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MACROSCOPIC K^+ CURRENTS IN SINGLE SMOOTH MUSCLE CELLS OF THE RABBIT PORTAL VEIN

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SUMMARY

1. Single smooth muscle cells isolated from rabbit portal vein were voltage clamped at room temperature using the whole-cell configuration of the patch-clamp technique. These cells exhibited a mean resting potential of −47·9 mV and a mean input resistance of 376 MΩ.

2. Using small tip diameter micropipettes (to avoid dialysis of the cells), depolarizing voltage-clamp pulses from a holding potential of −50 mV elicited two distinct outward currents: a quasi-instantaneous background current and a time-dependent current that did not appear to inactivate (delayed rectifier). Upon return to the holding potential, an outward tail current decaying back to the holding current was observed.

3. The time course of development of the tail current as estimated from envelopes of tail current protocols followed the kinetics of activation of the delayed rectifier elicited during the preceding test pulse. The tail current reversed close to the equilibrium potential for K^+ ions indicating that it is mainly carried by potassium ions.

4. Using large tip diameter micropipettes to internally dialyse the cells (EGTA = 0·1 mM; ATP = 5 mM), two additional outward currents having transient kinetics were revealed: a smooth transient outward current (I_{to}) and spontaneous transient outward currents (STOCs). I_{to} was found to be mainly selective for K^+ ions and exhibited voltage-dependent inactivation with half-maximal availability near −40 mV.

5. Removal of calcium from the bathing solution significantly reduced the background current and abolished both I_{to} and STOCs. The delayed rectifier current appeared to be insensitive to this procedure. The two types of transient outward currents were never recorded when EGTA was elevated to 5 mM inside the micropipette whereas the background and delayed rectifier currents were not affected. These results suggested that I_{to} and the spontaneous transient outward currents are activated by internal calcium.

6. External application of TEA (0·5–20 mM) blocked all four outward currents. Calcium replacement by barium significantly reduced the background current and I_{to}, and had small effects on the delayed rectifier current. When potassium was

replaced with caesium (130 mM) and TEA (20 mM) inside the pipette, none of the outward currents described was ever observed. In about 60% of the cells dialysed with this solution a small inward Ca^{2+} current was revealed.

7. External application of caffeine (5 mM) abolished STOCs in cells in which this activity was present under control conditions. In cells lacking this type of activity under control conditions caffeine induced and later abolished this type of current. Removal of ATP from the pipette solution significantly reduced the occurrence of the oscillatory type of current.

8. In conclusion, four components of outward currents have been described in rabbit portal vein cells which reflect the behaviour of distinct K^+ channels. The background current may be the one underlying the resting potential. The delayed rectifier K^+ current would be the main current responsible for repolarization of the membrane potential during an active response. The oscillatory type of transient outward current appears to be related to a sudden discharge of Ca^{2+} from internal stores, namely the sarcoplasmic reticulum. On the other hand, I_{to} seems to depend more on the resting level of intracellular free calcium. Both would also be important for repolarization when the intracellular Ca^{2+} concentration rises.

INTRODUCTION

The recent development of cell dispersion and patch-clamp techniques (Hamill, Marty, Neher, Sakmann & Sigworth, 1981) has provided new opportunities to characterize the electrical and pharmacological properties of a variety of different types of smooth muscle. Earlier work on smooth muscle preparations revealed a rich diversity in terms of basic electrical properties, sensitivity to receptor agonist and antagonists (Johansson & Somlyo, 1980; Bulbring & Tomita, 1987) and sensitivity to ion channel blockers (Godfraind, Miller & Wibo, 1986). Thus substantial differences exist according to species, the category of smooth muscle (vascular, visceral, myometrial, etc.) and even among smooth muscle of the same category (large *versus* small vessels, veins *versus* arteries, etc.; Hermsmeyer, 1980). A thorough description of the ionic channels responsible for some of this diversity seems essential for a full understanding of their function *in vivo* under normal as well as abnormal conditions. This is especially true in the case of vascular smooth muscle, which plays an essential role in the regulation of blood flow throughout the vascular bed.

A number of recent studies, using these new techniques, have described the properties of K^+ currents in a variety of smooth muscle cells. Time-dependent whole-cell K^+ currents have been described in cultured aortic cells (Toro & Stephani, 1987), rabbit pulmonary artery cells (Okabe, Kitamura & Kuriyama, 1987), toad stomach cells (Walsh & Singer, 1981, 1987), rabbit and guinea-pig stomach cells (Mitra & Morad, 1985), rabbit jejunal cells (Benham, Bolton & Lang, 1984), and cells from guinea-pig urinary bladder (Klockner & Isenberg, 1985). In some but not all of these preparations, K^+ current seems to be partially activated by influx of Ca^{2+} through voltage-dependent Ca^{2+} channels. Another type of K^+ current, described as spontaneous transient outward currents (STOCs; I_{oo}), has been observed in rabbit jejunum and ear artery cells (Benham & Bolton, 1986), and intestinal smooth muscle ball cells (Ohya, Kitamura & Kuriyama, 1987*a*). These transient K^+ currents seem

to be different from those described above since they appear to be induced by quantal release of Ca^{2+} from internal stores.

Single-channel measurements in cell-attached and excised membrane patches have characterized large-conductance calcium-activated K^+ channels in canine tracheal muscle (McCann & Welsh, 1986), toad stomach (Berger, Grygorcyk & Schwarz, 1984; Singer & Walsh, 1987), rabbit jejunum and guinea-pig mesenteric artery (Benham, Bolton, Lang & Takewaki, 1986), and rabbit portal vein (Inoue, Kitamura & Kuriyama, 1985; Inoue, Okabe, Kitamura & Kuriyama, 1986). In this latter preparation, three types of unitary K^+ activity were described (K_S, K_M and K_L) and classified according to different slope conductances in symmetrical potassium solutions (142 mM), voltage dependence, sensitivity to TEA and calcium concentration on both sides of the membrane.

The purpose of our experiments was to attempt to characterize the different components of whole-cell K^+ current which might correspond to the K_S, K_L and K_M channels (Inoue *et al.* 1985; Inoue *et al.* 1986) in isolated smooth muscle cells of rabbit portal vein. Such a characterization might help reveal the functional role of these various K^+ channels. Our results demonstrate the existence of four distinct whole-cell K^+ currents in this preparation: a time-independent background current, a slowly activating delayed rectifier K^+ rectifier, a smooth transient outward current (I_{to}) and finally a spontaneous transient outward current that is similar to the STOCs and I_{oo} reported by Benham & Bolton (1986) and Ohya *et al.* (1987*a*) respectively. To our knowledge, this is the first report describing four distinct whole-cell K^+ currents in a single vascular smooth muscle preparation. A preliminary report of these results has been presented (Leblanc & Hume, 1988).

METHODS

Preparation

Albino rabbits of either sex were killed by injecting T-61 (Hoechst-Roussel Agri-Vet Company, Somerville, NJ, USA) into the ear vein. After removal of the portal vein from the animal's chest, the vessel was placed in a cold oxygenated (100% O_2) dissection medium having the following composition (mM): NaCl, 130; $NaHCO_3$, 10; KCl, 4·2; KH_2PO_4 1·2; $MgCl_2$, 0·5; $CaCl_2$, 1·8; dextrose, 11; aspartic acid, 1; glutamic acid, 1; *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (HEPES–NaOH, pH = 7·35), 10. Layers of fat and connective tissue were carefully removed under binocular examination. The vessel was then cut into pieces of 4 × 4 mm in size and incubated for 60 min at room temperature in the same medium except that calcium was omitted (nominally Ca^{2+} free). Smooth muscle cells were dispersed in this latter medium in which 150–200 units/ml of collagenase type I (Sigma Chemicals Company, St Louis, MO, USA), 0·1% bovine serum albumin (Sigma) and 1 mM-ATP (disodium salt; Sigma) were added. The dispersion proceeded for 50–70 min at 35 °C with no agitation. After 50 min, appearance of smooth muscle cells was repeatedly verified under microscopic examination by gentle trituration of one piece every 5 min using a pasteur pipette. When a large number of elongated smooth muscle cells was observed, the enzymatic procedure was stopped and all pieces were agitated in the Ca^{2+}-free solution discussed above to disperse the cells, and the supernatant was stored in the cold (4 °C) until use.

Before each experiment, a sample of the stored supernatant was deposited on a Petri dish mounted on the stage of an inverted microscope (model TMS, Nikon, Tokyo, Japan) equipped with a TV camera to continually monitor the cell appearance during the experiment. The cells were then perfused for 5–10 min with a solution containing a normal calcium concentration (1·8–2 mM) before starting the patch-clamp experiments. Some of the cells contracted irreversibly during the initial

perfusion. Patch-clamp experiments were only performed on the cells that remained in a relaxed state.

Solutions

All experiments were carried out at room temperature (21–23 °C). The standard external solution used had the following composition (mM): NaCl, 130; $NaHCO_3$, 10; KCl, 4·2; KH_2PO_4, 1·2; $MgCl_2$, 0·5; $CaCl_2$, 1·8 dextrose, 5·5; HEPES-NaOH (pH = 7·35), 10. The reason for employing a mixed bicarbonate-HEPES buffer is related to the observation that during the isolation procedure, using a HEPES buffer alone significantly reduced the isolation yield. Similarly, it was found that the appearance of the cells in the Petri dish during a patch-clamp experiment deteriorated more quickly in the absence of bicarbonate. Combining these two buffers seemed to prevent contraction of the cells and improved the dispersion yield. This solution was constantly bubbled with a 100% O_2. In this situation, the pH stabilized between 7·35 and 7·4. In experiments in which external calcium was omitted, an equal concentration of $MgCl_2$ replaced it to minimize possible changes in surface charges. In the experiments using tetraethylammonium chloride (TEA), for all concentrations above 0·5 mM, an equivalent concentration of NaCl was removed for osmolarity balance. Two internal pipette solutions were used for intracellular dialysis. The standard internal solution designed to study the outward currents had the following composition (mM): potassium gluconate, 110; KCl, 20; ATP-dipotassium salt, 5; creatine phosphate, 5; $MgCl_2$, 0·5; ethyleneglycol-bis-(β-aminoethylether)*N,N'*-tetraacetic acid (EGTA), 0·1; HEPES-KOH (pH = 7·2), 5. In some experiments, potassium channels were blocked using the following solution (mM): caesium aspartate, 110; caesium chloride, 20; TEA, 20; $MgCl_2$, 0·5; ATP-magnesium salt, 5; creatine phosphate, 5; EGTA, 0·1; HEPES–CsOH (pH = 7·2), 5. Both ATP salts, creatine phosphate, HEPES buffer and TEA are from Sigma Chemical Company (St Louis, MO, USA).

Electrophysiology

The whole-cell configuration of the patch-clamp technique (Hamill *et al.* 1981) was employed to voltage-clamp the smooth muscle cells. Two types of micropipettes were used. The single suction micropipette technique described by Hume & Giles (1983) was used in some experiments to avoid internal dialysis while voltage-clamping the cells. Small diameter pipettes having a tip diameter of < 1 μm (square bore capillary tubing; Glass Co. of America, Bargaintown, NJ, USA) were pulled on a one-stage vertical microelectrode puller (David Kopf Instruments, model 700D, Tujunga, CA, USA). When filled with 2 M-potassium gluconate–100 mM-KCl, these electrodes had DC resistances that ranged between 2 and 5 MΩ. Standard patch micropipettes with tip diameter of 1–3 μm for internal dialysis were pulled on a two-stage vertical micropipette puller (model PP-83, Narashige Scientific Instruments Laboratories, Tokyo, Japan) and then fire-polished using a microforge (Model MF-83, Narashige Scientific Instruments Laboratories, Tokyo, Japan). Pipettes' resistances ranged from 4–6 MΩ when filled with the internal solution described above. As soon as a gigaohm seal was formed between the membrane and the pipette by applying negative pressure, access to the cell interior was obtained by rupturing the membrane using either additional suction or by application of strong voltage pulses (1·5 V in amplitude and 0·1–10 ms in duration) applied through the pipette. Voltage-clamp command pulses were delivered through a patch-clamp amplifier (model Axopatch 1-B, Axon Instruments Inc., Burlingame, CA, USA) with a 50 MΩ headstage feedback resistor. Series resistance compensation was performed in all experiments.

Data analysis

Data were recorded on a videotape recorder (Model IR-2, Indec systems Inc., CA, USA) at a bandwidth of DC to 20 kHz, and were digitized off-line at sampling intervals ranging from 0·35 to 1·5 ms and stored either in a PDP 11 23 computer (Digital Equipment Corp., Maynard, MA, USA) or in an IBM AT computer for analysis. Current and voltage traces were displayed with a digital plotter (model 7470A, Hewlett-Packard, San Diego, CA, USA).

TABLE 1. Electrical properties of single cells from rabbit portal vein. Summary of the current-clamp experiments performed in nine cells to determine their passive electrical properties

Cell	V_m (mV)	R_{inp} (MΩ)	T_m (ms)	C_m (pF)	Length × width (μm)
1	−30	430	6	14	120 × 5
2	−55	470	11	23	203 × 14
3	−44	60	1·5	25	200 × 6
4	−35	290	5	17	135 × 5
5	−70	420	7	17	131 × 5
6	−32	300	2	7	81 × 4
7	−52	110	4	37	113 × 6
8	−50	300	3	30	188 × 8
9	−63	1000	21	21	138 × 6
Mean	47·9	376	6·7	21·2	145 × 7·0
S.D.	13·9	272	6·1	9·1	42·3 × 3·0
S.E.M.	4·6	91	2·0	3·0	14 × 1·0

RESULTS

Passive electrical properties

All experiments were carried out on spindle-shaped relaxed cells. The isolation yield varied from day to day but the technique usually produced a large number of elongated cells; a very small percentage of rounded cells also resulted from this procedure. These types of cells were not used in the present study.

Table 1 presents a summary of survey experiments carried out to estimate the passive electrical properties of these cells. In nine cells, the length ranged from 81 to 203 μm (mean ± S.E.M. = 145 ± 14 μm) and the width from 4 to 14 μm (mean ± S.E.M. = 7 ± 1·0 μm). The single suction microelectrode technique without internal dialysis was employed to current-clamp these cells. The resting potential varied from −30 to −70 mV with a mean of −47·9 ± 4·6 mV which is in the range of what has been reported by several investigators in isolated smooth muscle cells (Klockner & Isenberg, 1985; Mitra & Morad, 1985; Nakazawa, Matsuki, Shigenobu & Kasuya, 1987). Using small constant hyperpolarizing current pulses (causing no more than 10 mV change in the membrane potential, V_m), the membrane input resistance (R_{inp}), time constant of the decay of change in membrane potential (T_m) induced by the imposed current, and the calculated total capacitance (C_m) were estimated.

In most cells studied under current-clamp, we were not able to elicit an action potential upon electrical stimulation at room temperature in a bathing solution containing a normal calcium concentration. However, it was possible to induce active responses by either treating the cells with the K^+ channel blocker tetraethylammonium chloride (data not shown), or by raising the extracellular calcium concentration from 1·8 to 5·4 mM. Figure 1*A* shows a typical example of active responses elicited upon electrical stimulation of a cell bathed in 5·4 mM $[Ca^{2+}]_o$. Constant depolarizing current pulses (lower trace) of 10 ms in duration were applied from the resting potential. In response to a small constant-current pulse, the membrane potential repolarized over the time course of about 100 ms (first step). As the cell was depolarized further (near −7 mV), a small regenerative depolarization was observed. Further increasing the stimulus strength progressively reduced the

depolarizing notch and accelerated repolarization. These responses indicate that the active notch observed around 0 mV is related to the activation of an inward current probably associated with Ca^{2+} entry into the cell. When the cell was depolarized to higher levels, outward currents were turned on which accelerated repolarization. The difficulty in eliciting active responses in low external Ca^{2+} solutions in these cells is not surprising in view of the predominance of outward current prevailing in this preparation as will be described in the next sections.

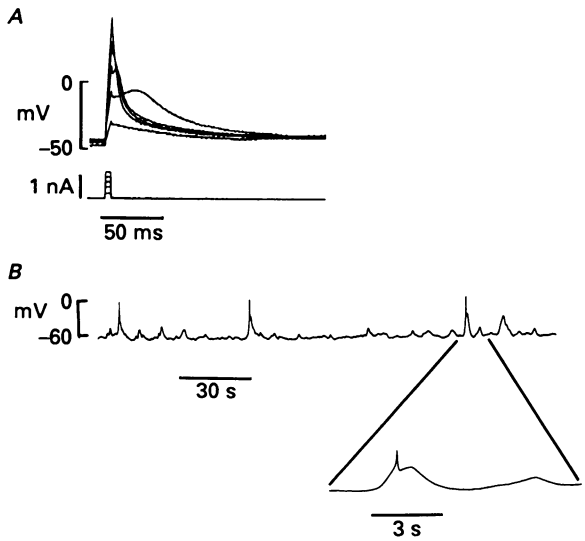

Fig. 1. Passive and active electrical properties of rabbit portal vein smooth muscle cells. *A*, current-clamp protocol to elicit an active response. Five consecutive square pulses of 10 ms in duration incrementing by a constant value were applied to the cell at a frequency of 0·2 Hz $[Ca^{2+}]_o$ = 5·4 mM. *B*, action potentials recorded in a spontaneously active cell. The inset shows a portion of the top trace at higher sweep speed. $[Ca^{2+}]_o$ = 1·8 mM.

On rare occasions, we identified cells which were firing spontaneously. An example of such behaviour is shown in Fig. 1*B*. The inset shows a portion of the top trace at higher sweep. As can be seen, these types of cells exhibit many irregular small depolarizations that do not reach threshold. Some of these depolarizations, however, do reach threshold and all-or-none action potentials lasting several seconds are generated. These events peak around 0 mV and repolarize in two phases: a fast component followed by a slow developing plateau. This type of active response markedly resembles the regenerative responses recorded from cells of the multicellular preparation using microelectrodes. The voltage-clamp experiments described in the following sections were only performed on cells that were quiescent. Spontaneously active cells will be studied in future experiments.

Outward currents in non-dialysed cells

In some experiments, we used the small suction micropipette method to voltage-clamp portal vein cells. This technique has been useful for studying the ionic currents in small isolated cardiac cell preparations (Hume & Giles, 1983; Giles & Shibata,

1985). It has the advantage that ionic currents can be examined without the necessity of internal dialysis of the cells. Another advantage lies in the fact that the small tip of the micropipette facilitates formation of a giga-seal on these thin cells and greatly enhances the number of successful experiments. It may be argued that the high concentration of potassium gluconate (2 M) inside the pipette will slowly contaminate by diffusion the intracellular milieu. This phenomenon is probably minimal in the present experiments for the following reasons: (i) the tip of the pipette is much smaller than the standard pipettes used for whole-cell patch clamp (< 1 μm tip diameter); (ii) a higher negative pressure is required to form a giga-seal (Hume & Leblanc, 1988); (iii) the reversal potential of outward potassium tail currents remains constant during the time course of experiments (J. R. Hume & N. Leblanc, unpublished observations); (iv) the K^+ currents observed in isolated cardiac cells measured using this technique are similar to those obtained using standard patch electrodes with internal dialysis (compare Hume, Giles, Robinson, Shibata & Nathan (1986) with Simmons, Creazzo & Hartzell (1986) in single frog atrial cells).

Figure 2*A* shows typical examples of membrane currents elicited by 500 ms voltage-clamp pulses from a holding potential of -50 mV. In all cells tested using this technique, two components of outward current were consistently observed. A quasi-instantaneous background component establishes immediately following the capacitative transient (jump step in membrane current). When the cell was depolarized to -30 mV and further, a time-dependent outward current component slowly developed. Figure 2*A* also shows that the activation of the time-dependent current became faster at more depolarized potentials indicating that its activation kinetics are voltage dependent. Upon return to the holding potential, a small outward tail current relaxation can be observed. The size of the tail current increased as the magnitude of the preceding depolarization was increased and reached a steady state following the depolarization to $+30$ mV. Another feature of this experiment is the apparent increase in the current noise as the cell is clamped towards more positive potentials. This behaviour was typical of all cells tested using either the suction micropipette technique or standard dialysing patch micropipettes (see Fig. 4*A*).

Figure 2*B* shows the current–voltage (I–V) relationships for the two current components involved. In this plot, the quasi-instantaneous (time-independent) current was measured as the initial current jump from the holding current. The total current was also measured at the end of the pulse. The I–V relationship for the background current is relatively linear in the range between -120 and 0 mV but rectifies slightly in the outward direction at more positive potentials ($+10$ mV and beyond). Linear regression of the data points of the linear portion of the I–V curve for the background component revealed a slope resistance of 204 MΩ. This value is well within the range of the input resistance measurements performed in current-clamp experiments (Table 1; mean $\pm$ S.D. $= 376 \pm 272$ MΩ). These results support the idea that the background component reflects at least partly the activity of an ionic channel active near the resting potential of the cell. The I–V relationship for the time-dependent outward current can be deduced from the difference between the current measured at the beginning and at the end of the voltage pulse. In this particular cell, the time-dependent current started to activate around -30 mV and also rectifies in

the outward direction. In all cells analysed, the onset of this component started in the range of −40 to −20 mV. Holding the membrane potential to more negative potentials (−80 to −90 mV) did not alter the activation range. These results suggest

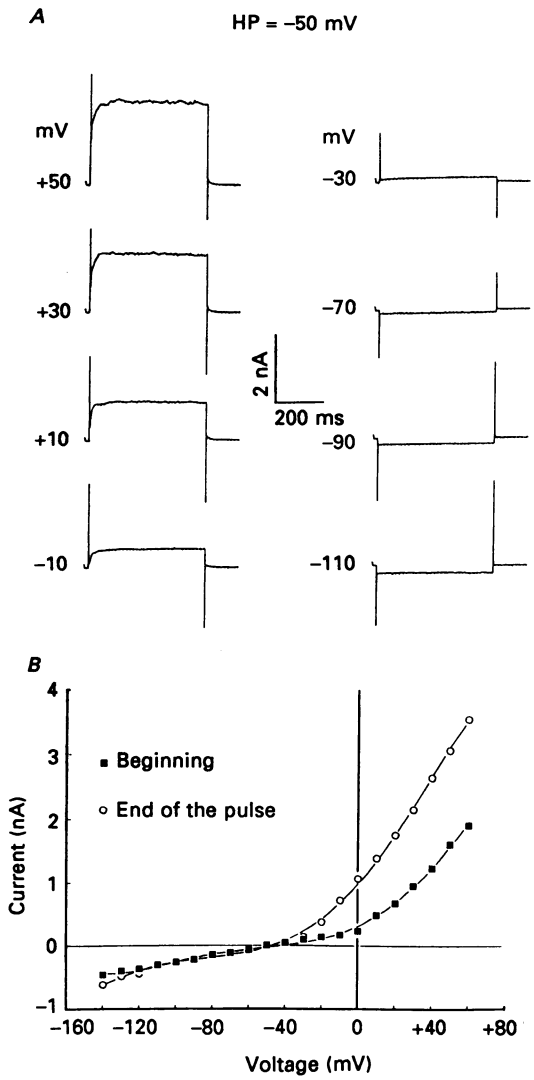

Fig. 2. Outward currents recorded in non-dialysed cells. *A*, the holding potential was −50 mV. 500 ms depolarizing and hyperpolarizing voltage-clamp pulses were applied to the cell. *B*, current–voltage relationships for the current measured at the beginning (■) and at the end of the pulse (○).

that the channel underlying this component is probably not open near the resting potential but may be involved in repolarization of the membrane when an active response is generated. Due to its apparent similarity with the delayed rectifier K^+ current described in many preparations, this component will be referred to as the delayed rectifier.

Tail current

To test whether the outward tail current reflected deactivation of one of the outward components activated current during the test pulse, an envelope of tails was carried out by varying the duration of the pulse. Figure 3*A* shows a typical example of such an experiment. The holding potential was held at −50 mV.

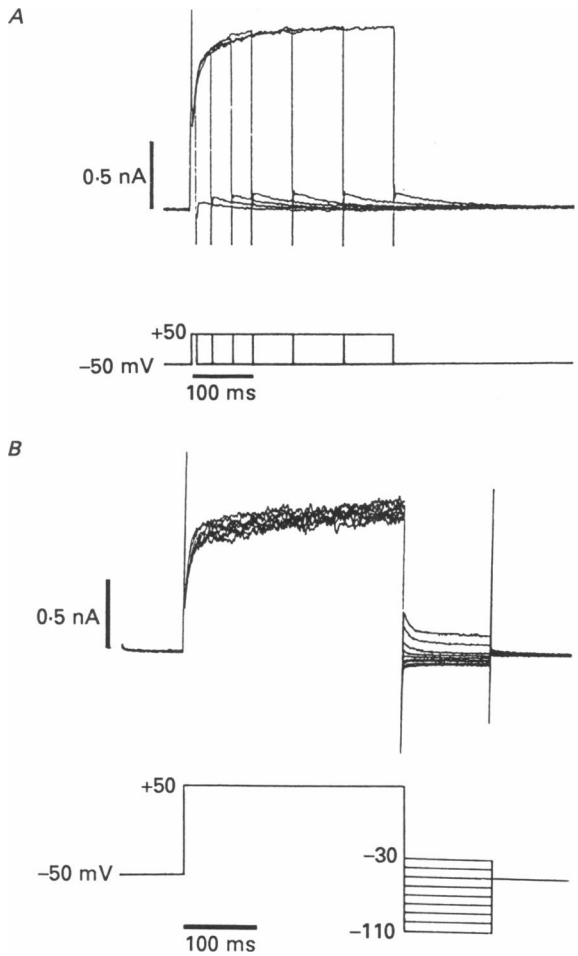

Fig. 3. Analysis of the tail current in non-dialysed cells. *A*, envelope of tails performed by applying consecutive voltage steps of increasing durations. *B*, tail reversal potential measurement estimated by a double-pulse protocol. In this cell, the tail inverted close to the calculated E_K (between −70 and −80 mV). In this and the following figures, the voltage traces shown represent the command signal applied to the amplifier.

Consecutive voltage steps to +50 mV of different durations were applied to the cell. The time course of development of the outward tail current closely followed the time course of activation of the delayed rectifier induced during the preceding test pulse indicating that the tail reflects deactivation of the delayed rectifier and can be used as a quantitative index of its size and time course.

To examine the ionic selectivity of the delayed rectifier, the reversal of the outward tail current was evaluated in four cells using a double-pulse protocol. The cell was held at −50 mV and depolarized to +50 mV for 300 ms. The membrane was then stepped back for 100 ms to various tests potentials to measure the reversal potential of the tail current. Fig 3*B* shows an example of one such experiment. In this cell, the tail reversed between −70 and −80 mV. Assuming an internal K^+ concentration of 140 mM ($[K^+]_o = 5{\cdot}4$ mM), the calculated E_K at 22 °C is −83 mV. The close correlation between the measured and estimated values suggests that this current is mainly carried by K^+ ions.

Outward currents in dialysed cells

When the cells were dialysed using larger tip diameter micropipettes containing 140 mM-K^+ and 5 mM-ATP, two additional components of outward current having transient kinetics were revealed. An outward current which activated quickly and decayed slowly was observed in about 65% of the cells studied (Fig. 4*A*). This current will be referred to as the transient outward current, I_{to}. Due to the overlap of the delayed rectifier K^+ current and due to its small amplitude in some cells, it was often difficult to establish its activation range. In some cells, it was readily discernable around −40 mV whereas in others, a small transient component was only discernable at more positive potentials (0 to +20 mV). This current is very similar to the 4-aminopyridine-sensitive current described by Okabe *et al.* (1987) in isolated pulmonary smooth muscle cells.

In another cell, we examined the voltage dependence of the inactivation of I_{to}. A double-pulse protocol with a 2 s pre-pulse to various levels (from −100 to +10 mV), 10 ms delay for resetting of the activation variable, and a 3 s test pulse to +50 mV was used. The current was fully available around −90 mV and was almost completely inactivated at +10 mV. Although the size and time course of this current is very similar to the one described by Okabe *et al.* (1987) in pulmonary artery cells, its voltage-dependent inactivation characteristics appear to differ significantly. They reported that inactivation started near −40 mV and depended markedly on the duration of the pre-pulse from 1 to 20 s. In rabbit portal vein cells, 2 s duration pre-pulses produced maximal inactivation of I_{to}.

Figure 5 shows the results of an experiment in which we examined the properties of outward tail currents associated with I_{to}. Figure 5*A* shows an envelope of tails obtained using a double-pulse protocol. The cell was depolarized at +50 mV for various times and stepped back to −10 mV for 100 ms. The time course of development of the tail current and deactivation followed closely the activation and inactivation time course of the transient current, indicating that it is at least qualitatively linked to the development of I_{to} induced during the preceding test pulse.

The data in Fig. 5*B* indicate that I_{to} is mainly carried by K^+ ions. The outward tail current associated with I_{to} reversed near −70 mV in this cell.

A second type of transient activity was seen in about 45% of the dialysed cells investigated. Such behaviour is shown in Fig. 6. In this example, the cell was clamped at −50 mV. Repetitive 500 ms duration depolarizing pulses to +50 mV were delivered at a frequency of 0·2 Hz following membrane rupture. The numbers

indicate the order of the imposed steps. It can be noticed that the first event following membrane rupture was almost free of oscillatory activity and represents the activation of the delayed rectifier K^+ current described earlier (Figs 2 and 3). As dialysis proceeded, spontaneous transient outward currents appeared and became larger as a function of time and eventually stabilized after about 1 min following

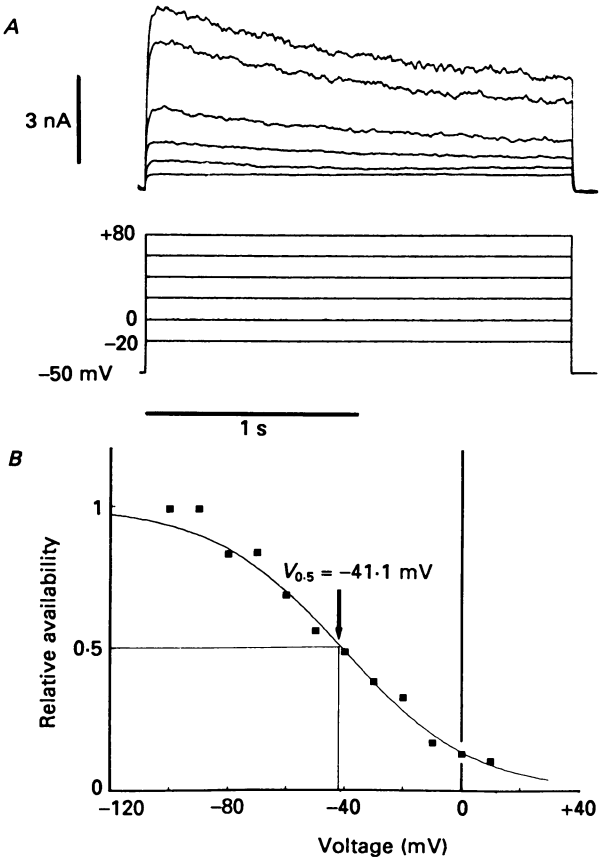

Fig. 4. Characteristics of the smooth transient outward current (I_{to}) recorded in dialysed cells. *A*, typical fast activating and slowly inactivating transient outward currents recorded in one cell. Two second pulses from −50 mV to various potential levels as shown. *B*, steady-state inactivation curve of I_{to} performed in a different cell. A double-pulse protocol was used. The holding potential was −50 mV. Two second pre-pulses to various levels (from −100 to +10 mV) followed by a constant test pulse to +50 mV were employed to assess the steady-state availability of I_{to}. Each data point plotted represents the amplitude of the peak transient outward current normalized to the highest value obtained as a function of the pre-pulse voltage. The line represents a non-linear least-squares fitting of the standard sigmoidal Boltzmann equation: $Y = 1/\{1+\exp[(x+41{\cdot}1)/22{\cdot}47]\}$.

whole-cell access. Although their behaviour varied from cell to cell, their amplitude increased with depolarization and the time to peak of the initial oscillation was generally faster at more positive potentials (see also Fig. 12*A*). At a constant voltage level, the largest peak was often observed right after the onset of the pulse. In three cells, similar spontaneous transient outward currents (STOCs) resembling those

reported by Benham & Bolton (1986) in jejunum and mesenteric artery smooth muscle cells were observed near the holding potential of −50 mV. Their amplitude and frequency were enhanced by depolarizing the cells from −50 to 0 mV. These results suggest that dialysis may be involved in promoting the development of this type of activity. Another feature of these currents is that their kinetics and activation seem to be linked directly or indirectly to a voltage-dependent process.

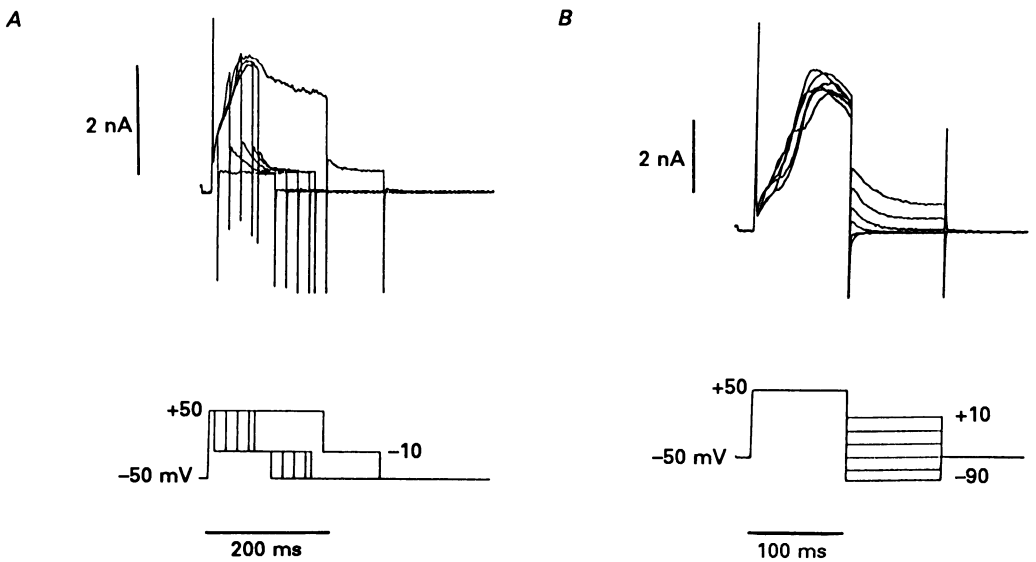

Fig. 5. Characteristics of the tail current associated with I_{to}. *A*, envelope of tails protocol during the onset and relaxation of I_{to}. In this example, after stepping from −50 to +50 mV for various durations, the membrane was repolarized for 100 ms to −10 mV to enhance the amplitude of the tail current. *B*, tail reversal potential measurement performed around the peak of I_{to} using a double-pulse protocol similar to the one shown in Fig. 3*B*. In this cell, the tail reversed close to −70 mV.

Sensitivity to external calcium

It is well known that many K^+ channels in smooth muscle are activated by calcium (Inoue *et al.* 1985; Benham *et al.* 1986; McCann & Welsh, 1986; Singer & Walsh, 1987). Therefore, we examined the effects of removing external calcium on the outward currents in dialysed cells. In all the experiments performed, external calcium was replaced by an equivalent concentration of magnesium to minimize possible changes in surface charges. The results of one such experiment are shown in Figs 7 and 8. In Fig. 7, the cell was held under voltage clamp at −60 mV. In 1·8 mM $[Ca^{2+}]_o$, a large quasi-instantaneous current jump preceded the onset of the delayed rectifier and transient outward currents at more positive potentials. The right column of traces shows an identical protocol performed in the same cell after 20 min exposure to 0 $[Ca^{2+}]_o$. Both the background current and I_{to} were strongly depressed by external calcium removal, whereas the delayed rectifier K^+ current remains as the dominating outward current. Notice also the reduction in the current noise level at +60 mV compared to control. Figure 8 shows the *I–V* relationships for the three outward currents obtained in normal external calcium and after calcium removal.

The inset of Fig. 8 describes how the currents were measured. The time-independent (background) current was measured by subtracting the amplitude of the instantaneous current step following the capacitative transient (*A*); the delayed rectifier was estimated by subtracting the current at the end of the pulse from the time-independent current (assuming that I_{to} is completely inactivated) (*B*); I_{to} was measured by subtracting the outward peak current from the current level at the end of the pulse (*C*). This figure shows that removing extracellular calcium selectively inhibited the time-independent current (*A*) and I_{to} (*C*) but barely affected the delayed rectifier (*B*). Similar findings were observed in three other cells.

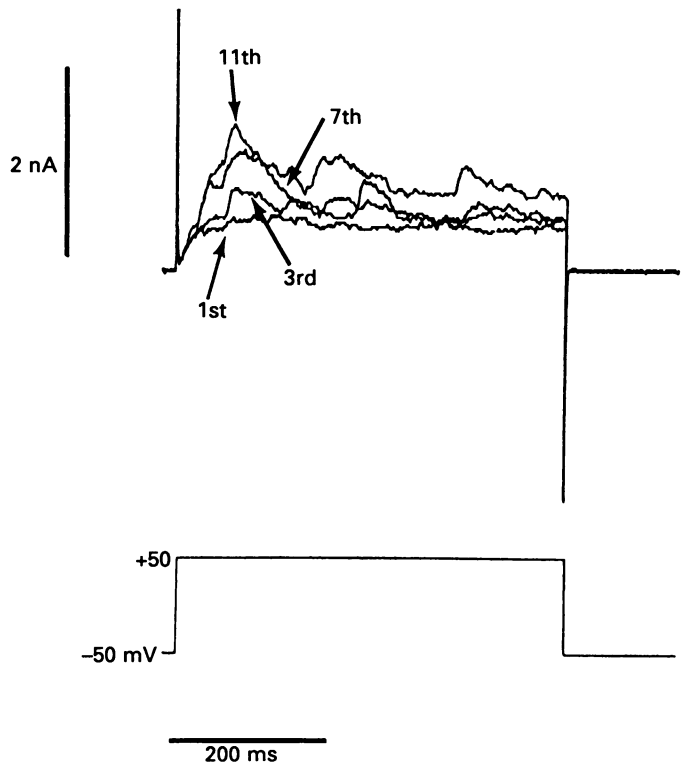

Fig. 6. Spontaneous oscillatory transient outward currents. In this cell consecutive voltage-clamp steps to +50 mV from a holding potential of −50 mV were applied at a frequency of 0·2 Hz as soon as the membrane was ruptured. The numbers indicate the sequence of the imposed steps. Note the appearance of transient rhythmic outward currents as dialysis proceeds in whole-cell mode.

These results show that at least two of the three outward currents are sensitive to a variation in external calcium. Whether the site of action is internal or external can not be assessed from the present experiments but it appears that I_{to} is activated by internal calcium since it was never seen when the concentration of EGTA inside the pipette was raised from 0·1 to 5 mM (five cells). A similar conclusion may also be drawn for STOCs, since they were also not observed in cells dialysed with higher EGTA concentrations. In these experiments, membrane currents resembled those observed in non-dialysed cells: background and delayed rectifier K^+ currents.

In three current-clamp experiments, external calcium removal quickly depolarized the cells (within 2 min) correlating with the inhibition of the time-independent background current (data not shown).

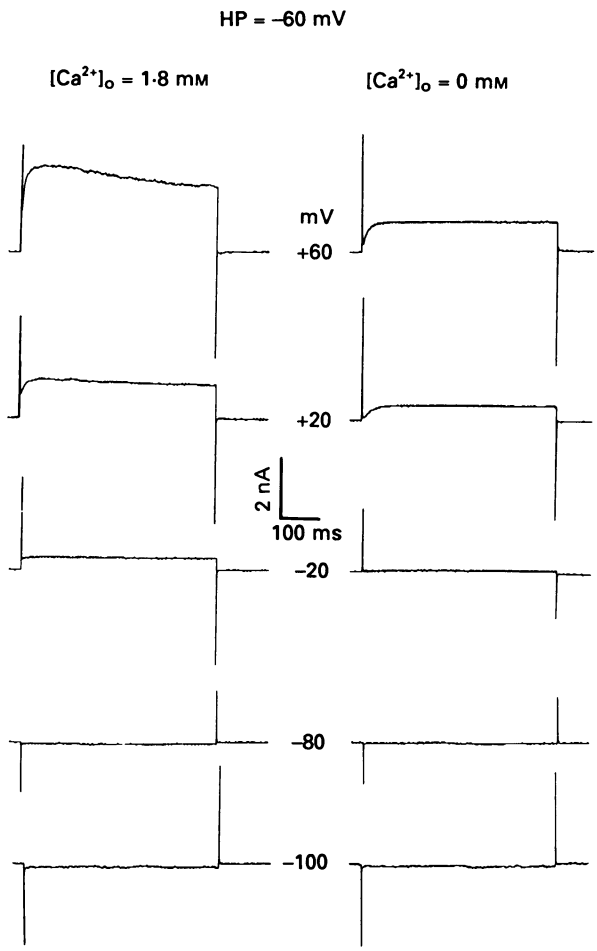

Fig. 7. Effects of calcium removal on the outward currents in dialysed cells. The left column shows control records obtained in normal extracellular calcium concentration. On the right-hand side are shown recordings obtained in the same cell during an identical voltage-clamp protocol performed after 20 min of exposure to a Ca^{2+}-free solution: 1·8 mM-$MgCl_2$ was added to this solution to minimize possible changes in surface charges (total Mg^{2+} concentration was 2·3 mM). Note the depression of I_{to} and of the initial instantaneous jump step in membrane current by 0 Ca^{2+} treatment. HP, holding potential.

Sensitivity to K^+ channel blockers

Tetraethylammonium chloride (TEA) has been shown to be an effective K^+ channel blocker in a wide variety of excitable cells (Hille, 1984). Recently, Inoue *et al.* (1985) have shown in excised membrane patches on rabbit portal vein smooth muscle cells that TEA (0·1–1 mM) blocked the small conductance K^+ channel (K_S:

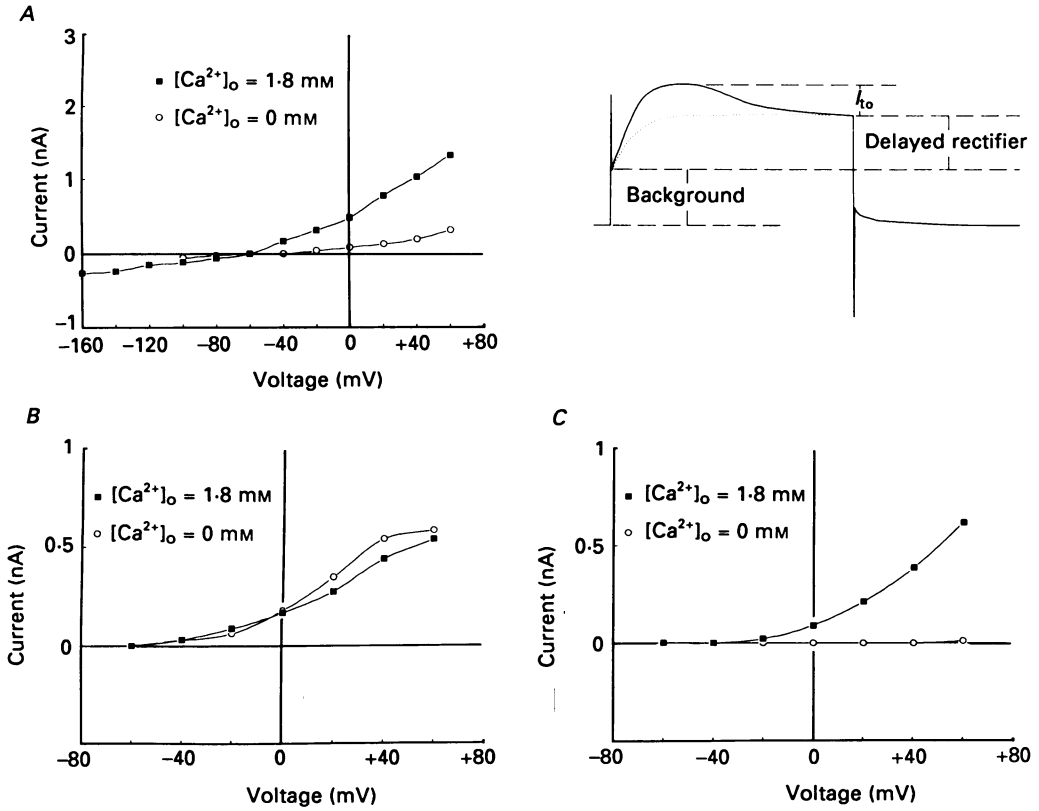

Fig. 8. *I–V* relationships of the outward currents obtained in the presence and absence of added calcium to the external solution. Data obtained from the experiments shown in Fig. 6. The filled squares and open circles were obtained respectively in the presence and absence of external calcium. *A*, quasi-instantaneous background current. *B*, delayed rectifier current. *C*, transient outward current (I_{to}). The inset describes how the three components were measured. This method of separating these components was also used in Figs 9 and 10.

92 pS with 142 mM-K^+ on both sides) when applied from the outside but had no effect on the large conductance channel (K_L; 273 pS) when applied on either side. In addition, Inoue *et al.* (1986) have shown that this compound also reduced the amplitude of the elementary current of the K_M channel (180 pS in symmetrical solutions) when applied from the inside (1–3 mM) but had a weak inhibitory effect when applied from the outside (3–10 mM). In contrast, TEA (1–30 mM) has been shown to block large Ca^{2+}-activated K^+ channels when applied from the inside in isolated patches of arterial and visceral smooth muscle cells (Benham, Bolton, Lang & Takewaki, 1985). We examined in eight cells the effects of external TEA (0·5–20 mM) on whole-cell K^+ currents. For 5 mM and higher, an equivalent concentration of NaCl was omitted for osmolarity balance.

The results of one TEA experiment are shown in Fig. 9. In panels *A*, *B* and *C* are plotted the current–voltage relationships for the background, the delayed rectifier and the transient outward current, respectively, obtained under control conditions

and after 10 min exposure to 20 mM-external TEA. It is clear that this concentration of TEA blocked all three currents. Interestingly in this cell, block of K^+ currents by TEA revealed a small inward current of 29 pA remaining at +10 mV which probably represents an inward calcium current.

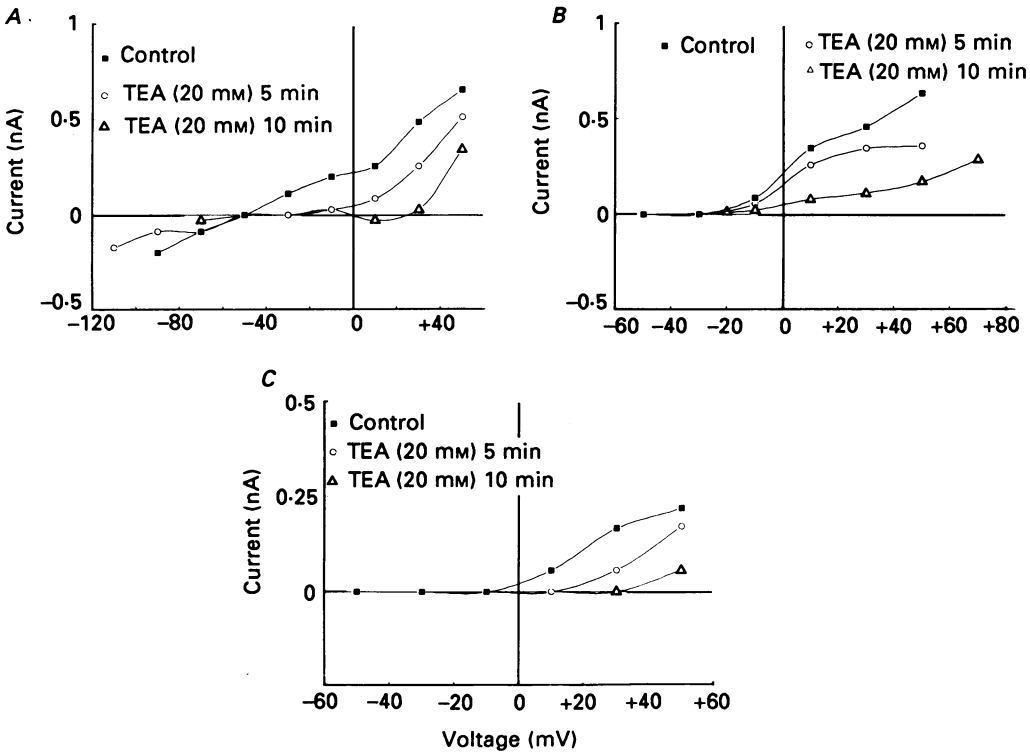

Fig. 9. *I–V* relationships of the outward currents obtained under control conditions and at various times during external application of tetraethylammonium chloride (TEA). Data obtained in the same cell under control conditions (■) and after 5 (○) and 10 min (△) in 20 mM-TEA. *A*, background current. *B*, delayed rectifier current. *C*, I_{to}. The three components were measured as described in the inset of Fig. 8.

In all cells tested, it was found that the effects of TEA on all three K^+ currents were slow (5–35 min depending on the concentration used) which suggests that the site of action is probably intracellular. Due to the difficulty of maintaining the seal for a long period of time (over 20 min), dose-response curves could not be performed. However, on a qualitative basis, the threshold effect of TEA on all currents occurred at about 0·5 mM and was maximal at a concentration of 10 mM.

In three cells in which STOCs were observed, TEA (10–20 mM) also effectively abolished these currents.

In five cells lacking STOCs, as for the calcium removal experiments (Fig. 7), TEA also reduced the current noise level seen at positive potentials. Even at concentrations as low as 0·5 mM, this effect was significant and rapid (less than 2 min). This fast action of TEA suggests that it might act from the outside. In view of the similarities between STOCs and the higher current noise level observed at

positive potentials, it is possible these phenomena share a mechanism of common origin. Therefore a possible mechanism would be that the current noise reflects the activity of Ca^{2+}-activated K^+ channels stimulated by membrane depolarization at low levels of intracellular Ca^{2+}. On the other hand, occurrence of large STOCs detected in some dialysed cells may simply reflect amplification of the current noise level observed in cells lacking STOCs. One hypothesis would be that the free Ca^{2+} level would be higher in these cells. This condition would favour a displacement toward negative potentials of the open probability *versus* membrane potential relationship of Ca^{2+}-activated K^+ channels (Benham *et al.* 1986; Singer & Walsh, 1987), thus allowing the occurrence of STOCs.

In current-clamp experiments, external application of 20 mM-TEA depolarized the smooth muscle cells by 10–24 mV ($n = 3$; data not shown). This observation correlated nicely with the observation that TEA also inhibits the background current and suggests that this latter component of membrane current underlies the resting potential in these cells.

External barium (10 mM) completely blocks in a voltage-dependent manner inward currents passing through Ca^{2+}-activated K^+ channels in tracheal smooth muscle cells. Similarly, outward currents through this channel are blocked when barium ions are applied to the cytoplasmic side (McCann & Welsh, 1986). Barium also reduces the open probability of this type of channel in arterial and intestinal smooth muscle cell membranes (Benham *et al.* 1985). We have investigated the effects of replacing external Ca^{2+} by Ba^{2+} on the outward currents. Figure 10*A* and *B* shows the *I–V* relationships obtained in normal calcium (■) and barium (○) for the background and delayed rectifier K^+ currents respectively. Calcium replacement (2 mM) by barium (2 mM) depressed the background current significantly and the delayed rectifier K^+ current was slightly reduced. Figure 10*C* shows the effect of calcium replacement by barium on the current–voltage relationship of I_{to} in a dialysed cell. The holding potential was −80 mV. In contrast to the calcium removal experiments (Figs 6 and 7), barium depressed but did not abolish I_{to}. Further experiments are needed in order to determine if the effects of barium on the three outward currents are due to a direct effect on the channel, or in the case of the background current and I_{to} (both of which are Ca^{2+}-dependent; Fig. 8) whether barium is less effective as a substitute for Ca^{2+} in activating these channels.

With caesium (130 mM) and TEA (20 mM) inside the micropipette, none of the outward currents described in this paper was ever observed. In some cells under these conditions a small inward current was revealed as shown in Fig. 11. In panel *A* are displayed membrane currents obtained by depolarizing voltage-clamp steps from a holding potential of −50 mV, and in panel *B* the corresponding *I–V* relationship. A small rapidly activating inward current which decays during the pulse can be observed. The bell-shaped *I–V* curve (Fig. 11*B*) with activation beginning around −30 mV and apparent current reversal near +60 mV, with a peak near 0 mV, is typical of the high-threshold dihydropyridine-sensitive calcium current described in many vascular and myocardial preparations. In a cell where the inward current was very small (less than 20 pA), application of the calcium channel agonist Bay K 8644 enhanced this current component by threefold (data not shown) supporting the concept that it is reflecting the activity of high-threshold calcium channels.

In eight out of fourteen cells dialysed with caesium and TEA, this inward current could not be recorded. In some of the cells in which it was detected, it was not present as the membrane was ruptured but slowly appeared over time (2–5 min) suggesting that ATP diffused inside the cell and might metabolically activate the channel (Irisawa & Kokubun, 1983; Ohya, Kitamura & Kuriyama, 1987*b*).

Although possible run-down cannot be ruled out, these observations support the idea that the inward Ca^{2+} current is very small in external solutions containing 1·8 mM-Ca^{2+} and that the outward currents previously described are dominant and largely determine the electrical activity of these cells.

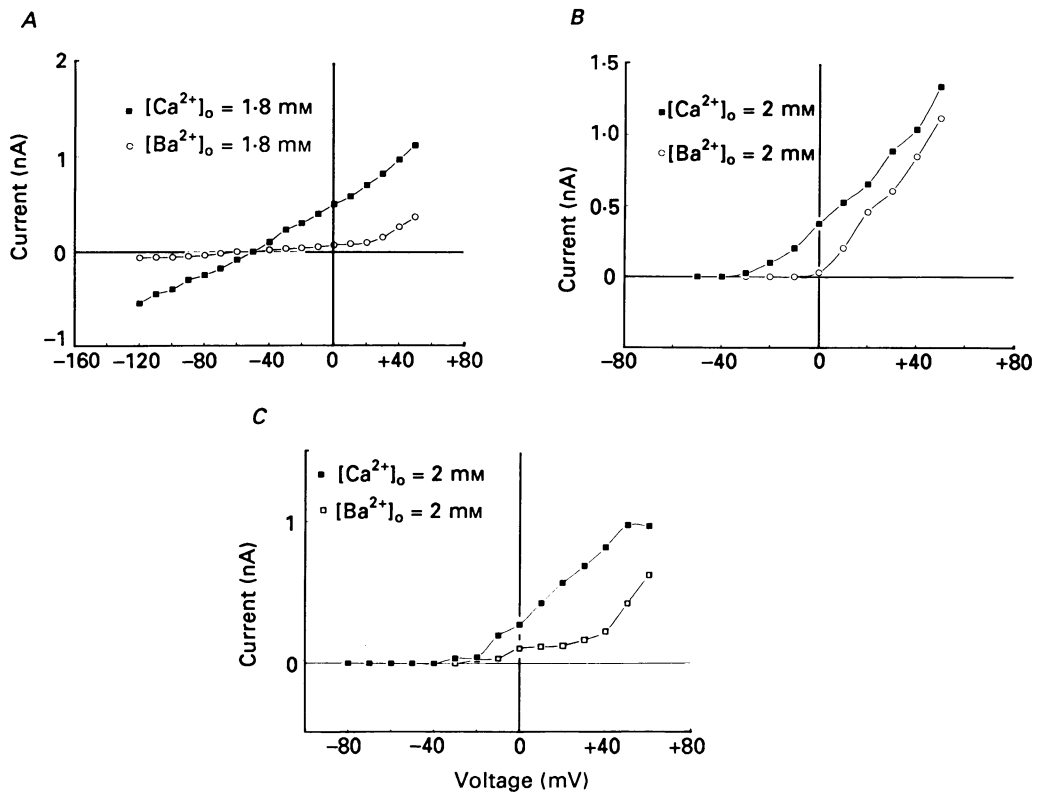

Fig. 10. *I–V* relationships of the outward currents obtained in normal external calcium and after calcium replacement by barium. The data shown in *A* and *B* are from the same cell and the suction microelectrode technique was used. In *C*, the data were obtained in a different cell voltage-clamped using a large dialysing micropipette. *A*, background component. *B*, delayed rectifier current. *C*, I_{to}. The three components were measured as shown in the inset of Fig. 8.

Effects of caffeine

In view of the observed sensitivity of the outward currents to a variation in external calcium concentration, it was of interest to examine the influence of conditions known to induce a change in intracellular free calcium on their behaviour. Caffeine is known to cause sudden release of Ca^{2+} from the sarcoplasmic reticulum in

vascular smooth muscle (Ito, Susuki & Kuriyama, 1977) leading eventually to a large depletion of this Ca^{2+} store. We tested the effects of caffeine (5 mM) on the outward currents in eight cells.

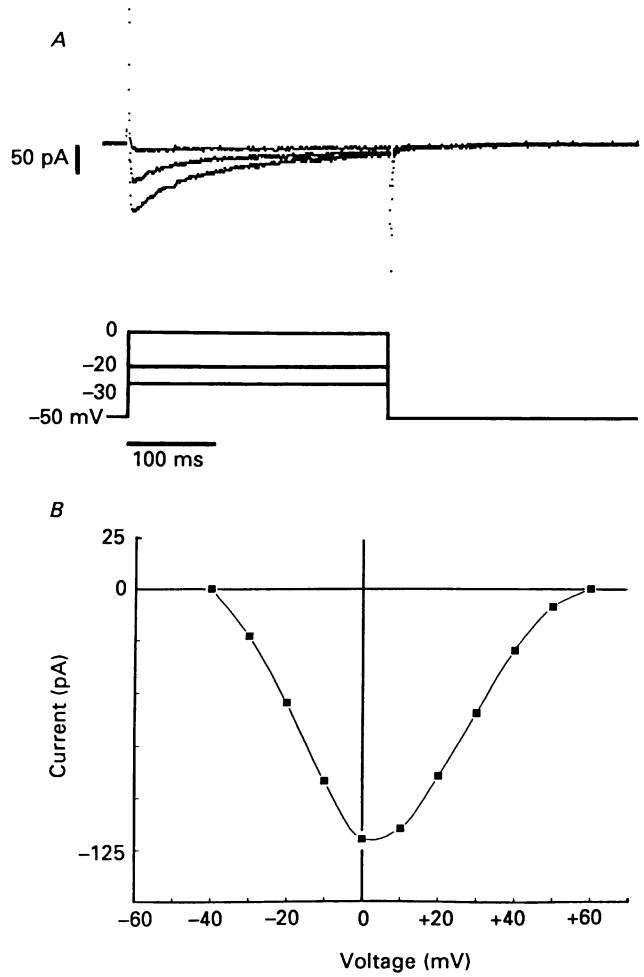

Fig. 11. Internal dialysis with caesium (130 mM) and TEA (20 mM) blocks all the outward currents and reveals a small net inward current. *A*, current records obtained at the three depolarizing test pulses shown from a holding potential of −50 mV. $[Ca^{2+}]_o$ = 1·8 mM. *B*, *I–V* relationship of the current shown in *A*.

In two non-dialysed cells, caffeine had minimal effects on the background and the delayed rectifier K^+ currents. In another non-dialysed cell, very small current oscillations were induced after 1 min of perfusion with caffeine, which were eventually abolished after 4–5 min of continued exposure.

Typical experiments showing the effects of caffeine on the outward currents in dialysed cells are shown in Fig. 12. In panel *A*, control outward currents were obtained by clamping the cell at −50 mV and depolarizing to different potentials for 500 ms. In this cell, this protocol induced the activation of the delayed rectifier and

at more positive potentials, spontaneous transient oscillatory outward currents (STOCs) were also observed. After exposure to caffeine for 10 min (lower set of current recordings), the transient oscillatory outward currents were abolished. However, a smooth transient outward current, I_{to} (see Fig. 5*A*) remained.

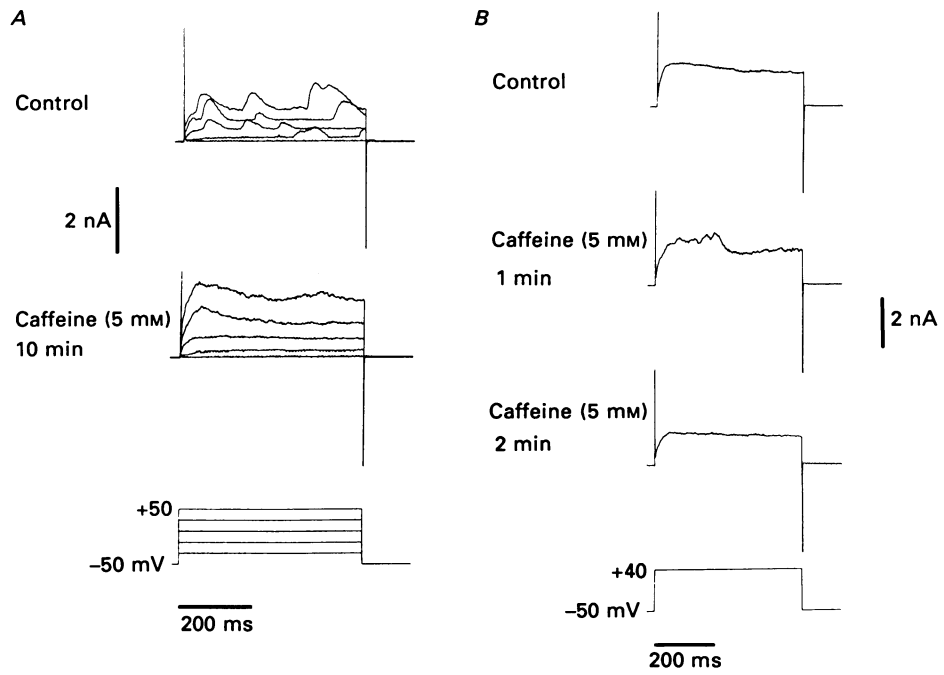

Fig. 12. Effects of caffeine (5 mM) on the oscillatory type of transient outward current. *A*, abolition of the rhythmic outward currents observed in normal external solution (top set of current recordings) and after 10 min exposure to 5 mM-caffeine (lower set of current recordings). *B*, inducement of the transient oscillatory current after 1 min in the presence of caffeine (top and middle current traces) and their subsequent abolition after 2 min incubation in the same medium (lower current trace).

The top current trace in panel *B* shows membrane currents obtained in control conditions in another cell before the application of caffeine. From a holding potential of −50 mV, a clamp pulse to +40 mV elicited net membrane current with contributions from the background current, the delayed rectifier K^+ current and I_{to}. One minute after the application of caffeine (middle current trace), transient oscillatory currents were initially induced but disappeared within 2 min exposure (lower current trace). These results are very similar to the effects of caffeine on spontaneous transient outward currents reported by Benham & Bolton (1986) in rabbit ear artery and jejunum smooth muscle cells. Although the effects of caffeine on I_{to} cannot be resolved from these experiments, the present results strongly support the hypothesis that Ca^{2+} release from sarcoplasmic reticulum (SR) is responsible for their generation.

Ohya *et al.* (1987*b*) have recently shown that intracellular ATP enhanced the transient outward current of smooth muscle balls of the rabbit ileum. Since both I_{to} and the transient rhythmic oscillations were only observed in experiments using

large tip diameter patch micropipettes (containing 5 mM-ATP, see Methods), we examined the effects of removing ATP from the micropipette solution on the transient outward currents (data not shown). In seven experiments, the transient oscillatory type of activity was recorded in only one cell and its amplitude was much smaller (< 500 pA) at $+50$ mV than that investigated in the presence of ATP. In three cells, only the background and the delayed rectifier K^+ currents were observed as typically seen in non-dialysed cells (see Figs 2 and 3). In the three other cells investigated, a background current, a delayed rectifier K^+ current and I_{to} were recorded. This procedure facilitated the analysis of I_{to} since its overlap with the spontaneous rhythmic type of transient outward current was common in the presence of ATP. These results show that the two types of transient outward currents can be dissociated by removing ATP from the pipette.

DISCUSSION

In the present report, four different macroscopic components of outward current were identified in isolated smooth muscle cells of the rabbit portal vein: (i) a time-independent background current; (ii) a time-dependent K^+ current (delayed rectifier); (iii) a smooth transient outward current (I_{to}); and (iv) oscillatory transient outward currents (STOCs). In the following sections, the major findings will be summarized and compared to the literature, and the physiological significance of each of these currents will be assessed.

Background current

To our knowledge, this is the first demonstration of the existence of a time-independent background current in vascular smooth muscle cells. Its current–voltage relationship is ohmic between -140 and 0 mV and rectifies in the outward direction at more positive potentials (Fig. 2*B*). This current appears to be either time-independent or exhibits kinetics which are too fast to be resolved by the technique employed. A reversal around the resting potential (~ -50 mV), the close correlation found between the input resistance obtained during current-clamp experiments (Table 1) and the slope conductance of the linear portion of its *I–V* relationship obtained in voltage clamp (Fig. 2*B*; $\sim 0{\cdot}2$ GΩ) suggest that it may be responsible for the generation of the transmembrane potential. It is blocked by the well-known K^+ channel blockers tetraethylammonium chloride (TEA; Fig. 9) and barium (Fig. 10*A*), is strongly depressed by external calcium removal (Figs 7 and 8), and is not affected by dialysing the cells with 5 mM-EGTA. Moreover, in current-clamp mode, these cells depolarized when calcium was omitted from the external solution or exposed to TEA (20 mM). These results are in agreement with the findings of Inoue *et al.* (1986) who described the existence of a K^+ channel with a slope conductance of 180 pS in symmetrical K^+ solutions (142 mM; K_M) in excised membrane patches of the same preparation. This channel is preferentially blocked by TEA from the inside and is exclusively sensitive to the external calcium concentration. From the fact that its open probability was voltage independent, they proposed that it may be the channel underlying the resting potential in these cells.

Since the measured resting potential is far from the expected equilibrium potential for K^+ ions, we must postulate that either the background current reflects the activity of a poorly selective ionic channel, or is due to the combination of inwardly directed leak channels and highly selective K^+ channels. More experiments at the single channel level are needed to address the issue of whether the K_M channel is indeed an important determinant of the background current.

Delayed rectifier

Using the suction microelectrode technique (no dialysis of the cell) an activating current that reached steady state and did not inactivate was also detected in portal vein cells. This outward rectifying current activates around -40 to -30 mV and is fully activated around $+20$ mV (Fig. 2). From analysis of the tail current seen upon repolarization to the holding potential, this current appears to be mainly selective for K^+ ions (Fig. 3*B*) and is calcium independent (Figs 7 and 8). Although the kinetics are faster, its behaviour mimicks the properties of the so-called delayed rectifier found in many heart preparations (McDonald & Trautwein; 1978; Gintant, Datyner & Cohen, 1985; Hume *et al.* 1986; Simmons *et al.* 1986; Shibasaki, 1987).

The delayed outward current has not been studied extensively in isolated smooth muscle cells. A component of macroscopic current that appears to be calcium insensitive has been observed but not characterized in rabbit ileal cells (Ohya *et al.* 1987*b*) and aortic smooth muscle cells in culture (Toro & Stephani, 1986). None of the channels described in isolated membrane patches of the same preparation (Inoue *et al.* 1985; Inoue *et al.* 1986) can be correlated with the delayed rectifier found in the present study, since it appeared to lack calcium sensitivity. There are similarities, however, between the delayed rectifier current described in our studies and a 50 pS K^+ channel identified by Benham & Bolton (1983) in longitudinal smooth muscle cells of rabbit jejunum. Both are blocked by TEA, insensitive to Ca^{2+}, exhibit voltage-dependent gating behaviour and exhibit little or no inactivation during step depolarizations.

Based upon the voltage dependence of its activation, this current is probably not involved in maintaining the resting potential in these cells. However, a small depolarization of the membrane will activate it and this will contribute to repolarization. Under conditions in which $[Ca^{2+}]_i$ is low, the delayed rectifier is probably the main current responsible for repolarization of the membrane potential.

Transient outward currents

An interesting finding of the present investigation was the appearance of two additional components of outward current when the cells were dialysed with large tip diameter patch pipettes. Both components were depressed by extracellular calcium removal (Figs 7 and 8) and abolished by internal dialysis with a high EGTA (5 mM) solution. From these results, it appears that they are activated by a rise in internal Ca^{2+}.

I_{to} activates quickly and inactivates slowly in a voltage-dependent manner (Fig. 4*B*). Apart from slight differences in its voltage-dependent availability, it markedly resembles the 4-aminopyridine-sensitive transient outward current found in pulmonary artery cells by Okabe *et al.* (1987). The activation of I_{to} does not appear to be related to the influx of Ca^{2+} through voltage-dependent Ca^{2+} channels, since

the I–V relationship did not exhibit any sign of downward inflexion at positive membrane potentials where Ca^{2+} influx through these channels would be expected to decrease as a result of reduction in Ca^{2+} driving force (Klockner & Isenberg, 1985; Walsh & Singer, 1987). Future experiments using calcium channel antagonists should clarify this question.

We propose that I_{to} is more dependent on a tonic component of intracellular Ca^{2+} since it can still be recorded in the presence of high concentrations of caffeine (Fig. 12) and does not appear to be related to a transient influx of Ca^{2+} through voltage-dependent Ca^{2+} channels.

Spontaneous transient oscillatory types of outward current were also detected in 45% of the dialysed cells. In many aspects, this type of activity is very similar to the spontaneous transient outward currents (STOCs; I_{oo}) demonstrated in vascular and intestinal smooth muscle cells (Benham & Bolton, 1986; Ohya *et al.* 1987*a*). They are also activated by internal Ca^{2+} but a clear distinction from I_{to} comes from the experiments performed in the presence of caffeine (Fig. 12). Caffeine is thought to induce Ca^{2+} release from SR stores and eventually cause its depletion (Ito *et al.* 1977). This compound abolishes STOCs in cells in which they are present under control conditions (Fig. 12*A*), and is able to transiently induce them and later abolish them in cells in which they were previously lacking (Fig. 12*B*).

Removal of ATP from the pipette reduced significantly their appearance whereas I_{to} was not affected by this procedure. We propose that a cyclic release of Ca^{2+} from SR activates large Ca^{2+}-activated K^+ channels (Inoue *et al.* 1985; Benham *et al.* 1986). The cyclic release of Ca^{2+} seems to be closely linked to a voltage-dependent mechanism because the frequency of discharge during a test pulse as well as the latency between the onset of the first oscillation are highly voltage dependent (see Fig. 12*A*). Intracellular ATP enhances the calcium current (I_{Ca}) in cardiac (Irisawa & Kokubun, 1983) and visceral smooth muscle cells (Ohya *et al.* 1987*b*). Part of the effect of ATP on STOCs may be related to the increase of I_{Ca} causing release of calcium from SR by a calcium-induced calcium release mechanism (Fabiato, 1983). However, if this was the sole mechanism, one would expect that the frequency and amplitude would eventually decrease as the equilibrium potential for Ca^{2+} ions is approached. This was not the case for many observations made in several cells (see for instance Figs 6 and 12*A* where the cells were clamped at +50 mV). In line with this argument is the fact that concentrations of Cd^{2+} that blocked the calcium current in rabbit jejunum cells had no effect on STOCs (Behnam & Bolton, 1986). However, Ca^{2+} replacement by Mn^{2+} (2·5 mM) blocked these currents in rabbit ileum cells (Ohya *et al.* 1987*a*).

Several mechanisms could participate in the onset and relaxation of STOCs. The contribution of an electrogenic Na^+–Ca^{2+} exchange carrier, the release of loosely associated membrane-bound Ca^{2+} by membrane depolarization causing further release of Ca^{2+} from SR (Fabiato, 1983), and a direct effect of ATP on SR Ca^{2+} channels (Rousseau, Smith, Henderson & Meissner, 1986) are among hypotheses that will be tested in future studies.

The physiological significance of the two types of transient outward currents is difficult to assess. Perhaps in response to stimulation induced by a receptor agonist such as noradrenaline (which is thought to cause release of Ca^{2+} from internal stores) causing an elevation of Ca^{2+} levels may lead to a significant activation of these

currents. However, we can not rule out the possibility that this activity reflects the behaviour of Ca^{2+} overloaded cells. This was also suggested by Benham & Bolton (1986) as a possible explanation for the appearance of STOCs in visceral and vascular smooth muscle cells. Pathophysiological conditions causing perturbations of Ca^{2+} homeostasis should activate these channels when the cells are depolarized.

In conclusion, we have demonstrated the existence of four distinct components of macroscopic outward current in single smooth muscle cells isolated from the rabbit portal vein. These components were identified as K^+ channels and separated according to their kinetics, voltage dependence, sensitivity to K^+ channel blockers and variations in the concentration of calcium on both sides of the membrane. Many questions remain unanswered as to how these currents are induced and regulated, and their physiological role. A thorough characterization of each of these current components and their biochemical regulation as well as regulation by humoral and neural substances will help to resolve some of these questions.

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