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INACTIVATION OF CALCIUM CHANNEL CURRENT IN RAT UTERINE SMOOTH MUSCLE: EVIDENCE FOR CALCIUM- AND VOLTAGE-MEDIATED MECHANISMS

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SUMMARY

1. Ca channel currents were recorded in Cs-loaded myometrial strips from pregnant rats after addition of tetraethylammonium chloride and 4-aminopyridine (10 mM each) by means of a double sucrose-gap technique.

2. During a depolarizing pulse, the decay of Ca channel current was slowed down when external Ca was replaced by Ba or Sr. This decay represented an inactivation phenomenon, as assessed by the decreased amplitude of inward tail currents following progressively longer depolarizations, the absence of shift in peak conductance curves against membrane potential, and the stable value of the reversal potential when Ba current was increased during conditioning pulses.

3. Inactivation of Ca and Ba currents through Ca channels was studied using the double-pulse method. Conditioning pulses that produced maximal Ca current induced maximal inactivation; with stronger depolarizations, inactivation decreased but was not completely prevented at the expected Ca reversal potential. Increasing the amount of Ca entering the cell during the pre-pulse reduced both amplitude and kinetics of test Ca currents. These results were not observed with Ba as charge carrier suggesting the participation of different mechanisms in inactivation.

4. With Ca as charge carrier, increasing the external Ca speeded the rate of inactivation. This was not observed with Ba outside. Addition of Co (2·5 mM) reduced the amplitude of both Ca and Ba currents but slowed the inactivation of only the Ca current.

5. Recovery from inactivation was described as a two-exponential process only when the conditioning pulse elicited a Ca inward current. In all other cases, recovery from inactivation was represented as a single exponential curve.

6. It is suggested that inactivation of Ca channels in rat uterine smooth muscle is mediated by both internal Ca-dependent and potential-dependent mechanisms.

INTRODUCTION

The existence of an inward Ca current responsible for the action potential in smooth muscles, originally suggested by Brading, Bülbring & Tomita (1969) and

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Bülbring & Kuriyama (1963) is now well supported by experimental evidence obtained by several groups (Mironneau, 1973; Vassort, 1975; Inomata & Kao, 1976; Walsh & Singer, 1980; Bolton, Lang, Takewaki & Benham, 1985; Isenberg, & Klöckner, 1985). The decay of Ca current during a maintained depolarization (called 'inactivation') was first attributed to a voltage-dependent process, analogous to inactivation of Na channels in squid axon (Hodgkin & Huxley, 1952); this description has been widely used (Mironneau, 1974; Anderson, 1975; Vassort, 1975; Kao & McCullough, 1975; Muramatsu, Kumamoto & Fujiwara, 1978). However, several mechanisms for inactivation of Ca channels in excitable cells have been recently reviewed (Hagiwara & Byerly, 1981; Tsien, 1983; Eckert & Chad, 1984). Some Ca channels show little or no inactivation, but those that do inactivate appear to do so by a voltage-dependent process (Fox, 1981), by an intracellular-mediated process (Tillotson, 1979; Eckert & Tillotson, 1981; Ashcroft & Stanfield, 1982; Mentrard, Vassort & Fischmeister, 1984), or by a combined mechanism (Brown, Morimoto, Tsuda & Wilson, 1981; Lee, Marban & Tsien, 1985). As another possibility, the decay of Ca current during a maintained depolarization can result from Ca depletion in a restricted extracellular space (Almers, Fink & Palade, 1981). All these reports have prompted a detailed examination of Ca channel decay in smooth muscle cells. The obvious question is whether the mechanism of uterine Ca channel inactivation can be classified as one of the above mechanisms.

This paper presents evidence that Ca channel inactivation in rat uterine smooth muscle is controlled by membrane depolarization as well as intracellular Ca. It is shown that inactivation of the Ca channel persists when Ba replaces external Ca, and that this inactivation satisfies the criteria for a voltage-dependent process. When Ca is the permeant divalent cation, inactivation is characterized by both voltage- and Ca-mediated mechanisms. In both cases, voltage-clamp analysis of the Ca channel current in uterine fibres was made possible by both Cs loading and addition of tetraethylammonium chloride and 4-aminopyridine to minimize outward currents through K channels. Some aspects of this work have been reported in a preliminary communication (Jmari, Mironneau & Mironneau, 1986).

METHODS

Isolated longitudinal strips from pregnant rat myometrium (70–100 μm in diameter; 3–4 mm in length) taken at the end of pregnancy (18–19 days) were used in the present experiments. In order to reduce the outward K currents, uterine strips were dissected in a K-free, 125 mM-Cs solution and bathed for 30–60 min in this solution before setting in the double sucrose-gap apparatus (Mironneau, 1973). With this method, adequate membrane voltage control was limited by the presence of a significant series resistance, and uniformity of the voltage control was limited by the multicellular and inherent cable properties of the muscular strips (Bolton, Tomita & Vassort, 1981). Therefore, the following precautions and tests were applied: (1) the length of the test compartment (150–200 μm) was relatively short in comparison with the space constant of the preparation (2·5 mm in resting conditions, Kuriyama & Suzuki, 1976). The low strip diameter/test compartment ratio (0·5) minimized sucrose–ionic solution diffusion and allowed viable action potentials to be obtained (Fig. 1); (2) direct evidence of voltage-clamp control was verified in some experiments by measuring the voltage independently in the test gap with a micro-electrode (Mironneau, Savineau & Mironneau, 1981). Action potentials and membrane currents were displayed on an oscilloscope and stored on a tape recorder (Hewlett-Packard 3968A) for subsequent analysis by a microcomputer (Tektronix 4052).

Physiological solutions had the following composition: (1) reference solution (mM): NaCl, 130; $CaCl_2$, 2·1; $MgCl_2$, 0·24; glucose, 11. The solution was aerated with O_2 and buffered by Tris-HCl (8·3 mM) at pH 7·4. (2) Cs-loading solution (mM): NaCl, 10; CsCl, 125; $CaCl_2$, 2·1; $MgCl_2$, 0·24; glucose, 11. Loading with high Cs solution for 30 min was accompanied by a noticeable depolarization (20–25 mV). Preparations were held at their original resting potential by hyperpolarization. (3) Ba or Sr solutions were obtained by substituting Ba or Sr in the reference solution for Ca in equimolar amounts. (4) Ca-rich or Ba-rich solutions were obtained by the addition of $CaCl_2$ or $BaCl_2$, respectively, to the solution. In these cases, the $MgCl_2$ concentration of the reference solution was brought up to the final Ca or Ba concentrations so as to maintain constant the external divalent cation concentration and to minimize surface potential shifts. (5) Co (2·5 mM) was used to inhibit the Ca current; tetraethylammonium chloride (TEA) and 4-aminopyridine (10 mM each) were used as inhibitors of the K currents (Mironneau *et al.* 1981). All the solutions were maintained at 30 ± 1 °C and the flow in the test compartment of the double sucrose gap was maintained at 0·5 ml/min by means of a constant flow pump. The rate of stimulation was 1/15 s.

The variations in membrane potential, V (mV), were imposed from the resting potential which was estimated to be -50 mV. Positive values of V represent a depolarization, negative values a hyperpolarization. Positive membrane currents, I (μA), correspond to an outward current, negative values to an inward current. Under our experimental conditions, inward current through Ca channel is considered as a pure divalent cation current. Because Ca is not the only permeant divalent charge carrier used in this study, we refer to current through this channel as Ca channel current.

RESULTS

Measurements of Ca channel currents in uterine smooth muscle

To study mechanisms of Ca channel current inactivation, it was first necessary to demonstrate that time-dependent changes in membrane conductantance during depolarizing voltage pulses were due to a Ca current and not to other current components or to depletion of extracellular divalent charge carriers. In uterine smooth muscle, it has been previously shown that large K outward currents partially superimpose on the Ca inward current (Vassort, 1975; Muramatsu *et al.* 1978; Mironneau *et al.* 1981) and complicate the analysis of the Ca conductance. In this study, K outward currents were largely blocked by substituting Cs for intracellular K. During Cs loading, intracellular K was believed to be replaced by Cs since Cs can substitute for K on the Na–K pump, as previously indicated on cardiac muscles (Guérin & Wallon, 1979; Mentrard *et al.* 1984). The Cs loading (30 min or more) induced a progressive depolarization in the resting potential which reached -20 to -25 mV when measured with an intracellular micro-electrode (five different preparations). When the normal resting potential had been re-established by hyperpolarizing the preparation, action potentials with larger amplitude and duration were elicited with the double sucrose gap (Fig. 1*A*). For example, the amplitude of the action potentials was 79 ± 7 mV ($n = 15$) and the duration (measured when repolarization had reached 80 % of its value) was 230 ± 25 ms ($n = 15$) compared with that in control. The maximal rate of rise and of repolarization of the action potentials were $2{\cdot}3 \pm 0{\cdot}5$ and $0{\cdot}50 \pm 0{\cdot}3$ V/s, respectively ($n = 7$). After Cs loading, application of both TEA ions (10 mM) and 4-aminopyridine (10 mM) did not significantly enhance the action potential amplitude but an increase in duration was generally observed (Fig. 1*A*). An equimolar replacement of Ba for Ca resulted in the development of action potentials characterized by a long duration and a similar amplitude (Fig. 1*B*). Typically, the action potential duration (at 80 % of repolarization) was 1100 ± 290 ms ($n = 10$). The rate of rise and of repolarization of the Ba

action potentials were $2{\cdot}2 \pm 0{\cdot}36$ and $0{\cdot}3 \pm 0{\cdot}1$ V/s, respectively, in Cs-loaded cells ($n = 10$). The absence of a significant effect on the maximal rate of repolarization in Ba solution suggests that inward Ba current failed to activate Ca-activated K channels. The effectiveness of cellular Cs loading and K inhibitor application was also

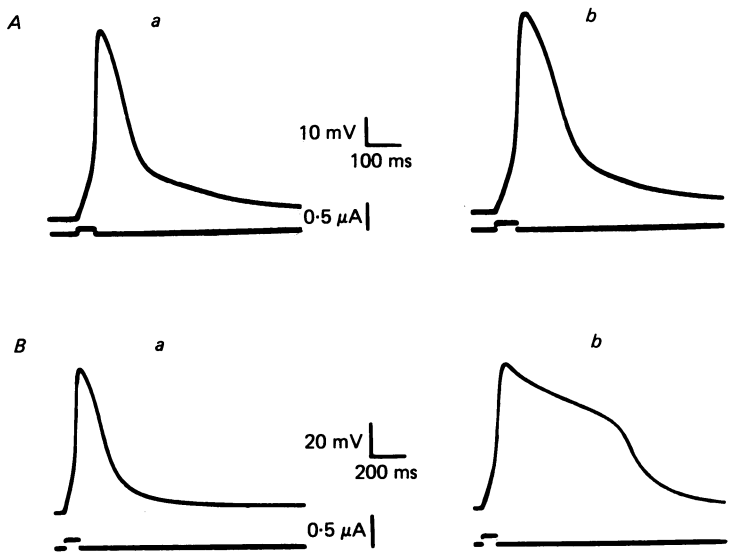

Fig. 1. Action potentials recorded from pregnant rat myometrial strips after Cs loading. *A*, the time course of the action potential in reference solution (*a*) is slightly modified after addition of tetraethylammonium chloride (TEA, 10 mM) and 4-aminopyridine (4-AP, 10 mM) (*b*). *B*, effect of Ba substitution for Ca on the action potential from another preparation. A maintained plateau is observed in Ba solution (*b*) when compared to reference with K inhibitors (TEA+4-AP, *a*).

illustrated by the flattening of both Ca and Ba current obtained after blockage of the channel by 2·5 mM-Co (Fig. 2). In the presence of Co the current–voltage relationship (Fig. 2*A*) was linear between -20 and $+60$ mV. For depolarizations above $+60$ mV, only a small rectification was observed. This rectifying current was thus considered to be mostly a leakage current. It was also noted that Co addition had no effect on the inward currents obtained during hyperpolarizing voltage pulses. For each depolarizing voltage, the inward current amplitude was estimated as the difference between the peak total current and the current recorded in Co-containing solution. When Ca was the divalent cation (Fig. 2*A*), the inward current displayed a rapid phase of decay followed by a much slower decline. As shown on the current–voltage relationship, the Ca current reached a maximum for depolarizations of $+40$ mV and approached zero for depolarizations near $+110$ mV. Outward currents through a Ca channel with Ca ions as charge carrier were never observed ($n = 10$). Ba current through the Ca channel decayed more slowly (Fig. 2*B*) and a net inward current component was observed at the end of the depolarizing pulse. As revealed by the current–voltage relationship, the peak Ba current was larger than the Ca current with a maximum near $+40$ mV. A clear reversal potential with Ba outside was obtained at $+115$ mV ($n = 3$).

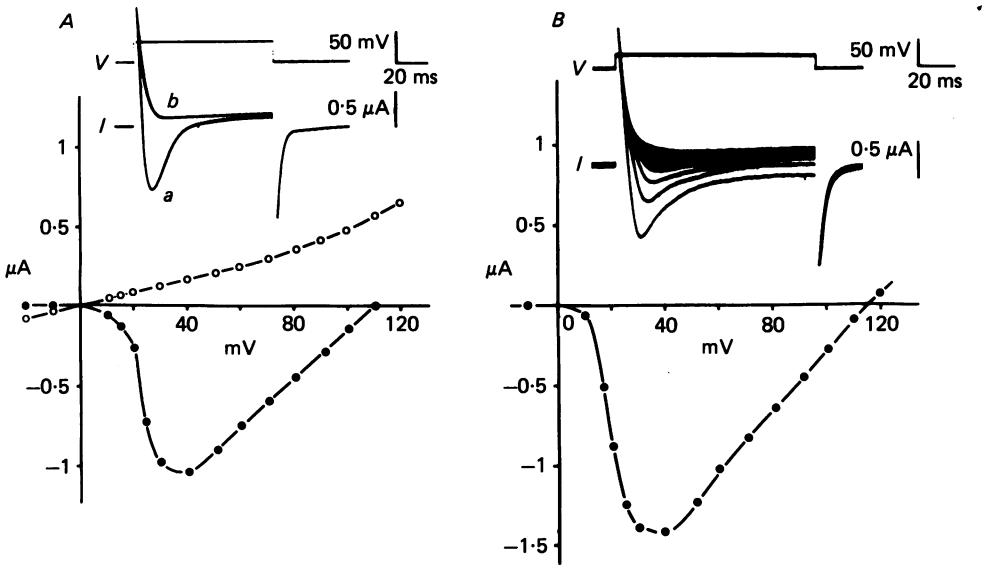

Fig. 2. Membrane current in Cs-loaded uterine preparations. *A*, current–voltage relationships established for the current in 2·5 mM-Co (open circles) and for the corrected Ca current in 2·1 mM-Ca solution containing TEA plus 4-AP (10 mM, filled circles). Inset: the corrected Ca current was estimated as the difference between the peak total current (*a*) and the current recorded in Co-containing solution (*b*). *B*, current–voltage relationship established for the corrected peak Ba current, as described in *A*. Inset: successive current traces during inhibition of Ba current by 2·5 mM-Co, showing no significant variation of the inactivation rate. *V*, potential; *I*, membrane current.

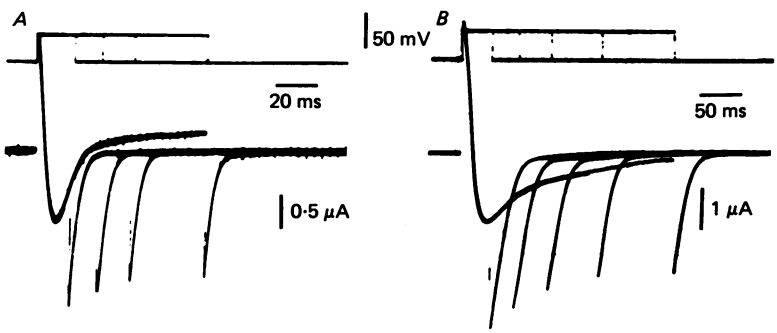

Fig. 3. Ca channel inactivation studied with inward tail currents on Cs-loaded uterine preparations in Ca solution, *A*, and Ba solution, *B*. Inward tails accompany the cessation of depolarizing pulses and decline with the same time course as the current during the pulse.

Two types of experiments were made in order to demonstrate that the decay of inward current was mainly due to a decline of the Ca channel conductance, i.e. an inactivation process. The first one concerned measurements of inward tail currents through Ca channels. As shown in Fig. 3, the amplitude of the inward tails declined with the same time course as the current during the pulse, regardless of whether the charge carrier was Ca (*A*) or Ba (*B*). This observation argued against the idea that the decay of net inward current was caused by a progressive activation of an outward

current through K channels, which would require that the tail current became progressively outward. The second concerned the possibility that depletion of charge carrier from a restricted extracellular space might contribute to the decay of inward currents in a multicellular preparation. Fig. 4 shows the measurement of the reversal

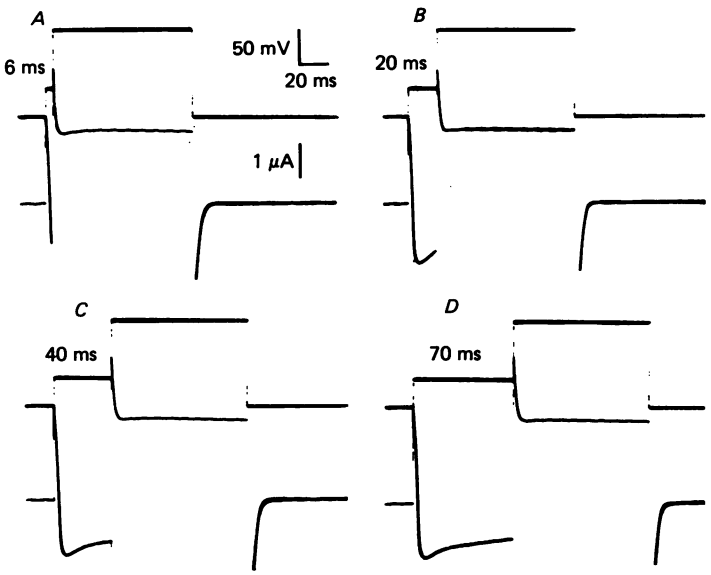

Fig. 4. Test for depletion of charge carrier. When Ba is used as charge carrier, a reversal potential is obtained at +115 mV. Increasing the pre-pulse duration from 6 to 70 ms (*A–D*) has no effect on the Ba reversal potential.

potential when Ba was used as the charge carrier. The reversal potential was found for a depolarization of +115 mV by applying a series of depolarizing voltage steps from a −50 mV holding potential. Then, depolarizing pre-pulses from 6 to 70 ms in duration were applied to promote a maximal inward current before repeating the reversal potential measurements. The reversal potential of the Ba current remained at the same potential value in this and three other preparations. These results suggest that the decay of the Ca channel current in these experiments was not caused by changes in the extracellular ion concentration.

Ca channel inactivation in the presence of extracellular Ca

Ca channel inactivation was studied using a two-pulse voltage-clamp protocol (Tillotson, 1979; Eckert & Chad, 1984). In this procedure, inactivation induced during an initial pulse (conditioning pulse or V_1) was estimated by the reduction of the peak current associated with the second depolarization (test pulse or V_2). The conditioning and test pulses were separated by a rest interval long enough to allow non-inactivated channels to deactivate, but short enough to allow little recovery from inactivation. In the absence of a conditioning pulse, the test depolarization (+35 mV) elicited a large Ca current (Fig. 5*A*). The test current was substantially

reduced by a 65 ms conditioning depolarization of +40 mV. Further increase in the depolarizing pre-pulse to +140 mV led to a smaller reduction in the test current. The observation that strong conditioning depolarizations produced less inactivation than moderate depolarizations was confirmed in forty-six other Cs-loaded uterine prepa-

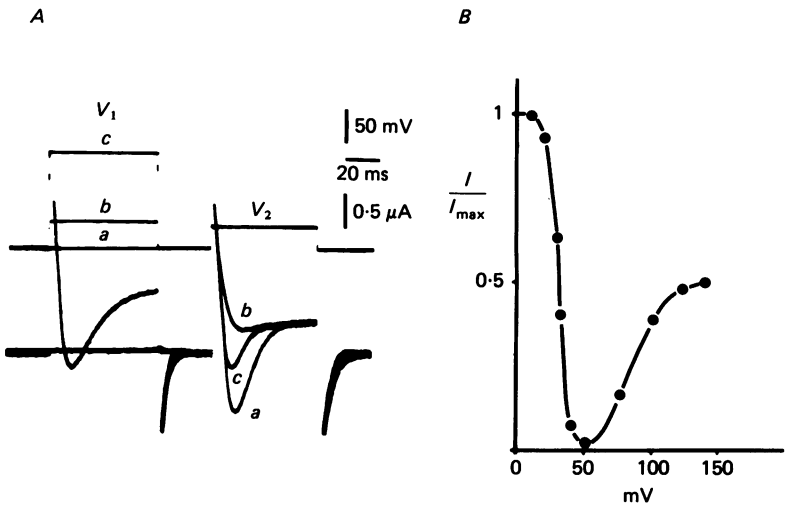

Fig. 5. Inactivation of Ca current in uterine preparation studied with the two-pulse protocol. *A*, a conditioning pulse of variable amplitude (V_1) is followed after a fixed 30 ms interpulse interval by a test pulse (V_2) of fixed amplitude and duration (35 mV, 60 ms). *a*, no pre-pulse; *b*, $V_1 = 40$ mV; *c*, $V_1 = 140$ mV. In *c* the outward current during the pre-pulse is off scale. *B*, relative inactivation of the Ca current by conditioning pulses of different amplitude. Peak test current was normalized by its value in absence of a conditioning pulse (I/I_{max}).

rations. Conditioning hyperpolarizations had no effect on the amplitude of the test current in all the preparations studied.

The diminution of the test current was taken as an index of the Ca current inactivation: relative inactivation was expressed by plotting the test current during V_2 against the level of the conditioning pulse V_1. The amplitude of the test current was normalized by its value in absence of a conditioning pulse (I/I_{max}). Fig. 5*B* shows that the relative inactivation was progressively enhanced in the voltage range from resting potential to +50 mV, as expected for a conventional inactivation process. However, the conditioning pulse induced progressively less and less inactivation as its level became increasingly more positive. Comparison with Fig. 2*A* shows that there is a good correspondence between the amplitude of the Ca current and the degree of inactivation of the Ca channel as a function of the membrane potential. Both curves presented a maximum near +40 mV; at strong depolarizations the inactivation was gradually reduced as the Ca influx decreased. The correspondence disappeared for membrane depolarizations higher than +120 mV since inactivation remained significant even though the Ca inward current became negligibly small. A possible explanation for such an observation might be that a voltage-dependent inactivation component became increasingly important at positive membrane po-

tentials. These results indicate that the degree of inactivation of the Ca channel is affected by the total amount of Ca entering the cell during the conditioning pulse.

The interpretation of two-pulse experiments could be complicated by the fact that the increase in intracellular Ca concentration in uterine smooth muscle was also dependent on a release of Ca from intracellular stores (Lalanne, Mironneau, Mironneau

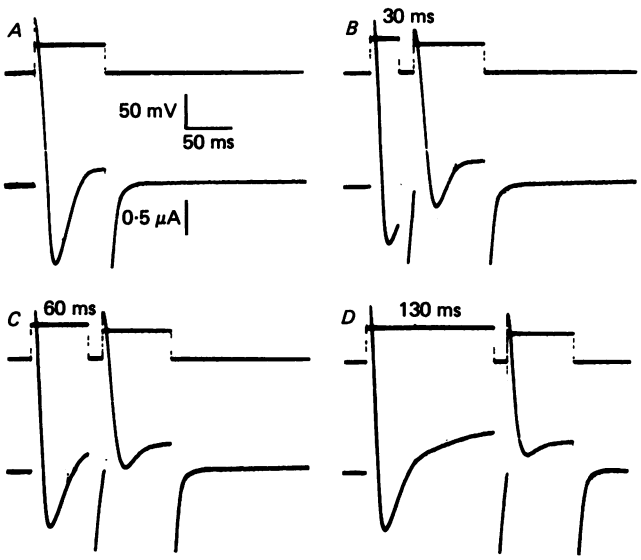

Fig. 6. Influence of pre-pulse duration on test Ca current. (*A*), test current in the absence of pre-pulse; the pre-pulse duration is successively increased to 30 (*B*), 60 (*C*) and 130 (*D*) ms. Both amplitude and rate of inactivation are modified as a function of the Ca current during the pre-pulse.

& Savineau, 1984). Application of 60 ms depolarizing pulses at our usual stimulus rate (1/15 s) induced a large twitch contraction at potentials where inward current was maximal, but very little contractile activity was measured at +120 mV (Mironneau, 1973) indicating a slight internal Ca release.

Dependence of Ca channel inactivation on Ca current intensity

As shown in Fig. 6, the amplitude of the Ca test current decreased as the duration of the pre-pulse increased from 30 to 130 ms. The relationship between the degree of inactivation (I/I_{max}) of the Ca current and the pre-pulse duration was roughly exponential over the range investigated (Fig. 7). Moreover, the time constant of the early component of inactivation increased significantly as a function of the duration of the conditioning pulse. Although the magnitude and the time course of the Ca current varied from fibre to fibre, qualitatively similar results were obtained in seven preparations. The effects of varying the external divalent cation concentration on the time course of Ca channel current are illustrated in Fig. 8. In order to minimize possible variations in surface charge, the final concentration of divalent cations in the different solutions was maintained at 10·24 mM by adjusting the external Mg concentration. Raising the external Ca from 2·1 to 10 mM produced an increase in the rate of inactivation as the inward current was increased (Fig. 8*A*).

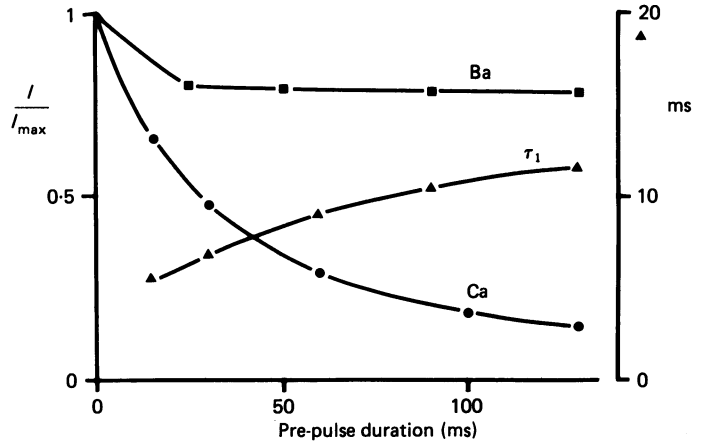

Fig. 7. Relative inactivation of Ca and Ba currents as a function of the pre-pulse duration. The inactivation time constant τ_1 (early component) of the Ca current increases with the duration of pre-pulses.

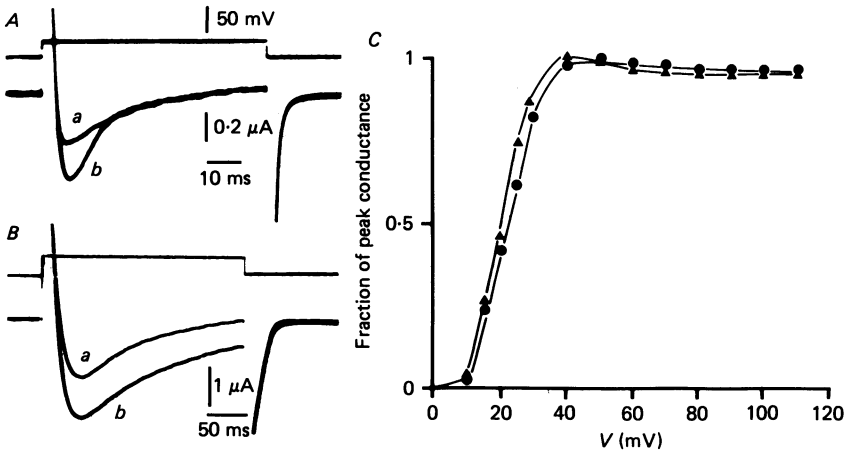

Fig. 8. Inactivation time course of Ca channel current and peak conductance. *A*, Ca currents recorded in the presence of 2·1 mM-Ca (*a*) and 10 mM-Ca (*b*). Inactivation rate varies with changes in extracellular Ca and current density. *B*, Ba currents recorded in 2·1 mM-Ba (*a*) and 5 mM-Ba (*b*). *C*, peak conductances calculated from Ca current (circles) and Ba currents (triangles). These are expressed as a fraction of maximal conductance and plotted against membrane potential.

Ca conductance as a function of membrane potential

Peak membrane conductance for Ca current, during a series of depolarizations, was calculated as peak current divided by the difference between membrane depolarization and apparent reversal potential. Fig. 8*C* shows the Ca peak conductance calculated in this way, plotted against membrane potential. The Ca peak conductance at each potential was normalized with respect to the maximal conductance, that occurred for depolarizations of approximately +50 mV. The Ca peak conductance was a steep function of voltage, and increased markedly over the potential range from 0 to +50 mV.

Ca channel inactivation in the absence of extracellular Ca

When Ba, Sr or Na were the permeant cation in the bathing solution, the inward current inactivated more slowly (Fig. 2*B*; Mironneau, Eugène & Mironneau, 1982). It may be argued that Ba reduced the outward K currents, but in the present experiments these currents were already inhibited by Cs loading and addition of TEA

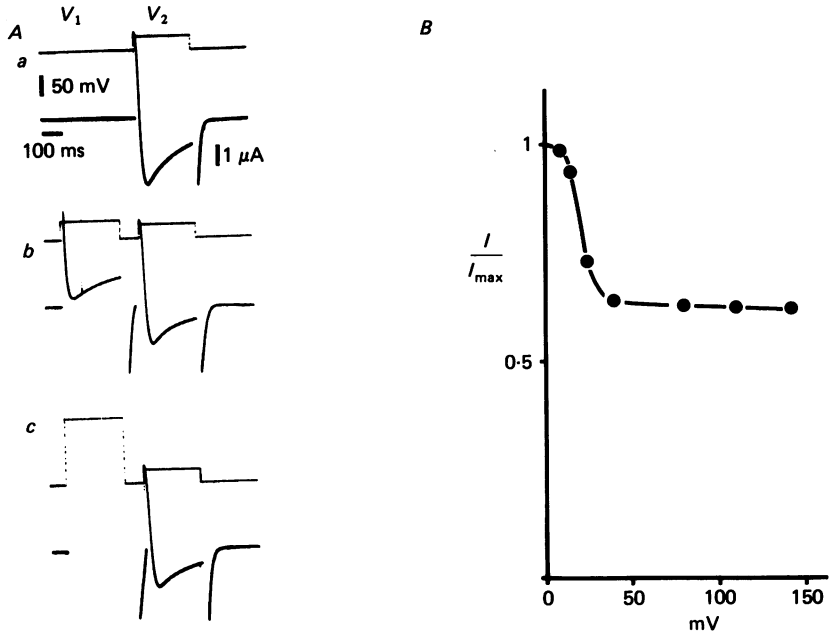

Fig. 9. Inactivation of Ba current studied with two-pulse protocol. *A*, a conditioning pulse of variable amplitude (V_1) is followed after a fixed interval by a test pulse (V_2) of fixed amplitude (30 mV) and duration (250 ms). *a*, no pre-pulse; *b*, $V_1 = 40$ mV; *c*, $V_1 = 140$ mV. In *c*, the outward current during the pre-pulse is off scale. *B*, relative inactivation of the Ba current by conditioning pulses of different amplitude.

plus 4-aminopyridine. Fig. 9*A* shows that a 250 ms conditioning pulse of +40 mV was accompanied by a minimum test current. A further increase in pre-pulse depolarization to +140 mV did not further modify the amplitude of the test current. The relative inactivation of the Ba current (I/I_{max}) was gradually increased to a maximum as the conditioning pulse approached +40 mV and then remained constant for higher depolarizing pre-pulses (Fig. 9*B*). Similar results were obtained in six other experiments. The degree of inactivation of inward current in Ba solution was also studied as a function of the pre-pulse duration. Fig. 10 shows that there was no modification in either the amplitude or inactivation rate of the test current when the duration of pre-pulse increased from 50 to 130 ms. This is also illustrated in Fig. 7. Finally, an increase in the extracellular Ba concentration from 2·1 to 5 mM led to an approximately twofold increase in the maximal amplitude of inward current without significant variation in the inactivation rate (Fig. 8*B*). Fig. 8*C* compares peak conductance data obtained in Ca and Ba solutions and shows no significant shift of the Ba curve against membrane potential.

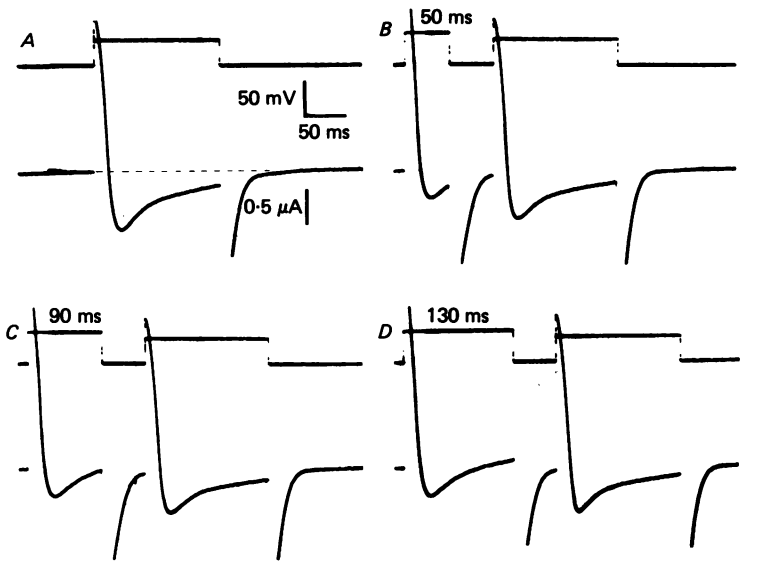

Fig. 10. Influence of pre-pulse duration on test Ba current. *A*, test current in the absence of pre-pulse; the pre-pulse duration is successively increased to 50 (*B*), 90 (*C*) and 130 (*D*) ms. Increasing the Ba current during the pre-pulse has no effect on both amplitude and rate of inactivation.

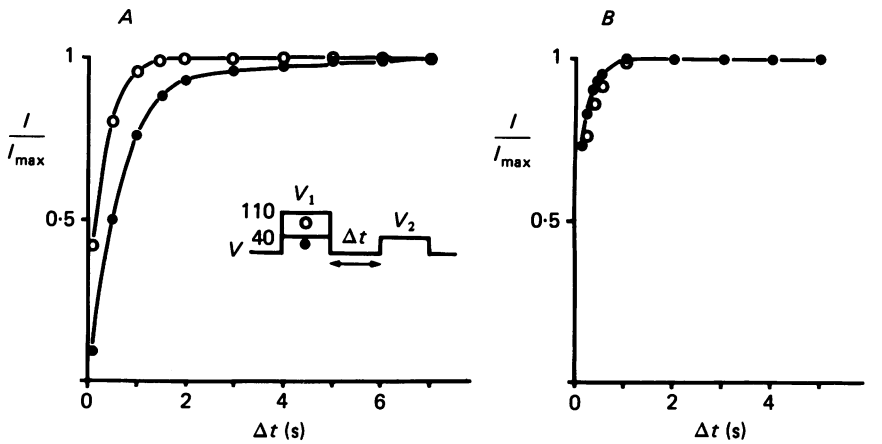

Fig. 11. Time course of recovery from inactivation of Ca, *A*, and Ba, *B*, currents established with the protocol shown in the inset. Peak test current was plotted against the duration of interpulse ($\triangle t$) at which the membrane was held at its resting potential. In Ca solution, the curve obtained with a pre-pulse of +40 mV is well fitted by a two-exponential function ($\tau_1 = 740$ ms, $\tau_2 = 2400$ ms); with a +110 mV pre-pulse, only one time constant is calculated ($\tau = 300$ ms). In Ba solution, the data are well fitted with a single-exponential function. The time constants for pre-pulses of +40 and +110 mV are 320 and 400 ms, respectively.

Recovery from inactivation

The time course of recovery from inactivation was investigated by a double-pulse method in solutions containing Ca or Ba as the charge carrier. A conditioning pulse was followed by a test pulse after an interval of increasing duration at resting potential. The test pulse was adjusted to elicit a maximal inward current. This amplitude was generally near +40 mV. Experiments were performed with conditioning pulses at +40 and +110 mV. Relative peak inward current measured during the test pulse was plotted against the recovery interval and expressed the recovery from inactivation. In Ca solution, the increase in current amplitude was observed simultaneously with an acceleration of the inactivation phase (not shown). When the two pulses had a similar amplitude (+40 mV), recovery from inactivation as a function of the pulse interval was described by a two-exponential process (Fig. 11*A*). The values of the time constants were 740 and 2400 ms, respectively. Complete restoration was obtained after 5–7 s in twelve experiments. When the pre-pulse amplitude was increased to +110 mV, the recovery curve in Ca solution was best described by a single exponential function with a time constant of 300 ms and complete restoration was obtained after 1·5 s. After equimolar substitution of Ba for Ca ions, removal of inactivation was investigated with a longer conditioning pulse (200 ms) in order to increase the degree of inactivation of Ba currents. Recovery from inactivation of the Ba current revealed two major differences (Fig. 11*B*). First, it was faster than in Ca solution because complete restoration was obtained in 1 s or less. Secondly, the time constants of reactivation had relatively similar values whatever the amplitude of conditioning pulses ($n = 3$).

DISCUSSION

The main conclusion of this paper is that inactivation of uterine Ca channels depends on both membrane potential and intracellular Ca. These results agree with data previously obtained in snail neurones (Brown *et al.* 1981) and in cardiac cells (Kass & Sanguinetti, 1984; Lee *et al.* 1985). The present findings were revealed because of special care to isolate the Ca channel current from contaminating K outward currents. The effectiveness of both Cs loading and addition of TEA ions and 4-aminopyridine to the external solution was demonstrated by the flattening of current traces during long depolarizations as well as the linearity of the current–voltage relationship in the presence of Co from −20 to +60 mV (Fig. 2*A*). A slight time- and voltage-dependence of the currents obtained after addition of Co appeared only for depolarizations larger than +60 mV; these currents could be considered to be mostly leakage currents, although Cs outward currents have been shown to be carried through cardiac Ca channels (Lee & Tsien, 1984). The possibility that outward currents might be increased by Ca influx, as previously described for Ca-activated K currents in uterus (Mironneau & Savineau, 1980), is unlikely because outward tail currents were never observed after repolarizing to resting potential or to more positive potentials. More complete isolation of the Ca channel current was also indicated by comparing the reversal potential obtained in this study (+120 mV) with previous values (+65 mV, Anderson, 1969; +75 mV, Vassort, 1975). In Ba

solution, an outward reversal current could be observed with depolarizations larger than +120 mV. However, the similar values of reversal potential with Ca or Ba outside suggests that the slowed decay of Ba current cannot be attributed to a blocking action of Ba on an outward current through K channels. The possibility that these changes in current kinetics might be due to changes in external surface charge when Ba replaced external Ca was also considered. Changes in external surface charge are known to shift voltage-dependent gating mechanisms along the voltage axis, and these shifts occur for both activation and inactivation parameters (Frankenhaeuser & Hodgkin, 1957; Byerly, Chase & Stimers, 1985; Inomata & Kao, 1985). When Ba replaced external Ca, peak conductance curves as a function of membrane potential showed only minor modifications (Fig. 8*C*) suggesting that external charge changes were too small to account for the differences between Ca and Ba current decay. The current reversal potential remained stable under experimental conditions where Ba current was largely increased during conditioning pre-pulses (Fig. 4). As depletion of charge carrier from a restricted extracellular space would at most produce a small change in the reversal potential (Kass & Sanguinetti, 1984), the absence of any variation suggests that depletion cannot account for the uterine Ca channel decay during depolarizing pulses.

Inactivation of the uterine Ca channel current persisted when external Ca was replaced by Sr or Ba, or when external Na carried inward current (Mironneau *et al.* 1982). Increasing the conditioning depolarization towards the expected reversal potential of the current did not reduce the degree of inactivation of Ba currents and had no effect on recovery from inactivation. Similarly, increasing the duration of a given conditioning pulse and consequently the amount of divalent charges entering the cells had no further effect on both amplitude and kinetics of test Ba currents. Thus, the inactivation of Ca channel current is not proportional to the magnitude of the current flowing through the channel when Ba carries the charge. Similar arguments have supported the concept of a voltage-dependent inactivation mechanism for Ca current in other excitable cells (Fox, 1981; Fukushima & Hagiwara, 1985). On the contrary, several findings favour the interpretation of a Ca-mediated inactivation. A strong correlation was observed between the magnitude of Ca inward current during a conditioning pulse and the degree of inactivation revealed by a subsequent test current. Conditioning depolarizations that produced maximal Ca current induced the greatest amount of inactivation. As the conditioning depolarization increased towards the expected reversal potential, and the driving force for Ca influx became smaller, inactivation decreased but was never completely reversed (Fig. 5). This may be interpreted as a contribution of voltage-dependent inactivation. Moreover, when Ba replaced external Ca, the current failed to inactivate completely in the voltage range from +50 to +150 mV (Fig. 9) and the inactivation remained stable. The absence of complete inactivation can be due to a substantial recovery of the Ba current during the longer repolarizing interval (100 ms) between the two pulses. From analysis of kinetic data, it is worthy of note that elevation of external Ca to 10 mM increased Ca influx and speeded inactivation. Similarly, increasing the Ca current during the conditioning pulse reduced both amplitude and inactivation kinetics of the test current. A Ca channel inhibitor such as Co reduced the amplitude of both Ca and Ba currents; it markedly slowed down Ca current inactivation but

had minor effects on Ba current inactivation. These observations suggest that inactivation of Ca channels is partly related to local internal accumulation of Ca (but not other cations) in the vicinity of the plasma membrane. Recovery from inactivation was also affected by the external permeant cation. Thus, the recovery rate was much faster in Ba solution than in Ca solution. Recovery from inactivation was described as a two-exponential process only when the conditioning pulse elicited a noticeable Ca inward current. According to these results, it may be proposed that the recovery times would reflect the repriming of a voltage-dependent gating mechanism with Ba currents or when no Ca current flowed through Ca channels. On the other hand, when the Ca current was elicited during a conditioning pulse, recovery from inactivation would follow the time course of removal of Ca from the cytoplasm (Mentrard *et al.* 1984). Accumulation of divalent cations might change the screening by surface charges at the inner side of the membrane. Binding of Ca, but not Ba, might shift the kinetic parameters for voltage-dependent inactivation along the voltage axis towards less positive potentials. Although this hypothesis cannot yet be excluded, it seems unlikely since the voltage shift caused by Ca binding would need to be quite large to account for the early phase of Ca current inactivation (Kass & Sanguinetti, 1984)

The present results may be interpreted as evidence that the Ca channel current in uterine smooth muscle inactivates by a mechanism that is both voltage and Ca dependent. In this view, inactivation of Ba current through the same channel reflects a pure voltage-dependent mechanism. When Ca is the divalent charge carrier, the inactivation mechanism is modulated by Ca influx during depolarizing pulses. As indicated by Lee *et al.* (1985), discrimination between the different explanations of Ca channel inactivation may be possible with recordings from single channels, along a line of experiments proposed by Brown, Lux & Wilson (1984).

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