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EFFECTS OF CALCIUM IONS ON OUTWARD MEMBRANE CURRENTS IN RAT UTERINE SMOOTH MUSCLE

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SUMMARY

1. The outward membrane current underlying delayed rectification in uterine smooth muscle has been studied by means of a double sucrose gap apparatus with particular reference to the effects of the external calcium.

2. The outward current was reversibly reduced in calcium-free solution and in the presence of manganese (5 mM), or increased in high-calcium solution.

3. In reference solution, when depolarizing steps activated the outward current to its maximal value, the current tails measured at the end of the pulse were made up of two exponentially declining components. The slower of the two components was suppressed in calcium-free solution. The fast component reached full, steady-state activation at about +75 mV and the slow one at less positive potentials, i.e. +50 mV. Altering the external calcium did not shift the activation curves of the outward current along the voltage axis.

4. The reversal potential of the outward current was not affected by alterations of the external calcium concentration.

5. The outward current components can also be separated on the basis of their sensitivity to 4-aminopyridine (4-AP) and tetraethylammonium (TEA). The fast component was selectively blocked by externally applied 4-AP. TEA blocked both fast and slow components.

6. It is suggested that two sets of potassium channels contribute to the outward current in myometrium and that these channels can be separated pharmacologically.

INTRODUCTION

In a large variety of tissues calcium ions are known to modify the potassium conductance. The well known stabilizing effect of calcium on squid axons has been attributed by Frankenhaeuser & Hodgkin (1957) to an increase in the electrical field across the membrane when calcium ions partially neutralize fixed negative charges at the external surface of the membrane. This produces a shift of the potassium activation curve towards less negative potentials. More recently, electrophysiological studies of many cells have shown that some of the potassium channels in the plasma membrane seem to be controlled by the ionized calcium concentration inside the cell (nerve: Krnjević & Lisiewicz, 1972; Meech, 1974; Meech & Standen.

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1975; Thompson, 1977; Eckert & Lux, 1977; electroreceptors, Clusin & Bennett, 1977; photoreceptors: Hanani & Schaw, 1977; pancreatic cells: Atwater, Dawson, Ribalet & Rojas, 1979), while other potassium channels are controlled by the more familiar time- and voltage-dependent gating mechanism (Hodgkin & Huxley, 1952). In heart muscle, the complexity of outward current systems leads to different conclusions suggesting that the effects of calcium ions could be dependent on either a direct action on potassium channels (Bassingthwaighte, Fry & McGuigan, 1976; Isenberg, 1977; Siegelbaum, Tsien & Kass, 1977) or a shift in the potential-dependence of potassium activation and an increase in the potassium accumulation (DiFrancesco & McNaughton, 1979).

In taenia coli smooth muscle, a decrease in outward current intensity has been shown in the presence of manganese ions (Inomata & Kao, 1976). In uterus, Vassort (1975) has suggested a possible association between the calcium influx and the potassium activation.

The intention of the present work is to determine if any component of the outward current in rat uterine smooth muscle could be dependent on the internal calcium concentration. The experimental procedure has been to analyse the outward current (and the current tails) when the extracellular calcium concentration is altered or in the presence of calcium inhibitors. This indirect method (when compared to calcium injection) has the advantages of being reproducible and easy to carry out. Our main conclusion is that a component of the outward current in uterus may result from an interaction of internal calcium ions with potassium channels. This component can be pharmacologically separated from the time- and voltage-dependent component which is specifically blocked by 4-aminopyridine (4-AP). Moreover, tetraethylammonium ions (TEA) block both components of the outward current suggesting a similar potassium dependence.

A preliminary report of part of this work has already been published (Mironneau, Savineau & Rahéty, 1977).

METHODS

(1) *Apparatus*

A double sucrose gap (Rougier, Vassort & Stämpfli, 1968) has been used to current- and voltage-clamp small strips of rat myometrium. For voltage-clamp experiments the electronic circuit has been modified: the liquid surrounding the preparation is held at ground potential by the inverting input of the current to voltage converter. The output voltage is proportional to the total current flowing in the test node. An appropriate calibration allows a direct measurement of the current which was expressed in amperes.

(2) *Preparation*

Isolated strips from pregnant rat myometrium (60–80 μm in diameter; 3–4 mm in length) at the end of pregnancy (18–20 days) were used in these experiments. After a short healing over period (approx. 15–30 min) in reference solution, the preparation was ready for electrical recordings.

Cross-sections through the strips indicated that the number of cells in the test gap was between thirty and sixty (in twenty-five experiments).

(3) *Gap potential*

Before each voltage-clamp experiment, an estimate of the gap potential of the strip was obtained. The preparation in the test gap was perfused with a high-potassium solution (135·6 mM) and the electronic set-up was connected for current-clamp. Then, when the high-potassium solution was changed to the reference solution, the preparation repolarized to a stable value. The average gap potential was 45 ± 5 mV (mean value $\pm$ S.E. of mean of twenty-seven preparations). Because the short-circuiting factor of the system was about 0·9, and because the observed gap potential was very close to the resting potential recorded by intracellular micro-electrodes (Kuriyama & Suzuki, 1976), this gap potential may be accepted as representing the average resting potential of the cells in the test compartment.

The end compartments were perfused with a high-potassium solution and were depolarized to approx. 0 mV. This procedure is advantageous because (1) it abolishes any spontaneous activity in the end compartment, (2) it markedly reduces the membrane resistance of the part of the preparation in the end compartments, and may therefore produce more favourable conditions for voltage clamping.

(4) *Solutions*

Physiological solutions had the following composition.

(*a*) Reference solution (mM) NaCl 130; KCl 5·6; $CaCl_2$ 2·1; $MgCl_2$ 0·24; glucose 11. The solution was aerated with O_2 and was buffered by Tris-HCl (8·3 mM) at pH 7·4.

(*b*) High potassium solution was prepared by substituting NaCl for KCl in equimolar amounts (135·6 mM).

(*c*) The following inhibitors of permeability were used: manganese chloride (5 mM) as an inhibitor of the inward calcium current (Hagiwara & Nakajima, 1966; Anderson, Ramon & Snyder, 1971; Mironneau, 1973); tetraethylammonium chloride (TEA) and 4-aminopyridine (4-AP) as inhibitors of the potassium outward current (Hille, 1967; Armstrong, 1971; Meves & Pichon, 1977; Thompson, 1977). In some experiments, the possible interference between 4-AP and release of endogenous neurotransmitters was eliminated by use of phentolamine (3×10^{-6} M), propranolol (3×10^{-6} M) and atropine (10^{-4} M).

In order to obtain a calcium-free solution, EGTA (added as 1 mM) was used. All solutions were maintained at 30 ± 1 °C and the flow in the test compartment was maintained at 0·5 ml/min by means of a constant flow pump. The rate of stimulation was 1/30 sec.

(5) *Tests and precautions*

Adequate transmembrane voltage control is limited by the presence of a significant series resistance, and uniformity of the voltage control is limited by the multicellular and inherent cable properties of the muscular strips (Johnson & Lieberman, 1971; Ramon, Anderson, Joyner & Moore, 1975; Atwell & Cohen, 1977; Beeler & McGuigan, 1978). Therefore, the following precautions and tests were observed: (1) the length of the test compartment (150 μm) was relatively short in comparison with the space constant of the preparation. In resting preparations, the space constant was about 1·8 mm (Abe, 1971) and during the active state (when the membrane resistance was reduced to one-tenth) the space constant was approximately one third of its resting value (Bolton, 1975), suggesting that a longitudinal uniformity of voltage clamp may be obtained in the test compartment. On the other hand, the low strip diameter/test compartment length ratio (0·5) might minimize the sucrose-ionic solution diffusion and allowed viable action potentials to be obtained; (2) at the beginning and at the end of each experiment, the values of the series and membrane resistances were estimated using a small hyperpolarization (5–10 mV negative to the resting potential). If there is no important variation in the series resistance values (4–6 kΩ) during the experiment, this allows comparison of membrane currents in different experimental conditions. (3) Direct evidence of voltage-clamp control may be obtained by measuring independently the voltage in the test node with a microelectrode (Grosset & Mironneau, 1977). Fig. 1*A* illustrates the ionic current (I) elicited by a command depolarization of +40 mV (V_c) and the controlled voltage (V_e) at the tip of a micro-electrode inside the cell. The internal voltage remains almost constant during the time-course of the inward and outward currents and is similar in amplitude to the command depolarization. It

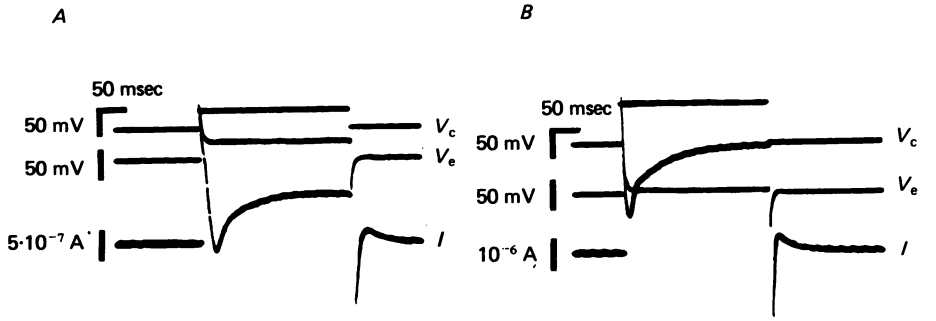

Fig. 1. *A* and *B* show simultaneous records of membrane current (I) and control potential (V_c) induced by command depolarization (V_c). In *A*, the micro-electrode is inside the cell and the true transmembrane potential can be measured. In *B*, the micro-electrode is just outside the membrane, the external surface of which is held at ground potential. These recordings show that there is no important variation in voltage during ionic currents.

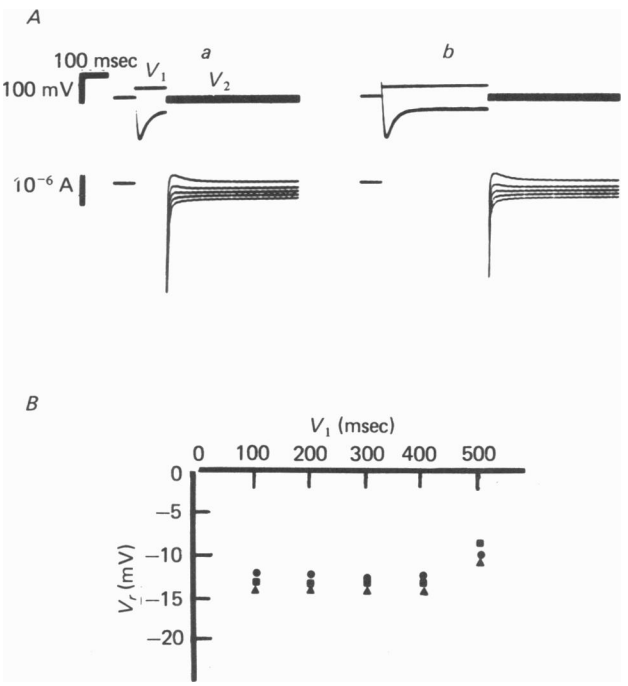

Fig. 2. *A*, reversal potential of the outward current measured with the double step method. The duration of the initial depolarization V_1 increases from 100 msec (*a*) to 370 msec (*b*). *B*, the plot of the reversal potential as a function of the duration of V_1 shows that V_r remains almost constant from 100 to 400 msec but decreases for longer depolarizations.

should also be noted that there is no variation in voltage during the decay of the outward current at the end of the depolarization. The same accuracy of the voltage control is obtained for a higher depolarization (+65 mV; Fig. 1*B*) when the micro-electrode is just outside the membrane and measures the external voltage which is the ground potential. This micro-electrode test was used routinely to select muscular strips suitable for voltage-clamp analysis.

(6) *Reversal potential of the outward current*

During long-lasting depolarizations, potassium ions may accumulate outside the membrane of muscular cells (Maugham, 1973; Vassort, 1975; Noble, 1976; Cleemann & Morad, 1979). Some information can be obtained in uterus by measuring the reversal potential as a function of the duration of the depolarization. In Fig. 2*A* the reversal potential of the outward current was evaluated as follows: after a large initial depolarization (V_1) whose duration can be changed, the preparation was repolarized to different test potentials (V_2) to determine the value of the reversal potential (i.e. when there was no tail current). Fig. 2 *B* shows that the value of the reversal potential of the outward current (about − 12 mV in three different preparations) is not modified when the duration of the depolarization V_1 is shorter than 400 msec. For a 500 msec step, a noticeable shift of the reversal potential toward less negative values of voltage can be observed.

(7) *Expression of the results*

The nomenclature used to express the results was as follows: V (mV), variation of the membrane potential, the resting potential being taken as zero (holding potential = E_r = 0). Positive values of V represent a depolarization, negative values a hyperpolarization; I (A) membrane current. Positive values of I correspond to an outward current, negative values to an inward current.

RESULTS

Effects of calcium-free solution and calcium antagonists on the outward current of myometrium

In uterine smooth muscle, calcium-free solution, manganese and D 600 are known to markedly decrease the inward current (Anderson *et al.* 1971; Mironneau, 1973). Fig. 3*A* shows that the outward current (measured at 400 msec) is reduced by about $20 \pm 5\%$ (n = 7) for a depolarization of +40 mV in a calcium-free solution. This effect was fully reversible. Manganese ions (5 mM) had similar effects, but were only partially reversible.

Referring to Noble & Tsien (1969), the equation for describing a time-dependent current is

$$i_x = \bar{i}_x(Y)^z,$$

where $\bar{i}_x$ is the fully activated current at each potential and Y the activation variable; z is a power which is greater than 1 suggesting that more than one membrane reaction is required to open a membrane channel to the passage of ions. There are two obstacles to simple analysis of current onsets: (1) the current onsets are not separable by semilogarithmic analysis into exponential form when $z > 1$; (2) an important leak current is observed during depolarizations in smooth muscle cells. For this reason, the current decay has been analysed since the deactivation of a time-dependent conductance is always exponential (Hodgkin & Huxley, 1952). A similar analysis of the deactivation current is routinely used in cardiac tissues (Noble & Tsien, 1969; Brown, Clark & Noble, 1976). Current tails on repolarization

after a pulse of +90 mV in amplitude and 400 msec in duration are shown in Fig. 3*B*. It can be seen that the current tail intensity is decreased in a calcium-free solution. A similar decrease was observed in seven experiments.

If we assume that the total amount of activation of the outward current by any given depolarization is equal to the amount of subsequent deactivation, the extrapolated peak values of the current tails give the activation for all the outward currents

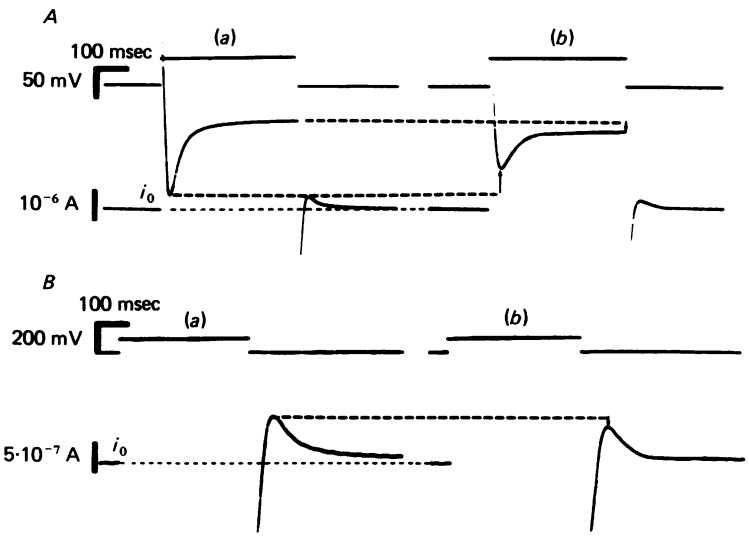

Fig. 3. *A*, effects of calcium-free solution on the membrane current obtained for a +40 mV depolarization. (*a*) reference solution; (*b*) calcium-free solution (5 min). The minimal outward current as well as the steady-state current are affected in the absence of external calcium. *B*, higher gain current records showing current tails only for a +90 mV depolarization in reference solution (*a*) and in calcium-free solution (*b*). i_0 = zero current level.

activated over the selected potential range. The experimental method was as follows: the holding potential being chosen to equal the resting potential, square pulses (400 msec) were applied to depolarize the membrane to different voltages. Then, the membrane was repolarized to the resting potential and the resulting current tails were analysed. Fig. 4*A* shows a typical semilogarithmic plot of tail currents against time following a +60 mV pulse. The results are plotted as the filled points in reference solution. The latter points were fitted by hand with a straight line whose time constant was 120 msec. The line was then subtracted from the filled points to give the remaining current change which should be attributable to a fast component. The open points are a good fit to a line whose time constant is 24 msec. It is the slower of the two components which appears to be suppressed in a calcium-free solution since only one component remains whose time constant is 35 msec. This larger time constant (when compared to that obtained for the fast component in reference solution) could be attributable to the fact that, even in the presence of EGTA (1 mM), a small quantity of calcium ions could remain present in the close vicinity of the plasma membrane. The terminology concerning the fast and slow

components is based on their apparent decay at positive potentials. In a number of experiments, the slow component appears to be activated by both long and short depolarizing pulses.

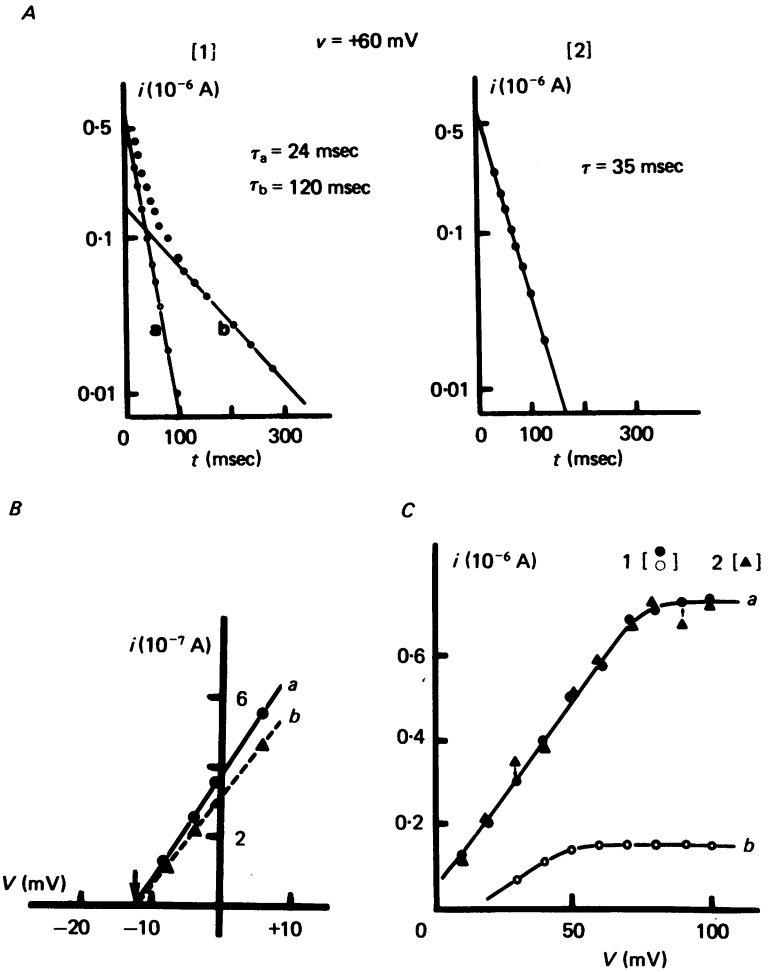

Fig. 4. *A*, effects of calcium-free solution on current tails recorded after a +60 mV depolarizing pulse. In reference solution (1) the current tail can be separated into two exponentially decaying components. In calcium-free solution (2) only one component remains whose time constant can be compared to that of the fast component in reference solution. *B*, reversal potential of the outward current (arrow) in reference solution (*a*) and in calcium-free solution (*b*) obtained from current tails plotting as a function of the holding potential. *C*, activation curves obtained by the method of current tail analysis. In reference solution (1) two activation curves can be separated corresponding to the fast component (●) and to the slow one (○). In calcium-free solution, the slow component is suppressed while the fast component remains unchanged (▲).

When the current tails were separated into two components, activation curves for the two systems were obtained by plotting maximal current tails at zero time as a function of voltage (Fig. 4*C*). The fast component is fully activated for potentials higher than +70 mV while the slow component seems to be fully activated for

lower potentials, i.e. +50 mV. In calcium-free solution, the slow component is suppressed while the fast component is not modified. It must be noted that if the extrapolation of the current at zero time can introduce some errors, the activation

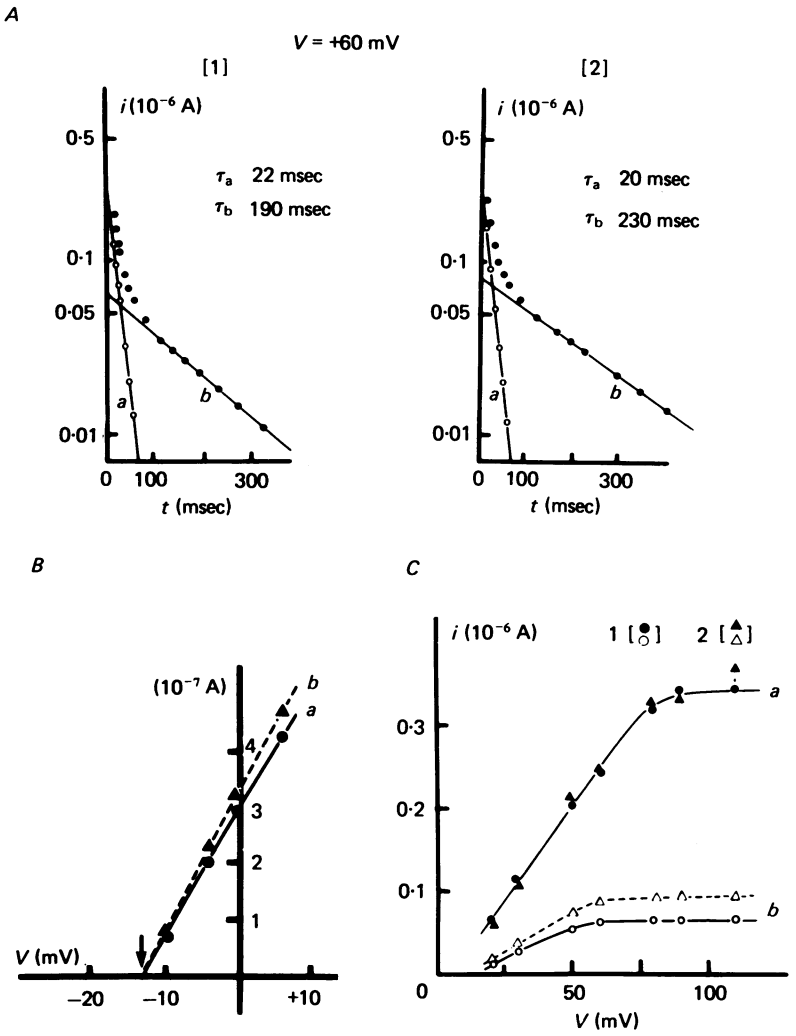

Fig. 5. *A*, effects of high-calcium solution on current tails recorded after a +60 mV depolarization. In reference solution (1) the current tail is separated into two experimentally decaying components. In high-calcium solution (2) only the slow component (*b*) is increased. *B*, reversal potential of the outward current in reference solution (*a*) and in high-calcium solution (*b*) obtained from current tails. *C*, activation curves obtained by the method of current tail analysis. In reference solution (1) two activation curves can be seen corresponding to the fast component (●) and to the slow one (○). In high-calcium solution the slow component activation is increased (△) while the fast component activation is unchanged (▲).

curves obtained in the same way but in different preparations ($n = 11$) show that the general shape of the curves in relation to their position on the voltage axis is, considering the inevitable errors involved, reasonably similar.

The reversal potential was extrapolated by plotting the outward current tails as a function of the holding potential (see Methods). Fig. 4*B* shows that there is no noticeable variation of the reversal potential of the outward current with or without calcium ions in the external medium (-13 ± 3 mV, $n = 10$) indicating that both current systems reverse at the same potential. These observations suggest that outward potassium activation during a depolarizing pulse is at least dependent on calcium influx (and therefore on internal calcium concentration) in uterine smooth muscle.

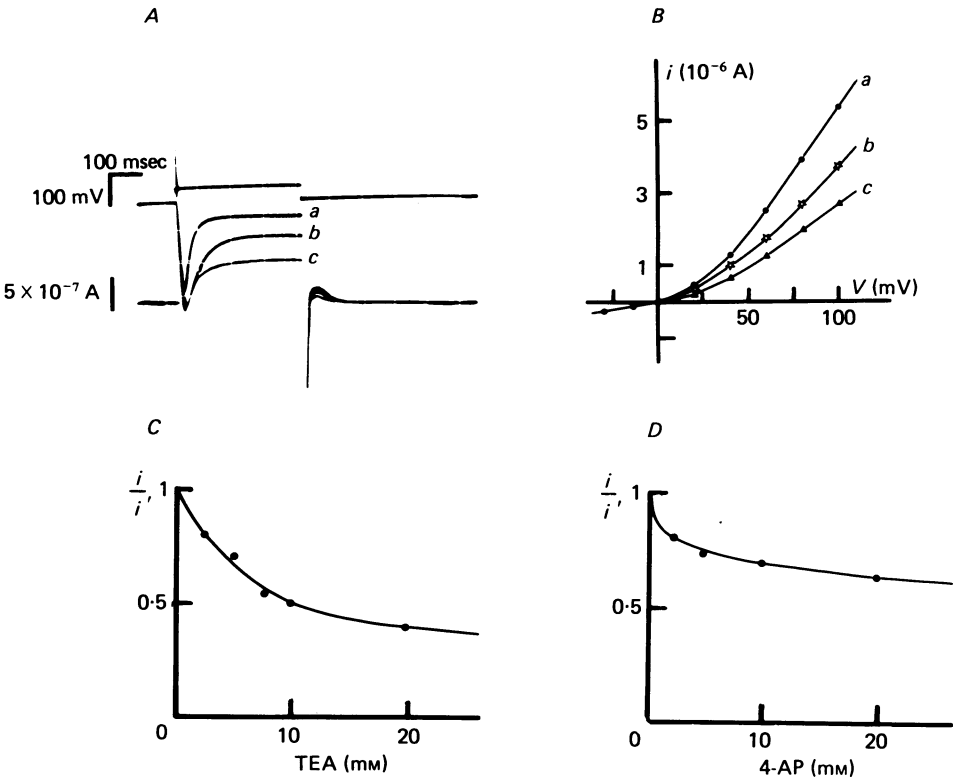

Fig. 6. *A*, effects of TEA (10 mM) and 4-AP (10 mM) on the outward current intensity. (*a*) reference solution; (*b*) in the presence of 4-AP (3 min); (*c*) in the presence of TEA (3 min). *B*, current–voltage relationships of the steady-state outward current in reference solution (*a*), in the presence of 4-AP (10 mM; *b*) and in the presence of TEA (10 mM; *c*). *C*, dose–response curve for TEA and outward current. The current is expressed as a ratio of the maximal current in the absence of TEA. *D*, dose–response curve for 4-AP and outward current. The current is expressed as in *C*.

Effects of high-calcium solution on outward current

It has been previously shown that an increase in the external calcium concentration (three times the normal concentration) leads to an increase in the maximal intensity of the inward current and in the internal calcium content (Anderson *et al.* 1971; Mironneau, 1974). Under these conditions, the outward current is increased by about $25 \pm 10\%$ ($n = 11$) at $+60$ mV and consequently, the current tail is

shifted upwards (Fig. 5*A*). A typical semilogarithmic plot of current tails produced when the muscular strip was repolarized to its resting potential after having been depolarized by +60 mV for 400 msec is analysed in Fig. 5*A*. The time constant of the fast component was unaffected in a high-calcium solution while the time constant of the slow component was noticeably increased from 190 to 230 msec. The reversal potential of the outward current was not modified in a 6·3 mM calcium solution when compared to reference solution in nine experiments (Fig. 5*B*). Fig. 5*C* shows

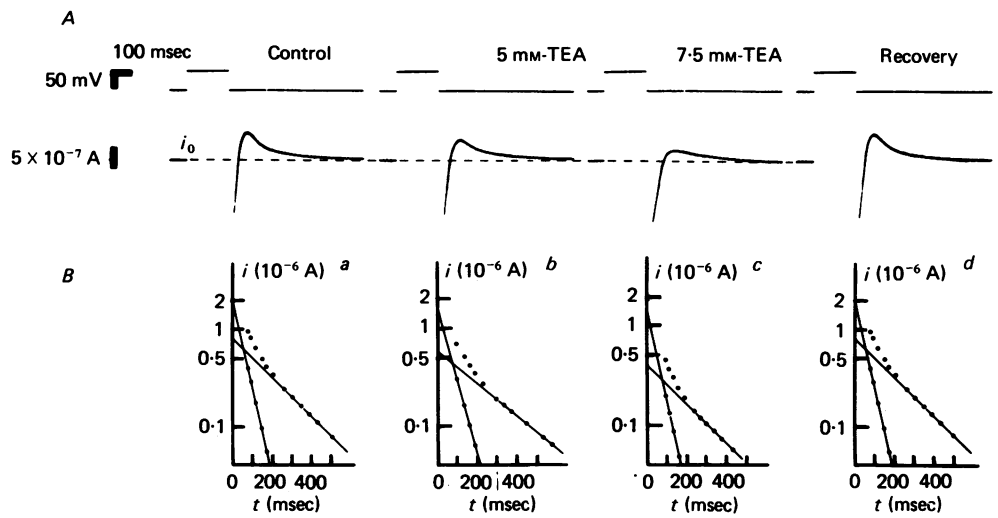

Fig. 7. *A*, effect of different TEA concentrations on high gain current records showing current tails only. *B*, semi-logarithmic analysis of the current tails shown in *A*. It is evident that TEA decreases the two decaying components.

that the fast component of the outward current activation curve seems to be insensitive to a high-calcium solution while the slow component is increased. During the plateau, this enhancement represents about 40% of the maximal activation in reference solution. However, if the upper curve is scaled down by a constant factor, the normalized slow component curve runs roughly parallel to the control curve, indicating that there is no shift of the activation curve along the voltage axis. Similar results were obtained in seven experiments.

Effects of TEA and 4-AP on both components of the outward current

Externally applied, both TEA (10 mM) and 4-AP (10 mM) depress the outward current (Fig. 6*A*) but TEA is much more effective than 4-AP. At +40 mV TEA causes a reduction of $55 \pm 10\%$ ($n = 15$) in steady state outward current while 4-AP induces a reduction of $35 \pm 7\%$ ($n = 9$). It will be noted that the downward deflection of the outward current in reference solution became really inward in the presence of potassium inhibitors. In Fig. 6*B*, the current–voltage relationships indicate that the outward current decreased at all potentials with TEA and 4-AP (10 mM). However, this concentration is not high enough to block completely the outward current. Plotting the amplitude of the outward current for a given membrane potential as the relative value of the amplitude of the current in the absence of

potassium current inhibitors resulted in cumulative dose–response curves for TEA (Fig. 6*C*) and 4-AP (Fig. 6*D*). The half-maximal response is 10 mM for TEA and much higher than 20 mM for 4-AP. The effects of TEA and 4-AP become maximal 2–3 min after application and can usually be reversed by washing for a few minutes.

The action of successive concentrations of TEA (from 2·5 to 10 mM) have been studied on current tails following a depolarization of + 50 mV for 250 msec (Fig. 7*A*).

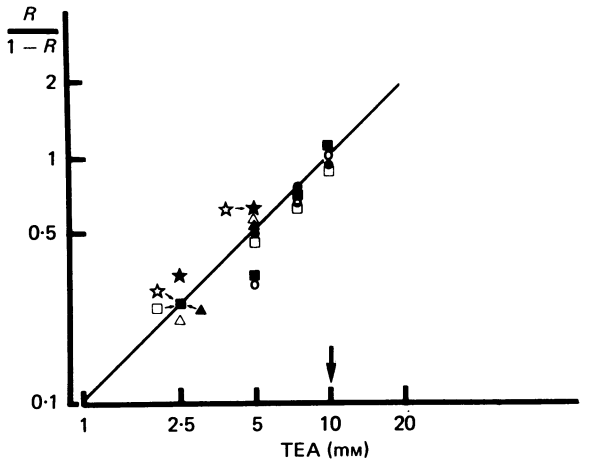

Fig. 8. Effects of TEA on current tail components. In ordinate, log $R/1-R$ where R is the reduction in each current tail (at zero time) when current tail in reference solution is equal to 1. In abscissa, log TEA concentration. Filled symbols represent results from the fast component in four different preparations; open symbols from slow component. All the points can be fitted with a straight line whose slope is 1; the half-maximal response is 10 mM-TEA (↓).

The current tails generated were analysed graphically, as shown in Fig. 7*B*, into their slow and fast components. It can be seen that both components are reduced by TEA without important variation of their time constants. The amplitude of each component at zero time in TEA solutions was expressed as the relative value of the amplitude of each component in reference solution. The reduction (R) was then calculated and log ($R/1-R$) plotted against the logarithm of the external TEA concentration. In Fig. 8, the results from four different preparations are plotted and all the points can be fitted with a straight line whose slope is 1. It appears also that the half-maximal response is about 10 mM (a value similar to that which has been calculated from the dose–response curve for the outward current). This value is very close to the apparent dissociation constant found in skeletal muscle (Stanfield, 1970) and in nervous preparations (Meech & Standen, 1975) for the delayed rectifier. The main conclusion to be drawn from these experiments is that it is not possible to distinguish between the two components of the outward current in myometrium on the basis of their sensitivity to TEA.

The effects of 4-AP have also been analysed in current tails on repolarization after a pulse of + 40 mV in amplitude and 300 msec in duration. Fig. 9*A* shows that, like TEA, 4-AP decreases the amplitude of the current tails as a function of the concen-

tration used. Unlike TEA, 4-AP has no effect on the slow component of the current tails and is only effective on the fast component. The time constants of the fast component appear to be largely increased in the presence of 4-AP. However, the time constants of the slow component are also modified but the effect is much smaller. The curve representing log $(R/1-R)$ against the logarithm of the external 4-AP concentration for the fast component of the current tails is shown in Fig. 9*B*. The

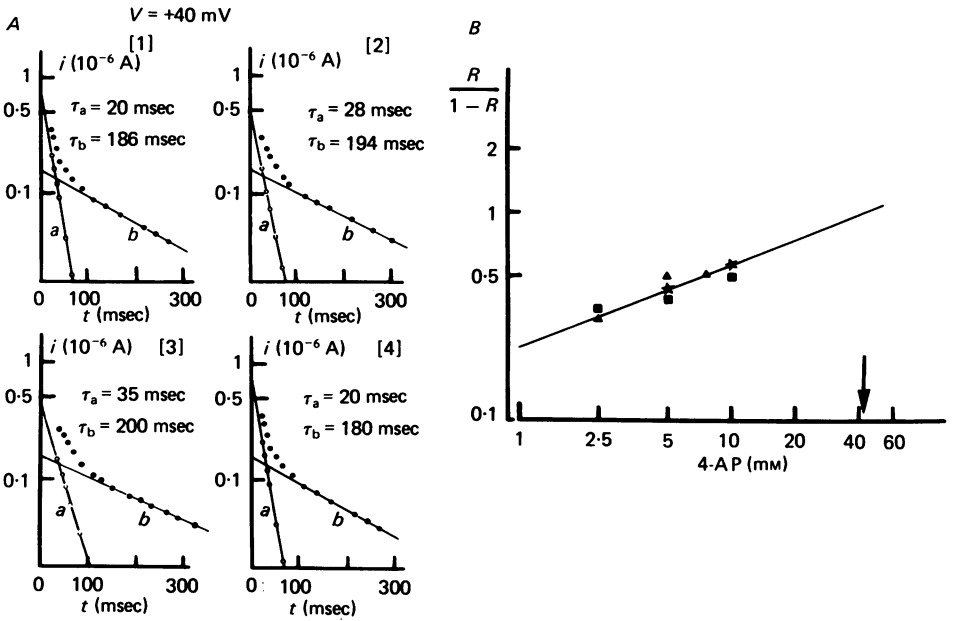

Fig. 9. *A*, semilogarithmic analysis of current tails in the presence of different 4-AP concentrations. (1) reference solution; (2) 5 mM-4-AP; (3) 10 mM-4-AP; (4) recovery. Note that the slow component is not modified by 4-AP. *B*, effect of 4-AP on current tail components. In ordinate, log $R/1-R$ where R is the reduction in fast component (at zero time) when fast component in reference solution is 1. In abscissa, log 4-AP concentration. Filled symbols represent results in three different preparations. The points can be fitted by a straight line whose slope is 3; the extrapolated half-maximal response is about 40 mM-4-AP (↓).

results from three experiments can be fitted with a straight line whose slope is 3. The extrapolated half-maximal response should be about 40 mM. These results indicate that the fast component of the outward current in myometrium can be pharmacologically separated from the slow one by using 4-AP.

DISCUSSION

The experiments reported here provide evidence for the existence in myometrium of a potassium conductance which is activated by internal calcium concentration as previously described in other tissues. There are several observations to support this. (1) The outward potassium currents seen during depolarizations under voltage-clamp are reversibly reduced by calcium-free solution and in the presence of manganese

(5 mM), or increased by high-calcium solution. (2) The current tails produced when the membrane was repolarized to its resting potential following a large depolarization, can be separated into two components, only one of which (the slow component) depends on the presence of external calcium.

These results cannot be explained simply by a surface-charge effect of calcium ions as proposed by Frankenhaeuser & Hodgkin (1957) in nerve since no shift in potassium activation curves was observed either in the presence or in the absence of calcium ions in the external solution. Our observations are in good agreement with those of Meech & Standen (1975) and Isenberg (1977) who suggest the existence of a calcium-mediated potassium current in nerve and heart. As proposed by DiFrancesco & McNaughton (1979) in heart muscle, an increase in potassium concentration immediately outside the membrane cannot be retained since in uterus no variation in the reversal potential of the outward current can be measured by increasing or decreasing the external calcium concentration. It seems justifiable to consider calcium-mediated potassium activation and voltage-dependent potassium activation as two functionally distinct systems in myometrium.

A similar classification of the outward current components based on pharmacological experiments is proposed on the following observations: (1) TEA blocks both fast and slow components of the outward current; (2) 4-AP selectively blocks the fast component.

The similarity of the effects of TEA on the two components of the current tails suggests that the potassium channels could have similar TEA receptors. It appears that the receptors associated with the two systems reversibly bind TEA with a half-maximal response of about 10 mM. This value is close to that found in frog skeletal muscle (8 mM; Stanfield, 1970) and in nerve cells (8 mM; Thompson, 1977; 10 mM; Meech & Standen, 1975). It will be noted that the rate at which the current tails declined was unaffected by TEA external concentrations suggesting that TEA acted only on potassium activation. More interesting is the action of 4-AP on current tails since it allows separation of the two components of the outward current. 4-AP selectively blocks the fast component which corresponds to the voltage-dependent potassium current. Similar action of 4-AP has been recently presented in skeletal muscles (Gillespie & Hutter, 1975) and in squid axons (Meves & Pichon, 1977). In uterus the voltage-dependent potassium system binds 4-AP with a half-maximal response of about 40 mM, a concentration which is 4 to 40 times higher than that obtained in nervous and muscular structures.

The fact that the effect is very rapid (3–4 min) suggests that the receptor is easily accessible to 4-AP and could be located on the outside of the membrane.

It is proposed that depolarization of uterine smooth muscle can activate two types of potassium channels. One channel is voltage-dependent and can be described with the Hodgkin–Huxley equations. This channel is selectively blocked by 4-AP. Activation of the other channel is dependent on the internal calcium concentration (which is partly related to calcium influx.) It is evident that all mechanisms which may regulate the level of intracellular calcium (e.g., Na-Ca exchange, Brading (1978)) could also modify the calcium-dependent potassium conductance.

In conclusion, it seems that the interaction between calcium influx and calcium-mediated potassium activation could play an important role in the regulation of the

action potential activity in smooth muscles. Its presence may be revealed by the appearance of regenerative activity following treatment with TEA in smooth muscles (Ito, Kuriyama & Sakamoto, 1970; Mekata, 1971; Vassort, 1975; Droogmans, Raeymaekers & Casteels, 1977) as in other muscle fibres (Mounier & Vassort, 1975).

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