp conditions. In the control (record *a*), the ionic current is composed of the early inward and the outward currents. Cobalt suppressed the early inward current completely but had no effect on the outward current.

The current-voltage relationships of the early inward current and the outward current are plotted in Fig. 4*B* before and after treatment with 1 mM Co^{++}. The early inward current (open circles) was completely suppressed, and the recorded current was in outward direction (filled circles), measured at the time corresponding to the peak of the inward current before Co^{++} treatment. This indicates that the outward current overlaps with the inward current before the application of Co^{++}. When the apparent inward current was corrected for the overlapping outward current, the maximal inward current occurs at about +10 mV and the current reverses its polarity at $+62 \pm 2.3$ mV ($n=6$) (thick dotted line). Twenty to thirty percent overlapping of the outward current occurs at the peak of inward current, and this amount of overlapping is close to that of the guinea pig *taenia coli* (Kumamoto & Horn, 1970) and

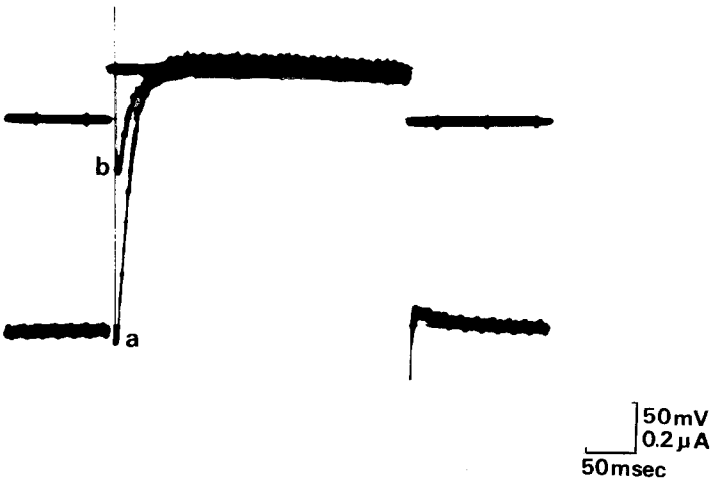

Fig. 4. (*A*) Effects of cobalt ion on the ionic currents. The currents elicited by a 50-mV depolarization step before (*a*) and after (*b*) application of 1 mM Co^{++} have been superimposed. (*B*) Current-voltage relationships before and after application of Co^{++} (1 mM). All currents except the thick dotted line were corrected for capacitive and leakage currents only. Circles and squares are ionic currents measured at the two points shown in the inset; open symbols (○, □) are controls, filled symbols (●, ■), 11 min after application of Co^{++} (1 mM), and open symbols with dots (⊙, ⊡), 6 min after recovery. The thick dotted line without symbols represents the inward current after correction for the overlapping outward current and the capacitive and leakage currents

the rat portal vein (Kumamoto *et al.*, 1977*b*); the overlapping increases with increasing depolarization.

Effects of TEA. Under current clamp conditions, TEA (30 mM) increased the spike height, decreased the maximum rate of fall of the spike and prolonged the duration of the spike (Fig. 5*A*). The resting membrane potential was not changed by TEA. Representative recordings of the current under voltage-clamp conditions are shown in Fig. 5*B*. Treatment with TEA increased the early inward current and reduced the outward current. The peak of the inward current was delayed by TEA. Such a change in time course indicates strongly that the increase in the inward current is the result of a decrease in the outward current which overlaps with the early inward current. The reversal potential of the inward current was shifted in the direction of depolarization by about 10 mV (open and filled circles in Fig. 5*C*). This shift corresponds to the augmentation of the spike amplitude under current clamp conditions (Fig. 5*A*). As shown in Fig. 5*B* and *C*, TEA suppressed the initial

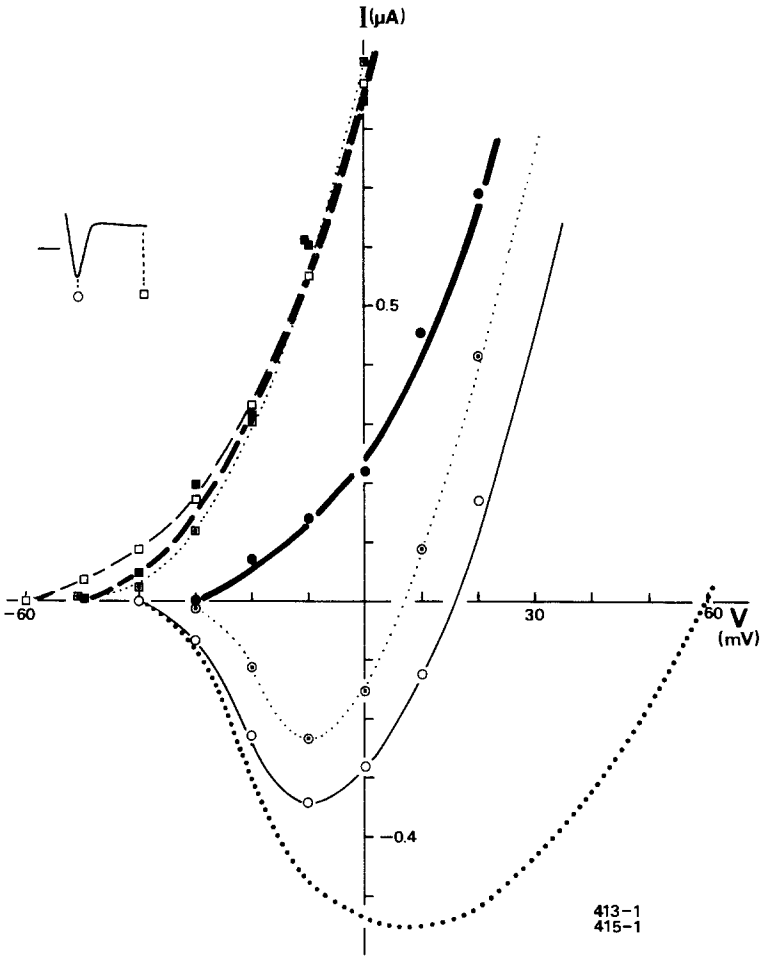

Fig. 4*B*

part of the outward current (triangles) more than the late part (squares). The suppressions of the initial and late parts of outward current by TEA were dose-dependent: about 40% and 27%, respectively, by 10 mM TEA ($n=3$), and 90% and 56%, respectively, by 30 mM TEA ($n=6$) at 0 mV. Recently, Vassort (1975, 1976) reported the existence of a fast outward current which is triggered by Ca influx and inhibited by TEA in the guinea pig myometrium. The present results with TEA also suggest the existence of such a fast outward current in the pregnant rat uterus and its possible overlapping with the early inward current.

Since the initial and late parts of the outward current are both sensitive to TEA, they are probably both carried by K- ions. Evidence supporting

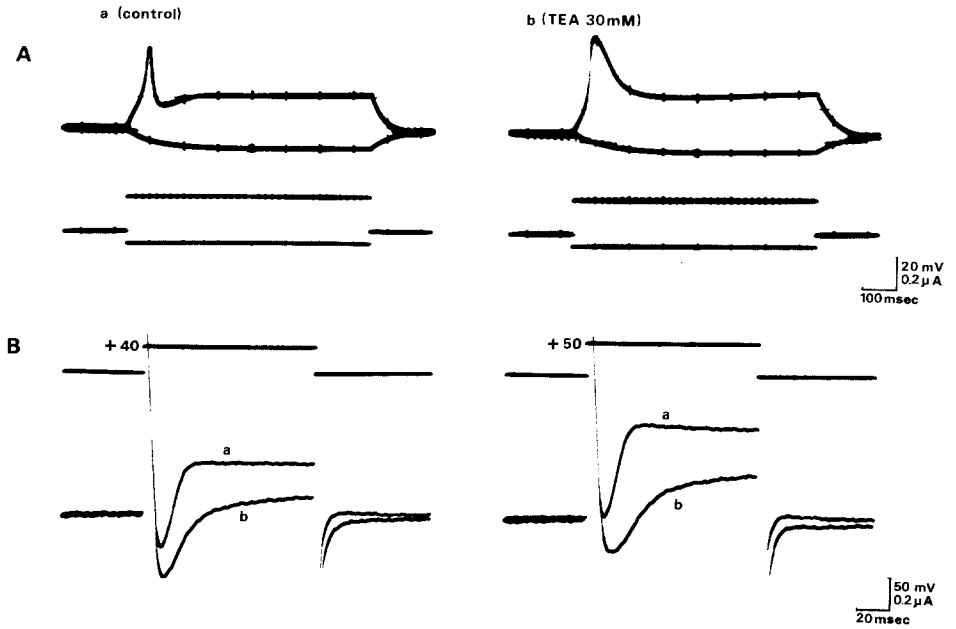

Fig. 5. Electrical activity of pregnant rat uterus before and after TEA. (*A*): Voltage recordings under current clamp conditions. (*B*): Membrane currents under voltage clamp conditions. The ionic currents before (*a*) and after (*b*) TEA were superimposed. Numbers represent the amplitudes of the depolarization steps. (*C*): Current-voltage relationships obtained from the records in *B*. Ionic currents were measured at three points as shown in the inset. Open and filled symbols are controls and at 6.5 min after application of 30 mM TEA, respectively

this view was provided by determinations of the reversal potentials using a double command pulse technique (Kao & McCullough, 1975). After a 50-mV depolarization pulse lasting for 50 or 100 msec, different test potentials were imposed, and the membrane potential at which the tail current associated with the test pulse became zero was determined. The reversal potential thus measured was -87 ± 2 mV ($n=10$).

Effects of conditioning voltage pulses in the presence of cobalt ions and/or TEA. Since the early inward current was overlapping with the outward current, the decrease in the inward current by conditioning hyperpolarization in normal Krebs solution may not be simply explained in terms of conductance changes for the inward current. To elucidate this mechanism, the effects of the conditioning pulses were compared before and after application of Co^{++}. After treatment with 1 mM Co^{++}, the early inward current was completely blocked, while the late outward current

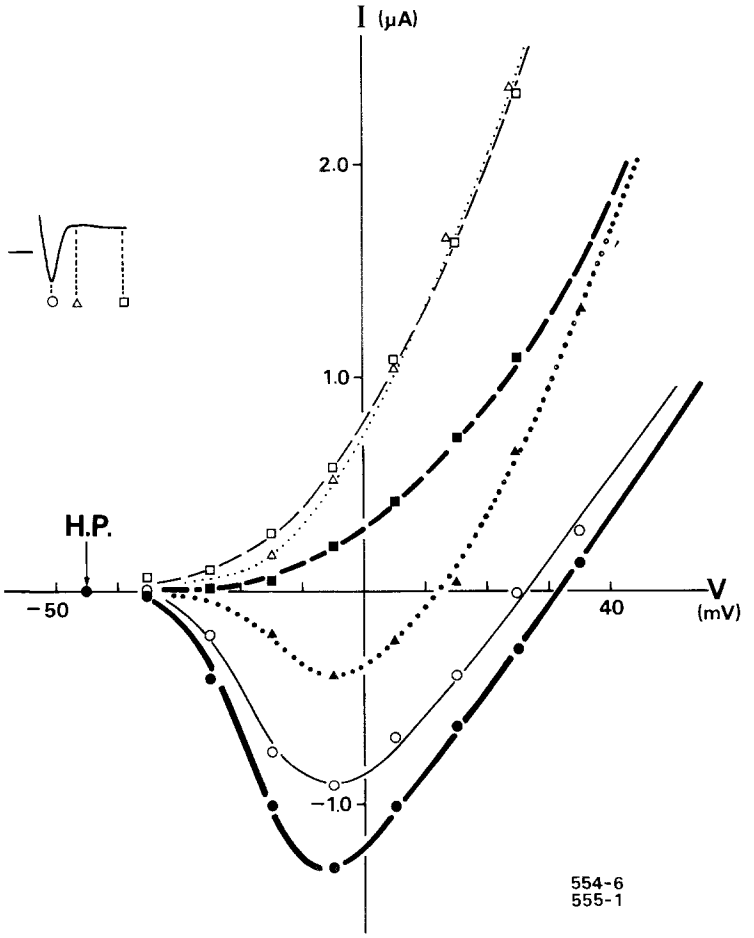

Fig. 5 C

was unaffected (Fig. 6 *A*). The amount of inward current corrected for due to the overlapping outward current is represented by the difference between the current before (*a*) and that after (*b*) treatment with Co^{++}. In contrast to the conditioning depolarization, the conditioning hyperpolarization had little effect on the corrected inward current, i.e., a correction equal to the difference between (*a*) and (*b*) in Fig. 6 *A*. When the current was measured directly at the peak of the downward deflection (open circles in Fig. 6 *B*), the lowest values were obtained near the resting membrane potential and increased with conditioning hyperpolarization and depolarization. However, after correction for the overlapping outward current, the current (filled circles) changed little with hyperpolariz-

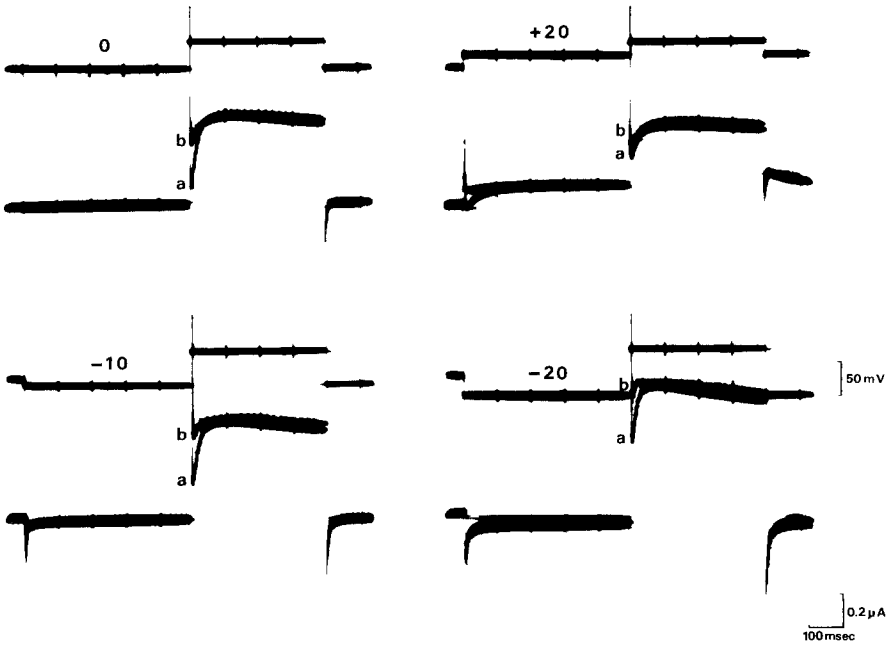

Fig. 6. Effects of conditioning pre-pulses in Co^{++}-treated preparations. (*A*): Membrane currents before and after 1 mM Co^{++} are labeled *a* and *b*, respectively. The membrane was held at the resting membrane potential (here −43 mV). Following the conditioning pre-pulses of 500 msec, the membrane was depolarized to −3 mV. The numbers on the voltage traces represent the amplitudes of the pre-pulses measured from the resting membrane potential. (*B*): The relationship between the amplitude of the early inward current (ordinate) and the potential of the conditioning pre-pulses (abscissa). The inward current was measured in three ways, as shown in the inset; open circle represents direct measurement of the total current at the peak of the downward deflection, open triangle after correction for capacitive and leakage currents, and filled circle after correlation for also the overlapping outward current. For further detail, *see* text

ing conditioning pulses (Fig. 6*B*). The apparent inward current corrected only for the leakage and capacitive currents was markedly decreased by conditioning hyperpolarization (open triangles in Fig. 6*B*). These observations indicate that the "true" inward current is not suppressed by conditioning hyperpolarization.

Further evidence was provided with TEA-treated preparations. The strips were pretreated with 30 mM TEA in order to suppress the outward current partially overlapping the inward current. The effects of the conditioning polarization with TEA (Fig. 7*A*) were similar to those seen in Fig. 6*A* where no TEA was used. Cobalt blocked the inward current completely but had no effect on the late outward current. Therefore,

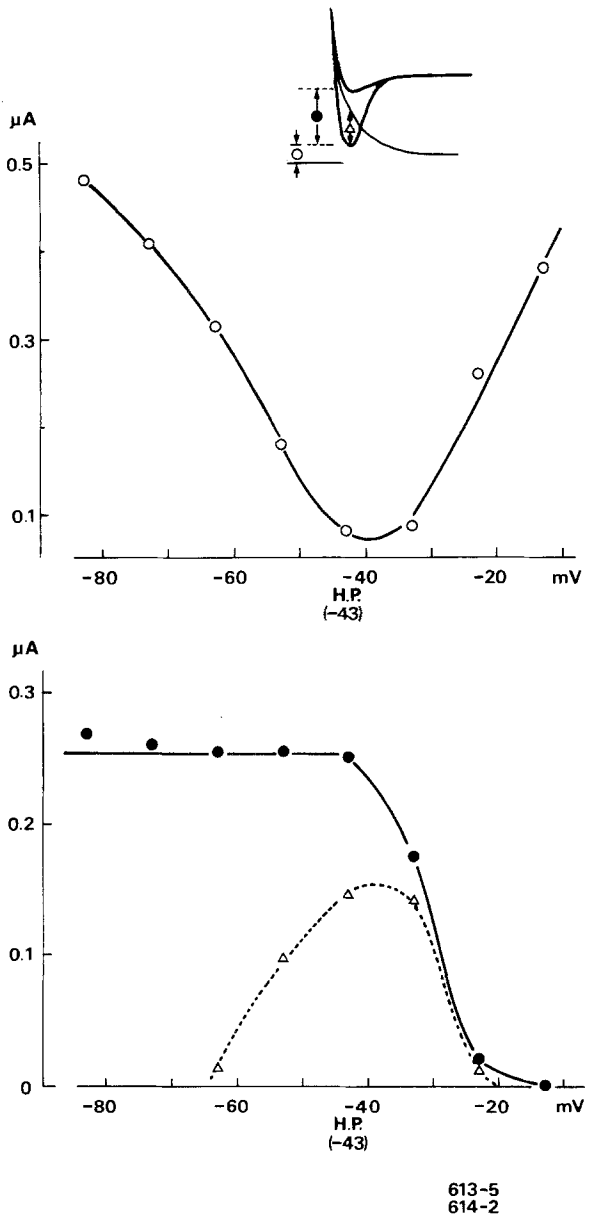

Fig. 6*B*

the difference between the two current traces shows the "true" inward current. When the inward current was measured and plotted in the same way as in Fig. 6*B*, the "true" inward current shows little or no change with the conditioning hyperpolarization (Fig. 7*B*). Conditioning hyperpolarization suppresses only the apparent inward current obtained by cor-

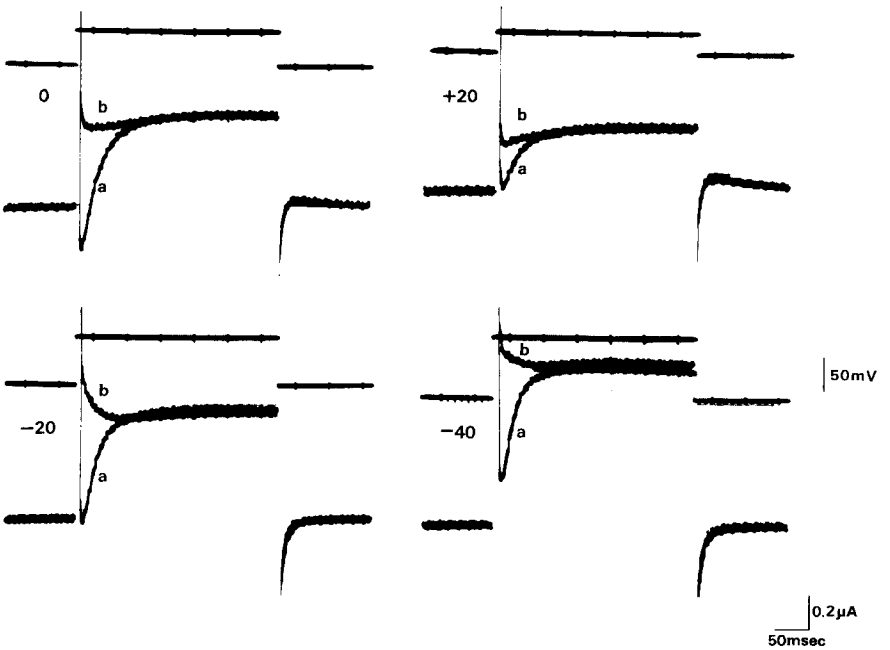

Fig. 7. Effects of conditioning pre-pulses on Co^{++}-treated preparation in presence of TEA. The preparation was soaked in 30 mM TEA prior to Co^{++} application. The membrane was held at the resting membrane potential (−50 mV). After conditioning pre-pulses of 400 msec, the membrane was depolarized to 0 mV. (*A*): Membrane currents before and after 1 mM Co^{++} are labeled *a* and *b*, respectively. (*B*): Relationship between the amplitude of the early inward current and the potential of the conditioning pulse. The symbols are the same as those used in Fig. 6. For further detail, *see* text

rection for only capacitive and leakage currents (Fig. 7*B*). Thus, the suppression of the apparent inward current with the conditioning hyperpolarization must be due to an increase in the outward current overlapping with the "true" inward current. The "true" inward current is suppressed only by the conditioning depolarization.

Discussion

Although voltage clamp analysis of the membrane ionic conductances of smooth muscle with the double sucrose gap technique has some limitations due to the complex geometry of the preparation (Anderson, 1969), large series resistance (Tarr & Trank, 1974), sucrose diffusion into the active area (Harrington & Johnson, 1973), liquid junction potentials

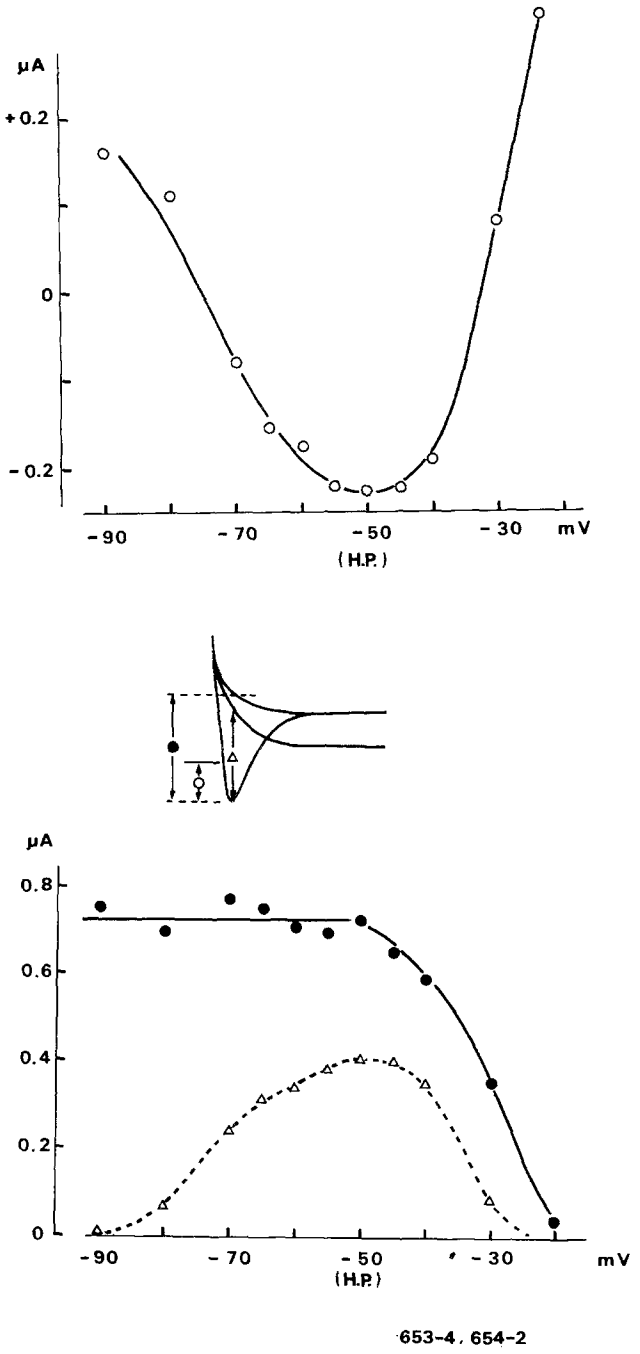
μA
+0.2
0
-0.2
-90
-70
-50
-30
mV
(H.P.)
μA
0.8
0.6
0.4
0.2
0
-90
-70
-50
-30
mV
(H.P.)
653-4, 654-2

Fig. 7*B*

(Julian, Moore & Goldman, 1962), and a large leakage current (McGuigan & Tsien, in the *appendix* of McGuigan, 1974), the results obtained are nontheless informative in a qualitative sense provided that the experimental conditions are adequately controlled.

Muramatsu *et al.* (1976) have shown that the outward currents in the pregnant rat uterine smooth muscle may have a relatively early activation and that the early transient inward current determining rate of rise and spike amplitude may be attenuated to a varying degree because it overlaps with this early part of the outward currents, in the same way as suggested for the guinea pig *taenia coli* (Kumamoto & Horn, 1970) and myometrium (Vassort, 1975, 1976) and the rat portal vein (Kumamoto *et al.*, 1977*b*). The present study confirms such an overlapping mechanism of the inward and outward currents in preparations from pregnant rat myometrium. This suggests that the early inward current observed is usually masked not only by the capacitive and leakage currents but also by overlapping potassium current. Therefore, the true magnitude of the inward current may be realized only after the correction for all of the overlapping currents.

The degree of overlapping could be estimated by the treatment with 1 mM Co^{++}, which abolished the early inward current without significantly affecting the late outward current, as reported previously by Muramatsu *et al.* (1976). As already mentioned, Vassort (1975, 1976) has proposed the existence of a fast, early outward current ("hump"), triggered by the inward current, and which in turn may overlap with the inward current in the guinea pig myometrium. The present results with TEA are consistent with the presence of such a fast outward current also in the rat uterine smooth muscle. However, it is difficult to separate the fast outward current from the late outward current utilizing Co^{++} and/or TEA. Because the time course of the early part of outward current is smoothly followed by the late outward current without showing a distinct hump and also because there is no significant difference between the slope conductances measured at the initial part and the late part of the outward current (Fig. 2*B*, filled triangles and squares), the fast outward current component must be very small, if indeed present, in this tissue. Therefore, the "true" inward current can be estimated by correction for the overlapping late outward current and the capacitive and leakage currents. It should be noted that relatively rapid inactivation of outward current has been observed in many different preparations (*see* Grundfest, 1966), and if a fast component is indeed present in pregnant rat myometrium but obscured due to rapid inactivation, then

the corrections applied here are underestimating the degree of overlap rather than any possible overestimation.

Conditioning hyperpolarization increased the outward current and decreased the apparent inward current. The depression of the inward current by conditioning hyperpolarization has been observed in the rat (Kao & McCullough, 1975; Anderson & Ramon, 1976) and guinea pig (Vassort, 1976) myometria and in the *Aplysia* (Geduldig & Gruener, 1970) and snail (Nehr, 1971) neurons. However, Anderson and Ramon (1976) did not attribute the apparent decrease in the transient inward current to an increase in the overlapping outward current in the estrogen-dominated rat uterus, because the amplitude of the transient inward current was not affected by TEA, used in an attempt to suppress the outward current. Vassort (1976) suggested that the conditioning hyperpolarization reduced the inward current as a result of inactivation of the Ca conductance and not due to effects on the overlapping fast outward current, as deduced from the experiments with 15 mM TEA which suppressed the fast outward current but had a lesser effect on the late steady-state current. Even twice this concentration, 30 mM of TEA, in our experiments, did not completely suppress the late outward currents. When the inward current was corrected for the overlapping late outward current utilizing 1 mM Co^{++}, depression of the inward current was not observed.

Increase in the outward currents by conditioning hyperpolarization may be due to a decrease in the resting membrane resistance, a change in the ionic concentration gradients across the membrane or an increase in available potassium channels. However, the first possibility can be ruled out by the fact that the relations between the hyperpolarizing current and the resultant potential change and those between the hyperpolarizing pulse and the leakage current are linear between -45 and -100 mV. As to the second possibility, Kao and McCullough (1975) have suggested that when small cells such as those of smooth muscle are kept at a voltage requiring an appreciable amount of current, the intracellular ionic concentrations might be changed. However, the duration of the conditioning pulses used in the present experiments would be too short to introduce significant changes in the ionic concentration gradients. Furthermore, the conditioning depolarization of the same duration did not produce an opposite effect, i.e., a decrease in the outward current. Therefore, the second possibility may also be excluded. The outward current increased with time and voltage of the conditioning hyperpolarization. It would thus appear that the conditioning hyperpolar-

ization makes more potassium channels available for activation. The potassium channels may be partly inactivated at the resting membrane potential and this inactivation would be removed by the hyperpolarization. The modulation of excitability at and below the resting membrane potential, e.g., between bursts of spontaneous electrical activity, may be attributed to changes in the availability of the potassium channels. This view is also supported by the fact that when the membrane returned to this resting potential from a preceding large hyperpolarization, an outward current was produced in the tail current and its magnitude was dependent on the duration and the voltage of the hyperpolarization (*see* arrow in Fig. 2*A*). This removal of inactivation by hyperpolarization is only slightly affected by TEA. Partial inactivation of the potassium channels at the resting membrane potential level has recently been demonstrated in the molluscan neurons (Conner & Stevens, 1971; Nehr, 1971; Nehr & Lux, 1972).

Thus, it must be concluded that the apparent depression of the inward current following conditioning hyperpolarization in rat myometrium is the result of an increase in the outward current overlapping with the inward current, and that the inward current itself is not altered by the conditioning hyperpolarization. After correction for this overlap, the voltage dependence of the inactivation curve for the inward current carrying mechanism looks very similar to that of other excitable membranes. The attenuation of the spike amplitude in other smooth muscles by hyperpolarization (Nagai & Prosser, 1963; Tomita, 1966, 1967) may be explained similarly.

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