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PATCH-CLAMP STUDIES OF SLOW POTENTIAL-SENSITIVE POTASSIUM CHANNELS IN LONGITUDINAL SMOOTH MUSCLE CELLS OF RABBIT JEJUNUM

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SUMMARY

1. The patch-clamp technique was used to study single channel currents in membrane patches of longitudinal smooth muscle cells of rabbit jejunum dispersed by collagenase treatment. Recordings were made from both cell-attached and isolated patches.

2. The predominant unit currents observed were outward at membrane potentials positive to the potassium equilibrium potential (E_K) and they were rapidly and reversibly blocked by tetraethylammonium (TEA). Their size varied as E_K was changed but was not noticeably affected by changing E_{Na}, E_{Cl} or E_{Ca}; it was little altered in calcium-free EGTA solution. Thus, these currents apparently result mainly, if not exclusively, from the movements of potassium ions through channels insensitive to the calcium ion concentration. The present study describes the properties of these potassium channels.

3. The unit conductance varied slightly with potential in most experiments; around zero potential it was about 50 pS. The conductance was dependent upon the potassium, but not the calcium, gradient. Sub levels of conductance of about two-thirds and, less commonly, one-third of the fully conducting channel state were sometimes seen.

4. Membrane patches were studied which showed one to about twelve levels of outward current which were presumed to result from the opening of up to twelve channels having the same characteristics. The probability of channel open state varied with membrane potential, increasing in the potential range -40 to $+40$ mV. Channel openings were rare negative to -40 mV. No inward currents through these potassium channels were observed as openings were not seen at membrane potentials negative to E_K.

5. When the probability of channel opening was low, channel openings occurred in bursts which could be separated by several seconds. Analysis of the openings of a single channel revealed that open times and short closed times were exponentially distributed with mean durations of 15–45 ms and about 6 ms at zero potential. In some patches regular cyclical openings of several channels occurred. In other patches openings of individual channels appeared to be independent events as they were reasonably fitted by a binomial distribution.

6. Following a step change from negative potentials, where channels were closed, to more positive potentials, channel openings increased during a period of 10 s to reach a steady state. No evidence of inactivation was observed.

7. These results suggest the existence of a population of potential-sensitive potassium-selective ion channels in the smooth muscle cell membrane which are closed at the resting membrane potential and which open upon depolarization with slow (seconds) kinetics; these may be involved in the slow potential (wave) activity of this muscle.

INTRODUCTION

Analysis of the ionic basis of electrical activity of intact smooth muscle tissues requires the accurate resolution and identification of membrane currents. Smooth muscle tissues consist of a multitude of small elongated cells, so that control of potential and the analysis of voltage-clamp recordings are greatly compounded by their multi-unit nature which creates uncertainties about ionic gradients and voltage control (e.g. see Bolton, Tomita & Vassort, 1981). An alternative approach to the problem of identifying ionic channels in excitable membranes is the study of the currents through single ionic channels by means of the patch-clamp technique (Neher, Sakmann & Steinbach, 1978; Hamill, Marty, Neher, Sakmann & Sigworth, 1981). To record from single ion channels it is necessary to isolate electrically a patch of cell membrane by means of a high resistance annular seal created around it by application of the polished tip of a small glass pipette. To form this seal the cell membrane needs to be very 'clean' which usually means enzymatic treatment to digest away adherent irregular material.

In recent years it has proved possible to disaggregate smooth muscle tissues enzymatically into their constituent cells (Bagby, Young, Dotson, Fisher & McKinnon, 1971; Fay & Delise, 1973). Initial studies utilized amphibian smooth muscle but later work (Momose & Gomi, 1980) extended this to mammalian muscle. We have utilized the collagenase dispersion technique of these authors to dissociate rabbit longitudinal jejunal smooth muscle into its constituent cells. We describe here the properties of the most prevalent type of ionic channel encountered in the membrane of these dispersed cells studied by means of the patch-clamp technique (Benham, Bolton & Kitamura, 1982, 1983).

METHODS

Adult rabbits (1–2 kg) of either sex were killed by cervical dislocation. Lengths of longitudinal muscle from the jejunum were peeled from the underlying circular muscle and placed in physiological salt solution (PSS).

Cell dispersal

Lengths of longitudinal muscle were cut into strips (2–3 mm wide and 5–10 mm long) and incubated at 35 °C for 10 min in calcium-free PSS. They were then transferred to a calcium-free PSS solution containing 0·05 % collagenase, 1 % trypsin inhibitor and 2 % bovine serum albumin (dispersal solution). After 10 min incubation the strips were mildly agitated by repeatedly sucking into a glass pipette. Incubation was repeated for a further 10 min with fresh dispersal solution. Further mild agitation was applied and then a final incubation in fresh dispersal solution.

During the final incubation the strips were agitated more vigorously by pipette for 2–5 min until a cloudy appearance of the solution indicated that a proportion of the cells had been dispersed.

All incubations were at 35 °C. Undispersed pieces of tissue were removed by coarse filtration and then the cells were separated by centrifugation at 100 *g* for 1 min. The supernatant was discarded and the cells resuspended in PSS containing 2·5 mM-calcium at room temperature.

Patch recording

Patch pipettes were prepared from haematocrit flint glass capillary tubes with a Kopf micro-electrode puller (Hamill *et al.* 1981). The pipette tips were about 1–2 μm diameter and were then heat polished in a microforge. They typically had a resistance of 3–8 MΩ when filled with normal PSS solution which was the usual filling solution used. The patch pipettes were held in a suction pipette holder which was firmly mounted on the head stage attached to a Leitz micromanipulator. Cells in normal PSS were placed on the microscope stage in a 1 ml. bath with a flat glass bottom. The cells adhered quite strongly to the glass bottom so they were not dislodged when the patch pipette was manipulated to touch the cell surface. Positive pressure was applied to the interior of the pipette until it was brought up to the cell surface to minimize pipette blockage. After contact was made, slight negative pressure was applied to achieve gigaohm seals (Sigworth & Neher, 1980). All experiments were carried out at room temperature, 21–25 °C.

Solutions

The PSS solution used was a modified Krebs' solution of the following composition (mM): Na^+, 137; K^+, 5·9; Ca^{2+}, 2·5; Mg^{2+}, 1·2; Cl^-, 134; HCO_3^-, 15·4; $H_2PO_4^-$, 1·2; glucose, 11·4. This solution was gassed with 95 % O_2, 5 % CO_2 and had a pH of about 7·2. In calcium-free PSS $CaCl_2$ was omitted and where indicated EGTA was added. High-potassium solution was prepared by substituting KCl for NaCl in appropriate amounts. Solution exchange in the bath was achieved by injection and withdrawal of equal volumes of solutions by means of syringes.

Drugs and reagents

Bovine serum albumin (Sigma), collagenase Type 1A (Sigma), ethylene glycol bis-(2-aminoethyl), tetra-acetic acid (EGTA, Fisons), tetraethylammonium chloride (TEA, Sigma), trypsin inhibitor (Sigma).

Analysis of results

Current recordings were collected on tape unfiltered with a frequency response flat to 2·5 kHz. Records were subsequently played back at $\frac{1}{8}$ speed through a Barr & Stroud low pass filter onto a Lectromed paper chart recorder. Data was then analysed by hand. Averaging was done by hand and by using a Neurolog signal averager, both methods giving similar results. Measurements made of mean open and mean closed times were taken after re-recording to further slow down the records. A channel opening was defined as a current excursion of > 75 % of the mean unit current and on this criterion 0·5 ms openings could be detected. Channel closing was defined as when the current fell to below 25 % of the mean unit current. Frequency histograms were constructed in 5 ms bins starting at 0·5 ms and fitting of the theoretical exponential curve was done by the method of least squares to a semi-logarithmic plot of the data. Mean open and closed life times were corrected unless otherwise stated for unresolved short events by assuming an exponential distribution and taking the point at which frequency fell to a relative value of 1/e.

RESULTS

Appearance of dispersed cells. The cell dispersal procedure yielded a population of longitudinal smooth muscle cells of heterogeneous appearance. Their morphological appearance is illustrated in Pls. 1 and 2 which shows relaxed, elongated cells with generally smooth cell membranes (*a*), cells in various degrees of contraction (*b*) and cells which were lysed (*c*). Lysed cells or those which were in a severely contracted state were not used for this study. Elongated cells, or those showing only a localized region of contraction were approached with the tip of the pipette with a view to obtaining a gigaohm seal (Pl. 2).

Relaxed cells were 200–400 μm or more long. Their cell membrane was often featureless under differential interference microscopy except that several fine processes could often be seen at their ends, and sometimes along the length of the cell. These cells generally attached themselves to the glass bottom of the bath by their ends and other parts and were not easily dislodged by the patch pipette. The edges of such cells were highly refractile (Pls. 1 and 2*a*). As such cells settled on the bottom of the bath they adopted a slightly oval cross-section such that their maximum diameter, where the nucleus could often be discerned, exceeded 5 μm. Such cells would often contract if carbachol (10^{-5} M) was added to the bathing solution or if they were prodded too hard with the patch pipette. The patch pipette was applied in the wider central region of the cell. Sometimes it was applied to the contracted region of partially contracted cells. We have not examined whether different regions of the cell membrane have different ionic channels.

Properties of patch currents

Patch currents. After a gigaohm seal of pipette to cell membrane had been achieved, only rarely were step changes in current seen in PSS of normal composition. In this solution, the cells probably have normal membrane potentials (at least -50 mV) so that, as will be described, channel opening would be a rare event. Also, the driving force on potassium ions (the predominant species involved in these channel currents) will be small as the resting membrane potential will be relatively close to the potassium equilibrium potential (E_K). Occasionally, as described (Benham *et al.* 1982) step changes in currents were seen. It may be that these were recorded from somewhat depolarized cells.

To elicit rectangular step changes in current, i.e. single channel unit currents, normally the medium bathing the cells was changed to one containing either 126 mM-potassium or occasionally 140 mM- or 40 mM-potassium. Distinct step changes in current then usually became apparent. If low resistance pipettes were used, individual current steps were difficult to distinguish, presumably because numerous channels were opening and closing. However, hyperpolarizing the membrane reduced the current 'noise' substantially, implying that channels had been closed by this procedure. Unless a gigaohm seal was achieved, and maintained, the recording was not sufficiently stable, nor the current steps sufficiently clear, to be useful for analysis. Examples of step changes in current, presumably indicative of the rapid opening or closing of individual ionic channels, are shown in several Figures which illustrate the behaviour of patches having one (Fig. 1*A*) to several (Figs. 1*B*, 3, 4 and 6) active channels.

In some cases the patch from which recording was made was attached to an intact cell. Sometimes, it was obvious visually after withdrawing the pipette that the cell and pipette tip were not contiguous so that recording was being made from an isolated patch. Sometimes, however, it was not possible to be certain whether the patch was isolated or not, since we were unwilling to risk disturbing recording to ascertain this. In any case, whether the patch was isolated or 'cell-attached' was generally not vital, since the difference between presumed $[K^+]$, in the cell, and $[K^+]_o$ in the bath, was small. We assume that where the patch was isolated that it was an 'inside-out' patch (Hamill *et al.* 1981).

Sometimes, in a deliberate attempt to obtain an isolated patch, the pipette was first sealed to the cell membrane and then withdrawn from the cell. After switching to a high-potassium solution a gigaohm seal then sometimes developed. On other occasions slight positive pressure applied to the pipette, following the usual negative pressure, resulted in a useful patch developing. Frequently, if the first attempt to obtain a gigaohm seal on a cell-attached patch was unsuccessful than approaches to further cells were made in high-potassium solution with fresh pipettes and were on occasions successful.

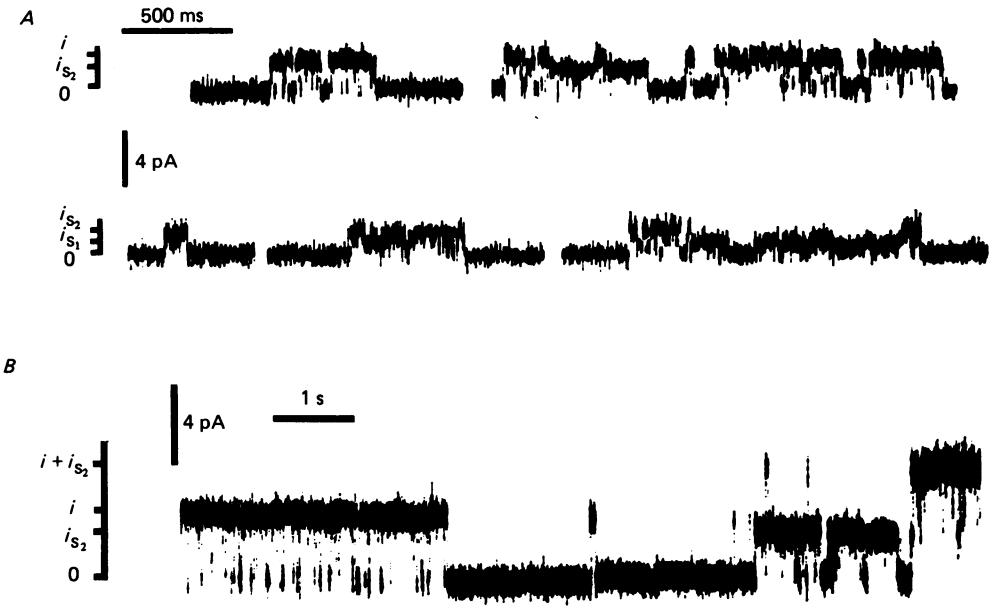

Fig. 1. Outward patch currents in smooth muscle cells of rabbit jejunum. *A*, full-size unit currents (i) and two sublevels (i_{S_1}, i_{S_2}) in an isolated patch. Bathing solution contained 126 mM-potassium. In *B* from an isolated patch, are shown full-size unit currents and a sublevel which shows some fluctuations in size. Bathing solution contained 126 mM-K^+. In these and in subsequent records the patch pipette contained normal PSS (5·9 mM-K^+, 2·5 mM-Ca^{2+}) and the temperature was 21–25 °C. Records in *A* and *B* were filtered at 500 Hz (−3 dB).

The form of the currents seen in cell-attached and isolated membrane patches was essentially similar. The current record showed rapid transitions from the zero current to constant levels of outward current and back in a way that has been interpreted as the opening or closing of single ionic channels (Neher *et al.* 1978; Hamill *et al.* 1981). Higher levels of outward current were generally simple multiples of the lowest current level and presumably indicated that several similar or identical channels were open at the same time (Figs. 3, 4 and 6). Occasionally, intermediate current levels occurred as will be described.

It was clear that on occasions several different types of channel were giving rise to currents both in different patches and in the same patch. Since the channel properties could be inferred only from the behaviour of the currents, uncertainty existed on some occasions whether the observed currents were flowing through the

potassium channels which are the subject of this paper, especially if the records obtained were incomplete. In different experiments the criteria for identification of potassium channel activity necessarily differed. Usually there was no difficulty in identifying potassium channel activity on the basis of the direction of current and its size related to the patch potential and E_K. The potassium channels also had characteristic kinetics as will be described. Currents through channels of different kinetics were observed upon, or without, blocking potassium channels, and these were often inward in some potential ranges. In this paper we propose to confine our attention to currents arising from a population of channels we believe we could unequivocably identify from experiment to experiment because they were by far the most commonly occurring and because of their characteristic properties which will be described.

Channel conductance and multiple conductance states. In both cell-attached and isolated patches, channel currents were invariably outward (upwards in the records shown). By stepping the potential for 5–15 s to different potentials, the effects on the size of channel current could be observed. The relationship between channel current size and potential was not linear but curved either in one direction or another (cf. Fig. 2*A* and *B*) when the inside of the membrane was exposed to high potassium concentrations (125 mM or 140 mM in isolated patches, maybe higher in intact cells) and the outside to 5·9 mM-potassium. Similar curvilinear current–voltage relationships have been observed for potassium channels in other excitable membranes when the membrane surfaces were exposed to 'physiological' concentrations of potassium (Pallotta, Magleby & Barrett, 1981). Negative to −40 mV channel openings were rare and channel current was so small that it was difficult to distinguish from background noise (Fig. 6). Because of the curvilinear nature of the current–voltage relationship there was uncertainty about the extrapolated value of the reversal potential. However it was clear that it differed from the calculated E_K (−77 mV in Fig. 2*A* and *B*) by at most 10 mV indicating that the contribution to channel current by ions other than potassium must be small.

In several experiments we examined the relationship between the channel reversal potential and the potassium concentration. We were particularly interested to discover if inward potassium currents could be obtained. In an isolated patch the channel currents were very much reduced in size when both sides of the patch membrane were exposed to normal solution containing 5·9 mM-potassium; the extrapolated reversal potential was zero as expected. However, inward currents through the potassium channels were not observed (Fig. 2*B*). The reason for this is not known, either the channels rectify or they fail to open at potentials negative to E_K. In another experiment we applied an intermediate concentration of K (about 70 mM, $E_K = -65$ mV) but we were unable to observe convincing inward currents. However, we have observed very clear reversal of other currents but these we believe to be through other types of channel and will not be further considered in this paper.

In the potential range 0 to −60 mV the currents under study were outward in cell-attached patches and could not therefore be carried by sodium or calcium ions which had an electrochemical gradient directed inwardly (with respect to the cell). This was also true for sodium in isolated patches. Calcium concentrations at either side of isolated patches were generally made equal, because in solutions containing

low calcium concentrations patch seals usually became unstable. In cell-attached patches, E_{Cl} may be the same or even negative to the holding potential, probably about −18 mV in 126 mM-KCl solution (e.g. Holman, 1958).

The current–voltage relationship was curvilinear indicating that the conductance of these channels varied with potential. Measured in the range 0 to −40 mV in nine patches in which fairly complete data were obtained, the conductance was 52±4 pS. The conductance was reduced to about 25 pS when 5·9 mM-potassium was present on both sides of the membrane (Fig. 2*B*).

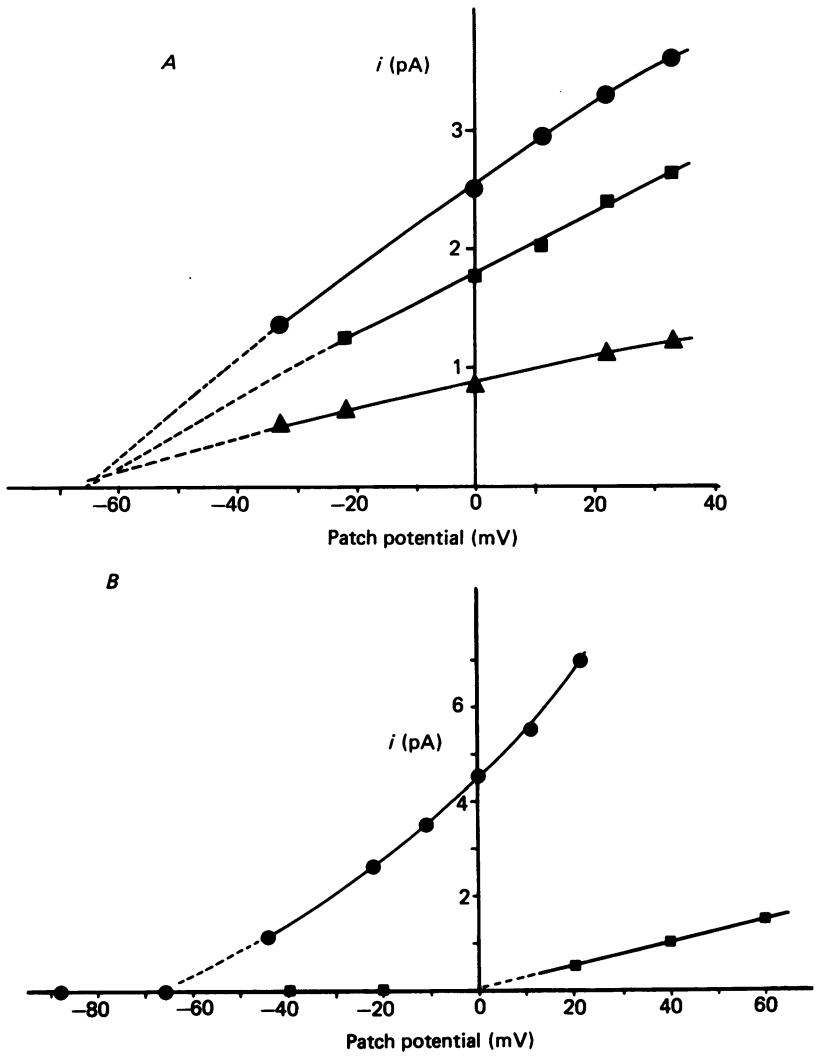

Fig. 2. *A*, current–voltage relationship of the full size currents (●) and the two sublevels (■, ▲) shown in Fig. 1*A*. No currents were discernible negative to about −40 mV but extrapolation of the lines suggests a reversal potential around −65 mV. Calculated E_K was −77 mV; the pipette contained normal physiological salt solution, the bathing solution contained 126 mM-K^+. The unit conductances are 40, 28 and 14 pS. *B*, current–voltage relationship of an isolated patch exposed on the inside to 126 mM-K^+ (●) or 6 mM-K^+ (■). Unit conductances are 50 and 25 pS respectively.

In half the patches a sublevel was apparent that was about two-thirds of the full conductance (Benham *et al.* 1982). In all but one of these patches this level was rare: less than 1 % of all openings. In one patch in which only one channel was active it was possible to observe a second sublevel of about one-third of the main level (Fig. 1*A*). In this patch the percents of openings to the one-third, two-thirds and full level were 25 %, 26 % and 49 % respectively. All three conducting states had the same apparent reversal potential upon extrapolation of the current–voltage relationship (Fig. 2*A*). Rapid transitions occurred between the main level (i) and the higher sublevel (i_{S_2}, Fig. 1*A*, top trace) and between the two sublevels (i_{S_1}, i_{S_2}). They were not seen between i and i_{S_1}. Transitions from zero current to i_{S_1} were, however, seen (Fig. 1*A*, lower trace).

Measurements of the size of sublevels were made on traces where it was possible to resolve 1 ms changes. Histograms were constructed of the frequency of steps to various levels. These frequency histograms showed three clear-cut peaks in the experiment illustrated in Fig. 1*A*, with little overlap between the distributions showing that the sublevels could be clearly distinguished from base-line noise. However, it was clear on some records that fluctuations in step size occurred which were not explicable in terms of fluctuations in random noise but indicated distinct and maintained (for a period) variations in the size of sublevels (Fig. 1*B*). The cause of these is not known.

It would seem unlikely that these sublevels of current represent two additional types of potassium channel because of the frequency of transitions between i_{S_1} and i_{S_2}; twenty-six of these were observed without return to the zero level in the experiment which gave rise to the trace shown in Fig. 1*A*. This was more than 5 % of all transitions observed in this patch. We therefore favour an explanation which involves reduced conductance states of a single type of channel, rather than one which involves separate channels. 'Rim' currents would also fall into the latter category (Neher *et al.* 1978). Sublevels of conductance have also been postulated for nicotinic acetylcholine receptor operated channels in myoballs (Hamill & Sakmann, 1981; Trautmann, 1982).

Action of Tetraethylammonium (TEA). TEA (5–10 mM) rapidly blocked outward channel activity when applied in the bathing solution to cell-attached or isolated patches. Thus, TEA appears to enter the smooth muscle cell rapidly to effect a block in cell-attached patches, as it is unlikely that it passes the patch seal in significant amounts (Hamill *et al.* 1981). Patch current was reduced to zero level within 10 s of applying TEA (Fig. 3). Recovery of outward currents could be achieved by washing out the TEA. Reversible block of these currents by TEA is further evidence that they are due to potassium channels. In Fig. 3*A–B* before application of TEA, four to six channels were open. 10 s after application of TEA no channel activity was seen (Fig. 3*C*). Scrutiny of the records revealed that block apparently occurred by gradual reduction of the conductance of individual channels. Activity recovered upon washout although in this patch not to the level seen before TEA application (Fig. 3*D*).

Channel activity was not affected by $[Ca^{2+}]_i$. Calcium-dependent potassium channels have been found using single channel recording in *Helix* neurones, rat myoballs and bullfrog sympathetic ganglion cells (Lux, Neher & Marty, 1981; Pallotta *et al.* 1981; Adams, Constanti, Brown & Clark, 1982). The activity of the potassium channels

studied in the present experiments showed only slight sensitivity to reducing calcium on the inside surface of isolated patches. In calcium-free solutions, patch recording generally became unstable within a few seconds of switching solutions and the patch was lost. Records of activity which were obtained showed only small changes, (e.g. Fig. 4). Average open time in the experiment of Fig. 4 showed a small decrease from

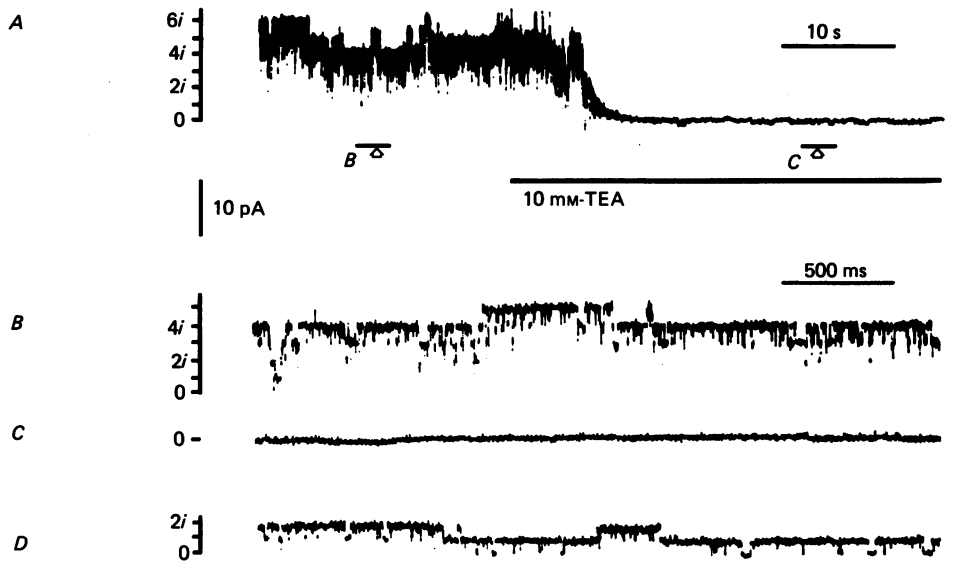

Fig. 3. Effects of 10 mM-TEA on channel currents in an isolated patch. *A*, onset of response to TEA. Portion of record before *B* and during *C*, TEA application on expanded time scale (positions shown on upper trace). *D*, activity 5 min after washing out TEA. The bathing solution contained 126 mM-K^+. Patch potential was zero. Filter in *A* was 250 Hz and in *B*, *C* and *D* 500 Hz.

45 to 38 ms in nominally calcium-free solution with 0·2 mM-EGTA. In two experiments, the time course of recovery of channel activity was examined upon returning to zero potential after a period during which it was held at −40 or −60 mV (see later). Recovery was slightly faster in low calcium conditions in each case but the effect was not great. In a 2·5 mM-Ca solution containing 1 mM-EGTA, the average number of channels open was 7·7, and 7·2 in normal 2·5 mM-Ca solution. These results were seen in patches where changing [K^+] produced rapid effects, as did application of TEA, indicating that there was no restriction of the access of the bath solution to the inner surface of the patch membrane.

Channel kinetics

Single channel kinetics. Channel behaviour was studied in patches in which no more than one channel was open at any one time during the period of observation. Individual channels showed burst-like activity (cf. Conti & Neher, 1980; Sakmann, Patlak & Neher, 1980) in which longer openings, separated by longer closed periods, were interrupted by rapid closings (Fig. 1*A*, see also Fig. 6). The durations of individual open and closed periods greater than 0·5 ms were measured. The frequency

distribution of open lifetimes is shown in Fig. 5*A*. It was well fitted by a single exponential and average lifetime was 16·4 ms. Closings within a burst were also exponentially distributed (Fig. 5*B*) with a mean duration of 5·8 ms. Fitting of the exponential was done by the method of least squares to a semi-log plot of the data. The longer closed periods between bursts lasted for up to 15 s with a mean duration of 3·2 s, but sufficient length of record could not be collected to be sure of their distribution. Providing only one channel was active in the patch these values will reflect the properties of a single channel.

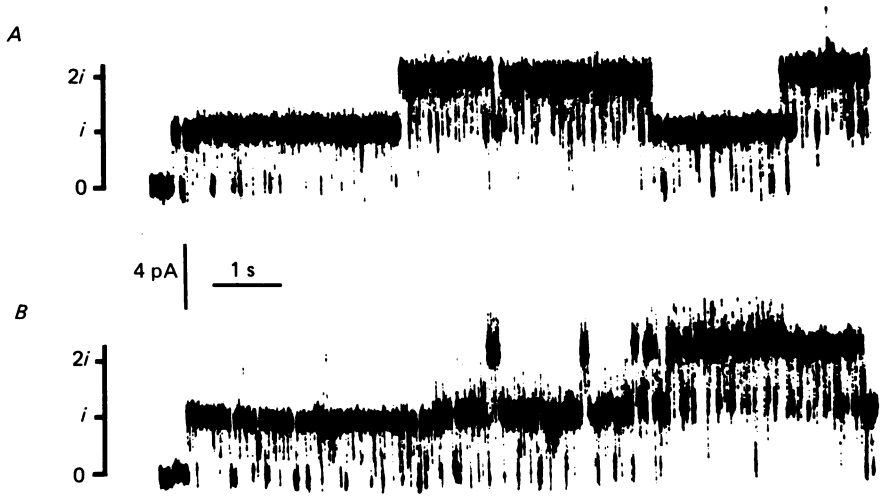

Fig. 4. Channel currents recorded in an isolated patch in calcium-free (with 0·2 mM-EGTA) solution (*B*) and in the same patch in 2·5 mM-calcium (*A*). Bathing solution contained 126 mM-K^+. Filtered at 500 Hz. More rapid closings are apparent in calcium-free conditions and average channel lifetime corrected for unresolved short openings fell from 45 to 38 ms. As several channels were active in this patch, average closed times were not calculated.

More commonly patches showed several levels of current, particularly at depolarized potentials (Figs. 3, 4 and 6). Records from some patches with only a few channels were analysed in detail (Table 1). The total open time of all channels was obtained by summing the time at each level weighted by the number of channels open at that level. All resolvable openings in the record were counted. Hence, the average channel lifetime, 19·93 ms, (uncorrected for unresolved short openings) could be calculated. This calculation does not depend on an assumption as to the number of active channels in the patch.

If the number of active channels in the patch was the same as the greatest number of channels seen to be simultaneously open (four) then the average time between openings of a single chanel (106 ms), and the fractional channel open time (0·188) could be calculated. It seemed worthwhile to compare total times spent at the different current levels with those predicted by the binomial distribution for four independent channels. Calculation from the total times at different current levels assuming a binomial distribution yielded an apparent probability of an open state of 0·189, which was extremely close to the fractional channel open time calculated

above. However, the following arguments cast doubt that only four active channels with a high fractional open time were present in the patch.

Since the average open time of a single channel, 19·93 ms, was known, when two channels are open the average sojourn time at the second current level will be half this, since the probability of channel closing is doubled. Similar arguments apply

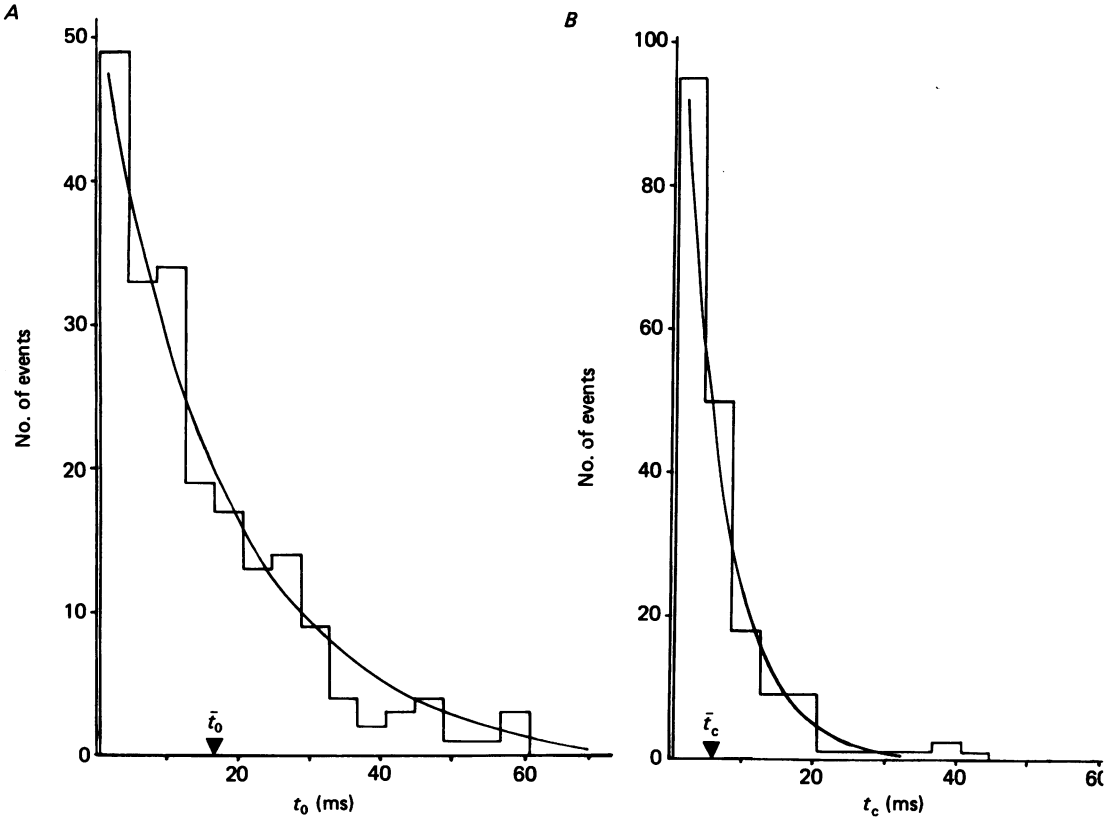

Fig. 5. *A*, distribution of open lifetimes, t_o, and *B*, short closed times, t_c, in an isolated patch in which only one channel seemed active. The distributions were fitted to exponential curves (by least-squares linear regression and semilog plots). Mean open lifetime, $\bar{t}_o$, was 16·4 ms and mean short closed time, $\bar{t}_c$, 5·8 ms. Longer closings had a mean closed time of 3200 ms so that about 2 % (i.e. $1-e^{-60/3200} = 0{\cdot}02$) of gaps between bursts would contribute to closings below 60 ms and be included in this figure. Bathing solution contained 126 mM-potassium. Patch held at zero potential. Mean lifetimes were obtained from the decline of the number of events to the proportion e.

when three or four channels are simultaneously open to yield the third and fourth current levels. Hence, the expected number of openings to a particular current level can be deduced from the times expected at the various current levels as predicted from the binomial distribution. These proved to be significantly ($P < 0{\cdot}005$) different from the observed numbers of channel openings to the various levels; better fits were not obtained assuming five and six channels were active in the patch.

It was also possible to estimate average sojourn times at each current level from

the time spent at that level and the number of openings to (equal to closings from) that level. These agreed quite closely with the estimates from the total open time for all channels and total number of openings in the patch. However, there was some evidence that at levels 3 and 4, when sojourn times were short, that ignoring unresolved short events gave higher estimates.

TABLE 1. Analysis of currents in a patch in which a maximum of four channels were simultaneously open and comparison of the kinetics with a binomial distribution. The average sojourn time at each level was calculated from the time spent at the level divided by the number of openings to that level

Current levels	Time spent at these levels (ms)		Number of channel openings* to these levels		Average sojourn time at these levels (ms)	
	Actual	Expected	Actual	Expected	Actual	Expected
4th	29	21	5	4·2	5·80	4·96
3rd	330	357	46	54	7·17	6·64
2nd	1589	2307	173	231	9·18	9·97
1st	8111	6626	398	332	20·38	19·93
zero	6388	7136	—	—		
Total	16447	16447	622	622		

Total number of openings (= closings)	622
Total open time for all channels	12·396 s
Average open time of one channel	19·93 ms
Total length of analysed record	16·447 s
Average time between openings of one channel assuming four channels in patch	105·77 ms
Fractional channel open time assuming four channels in patch	0·188

* Using a χ^2 test the actual values are significantly ($P < 0.005$) different from those predicted by a binomial distribution. Actual average sojourn times at the various levels were uncorrected for undetected short duration events.

Measurements were also made of openings to the various levels which were from, and returned to, the next lower level. This method would be expected to yield low estimates since longer sojourns at a particular level would be expected to have a greater chance of being interrupted by opening to a higher level, in which case they would have been ignored. Surprisingly the estimates (17·97, 8·10, 6·86 and 5·75 ms for levels 1 to 4 respectively) differed rather little from the theoretically expected values (Table 1) justifying our use elsewhere of this measure as an estimate of open channel lifetime. Another important conclusion to be drawn would seem to be that the probability of an individual channel opening is low i.e. there are numerous active channels in this patch.

In other experiments reasonable fits to a binomial distribution were obtained with records obtained at several potentials in the same patch. Hyperpolarization reduced, and depolarization increased the probability of channel opening. However, considerable fluctuations in the probability were observed in records made at different times at the same potential. Such fluctuations may also underlie the regular periodicity seen in the numbers of channels open in some patches which seemed too regular to arise from random factors.

Effect of potential on probability of channel opening. Patches were generally held at depolarized potentials close to zero and at these potentials several levels of current could usually be seen with rapid transitions between these indicating presumably the activity of several channels. As the differences between current levels were the same, this type of record presumably reflects the activity of several ion channels from tne same population (Figs. 3, 4 and 6). Hyperpolarizing the patch to −40 or −60 mV caused the current record to return to its zero level and channel openings were rare events (Fig. 6).

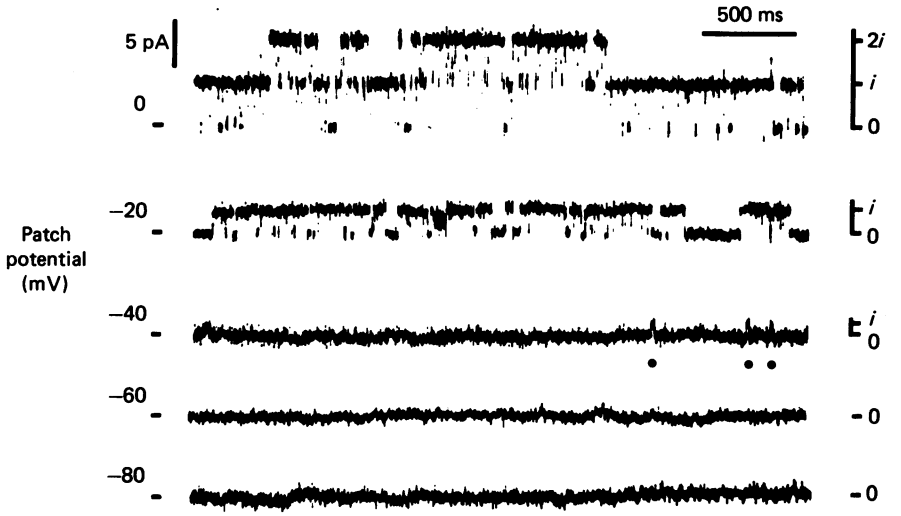

Fig. 6. Records of channel activity at different holding potentials in an isolated patch (bathing solution contained 126 mM-potassium). Records were representative sections of trace taken after activity had stabilized after the change in potential. Infrequent brief openings (●) were seen at −40 mV. Filtered at 500 Hz. Notice the brief closings within a burst which are not well resolved at this slow trace speed. These increase in frequency as predicted when two channels are open.

The steady state probability, P, that a channel is open at some potential can be defined as $P = I/Ni$ where I is the mean current over the period of observation, i is the unit current at that potential and N is the number of channels which may open during the period of observation in the patch (Reuter, Stevens, Tsien & Yellen, 1982). A typical plot of P against patch potential is shown in Fig. 7*A*. This plot was constructed by measuring the time spent at different current levels over 20–30 s at each potential. N was assumed to be the maximum number of channels which were observed to be open at any one time in the patch. An error in N will not affect the shape of the curve. Interestingly, P continued to increase positive to +20 mV and the relationship is much less steep than an exponential one.

Patches in which only a single channel was active were seldom encountered despite using high resistance pipettes so that we have been unable to date to study the effects of potential on long and short closed times and burst length. In patches in which several channels were active at depolarized potentials, we have examined the effects of potential on mean open time by the method of Table 1. We found a linear

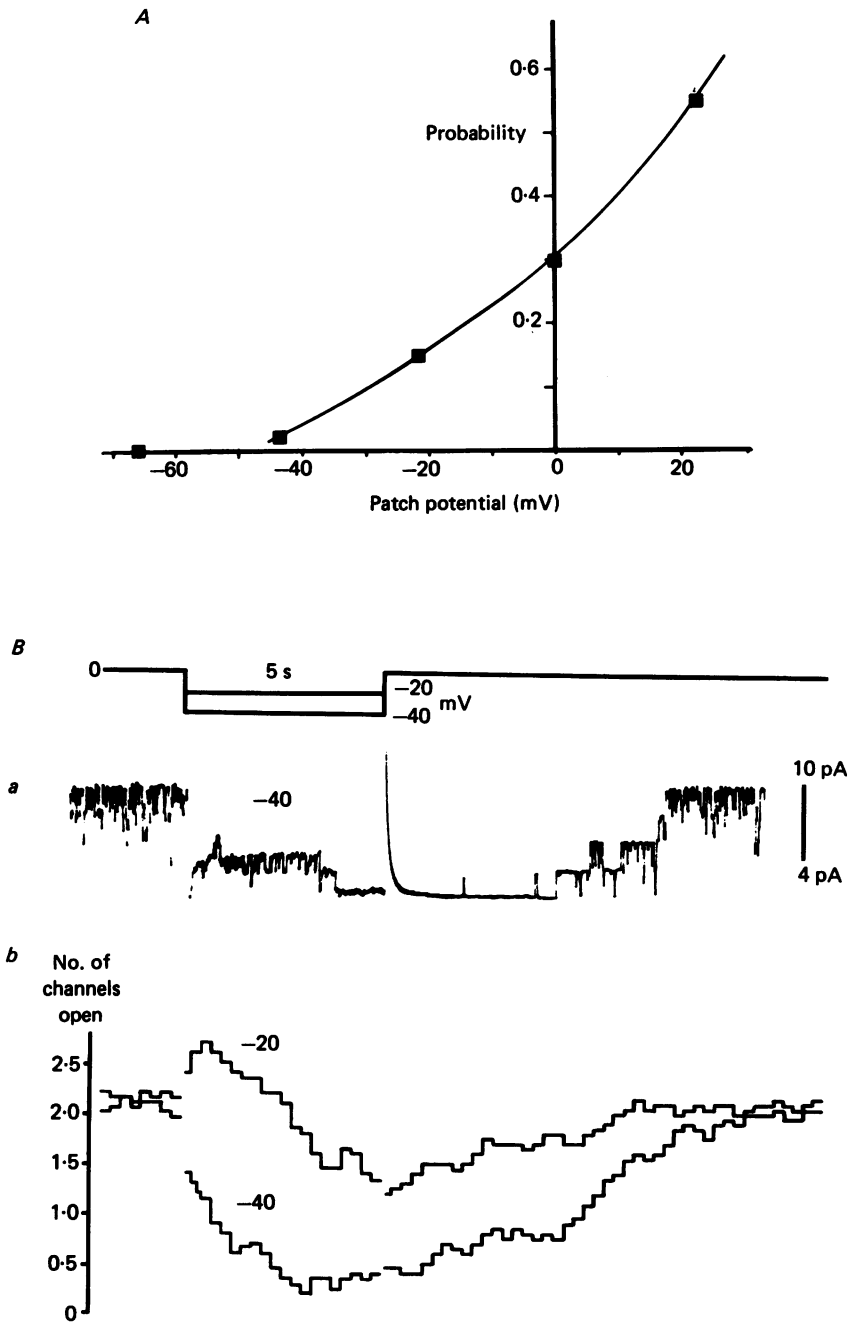

Fig. 7. For legend see opposite.

relationship between mean open time and potential, values of 22 and 49 ms being obtained at −40 and +20 mV respectively. Despite the poor signal to noise ratio at −40 mV (Fig. 6) which makes these measurements much more susceptible to random fluctuations than the measurement of P above, the shortening of channel lifetime with hyperpolarization was a clear effect.

Time-dependent changes in channel open time. It was noticed that hyperpolarizing the membrane reduced channel activity, and upon returning to more depolarized potentials channel activity resumed its previous level as shown in a single record in Fig. 7*B*. However, these changes in activity were not instantaneous, but took place over several seconds (see also Benham *et al.* 1983). Generally, however, single records, unless the patch contained a large number of active channels, were very much subject to random fluctuations in channel opening, and it was necessary to average up to ten to twenty records obtained by stepping between the same potentials to obtain a clear idea of the effect of potential on the probability of channel opening. In the experiment illustrated in Fig. 7*B* at zero potential the average number of channels open was about 2·1 giving a probability of a channel being open of about 0·3 (assuming seven active channels in the patch). Upon stepping to −20 mV, channel openings declined over 5 s to about half the previous level of activity after an apparent slight increase in the probability of opening. Stepping to −40 mV caused a slow reduction in the probability of channel opening to a steady low level. Upon stepping back to zero potential after 5 s, the probability of channel opening recovered to its previous level over a period of about 10 s. In this experiment, steps to −10 mV and −80 mV were also applied but at the latter potential no currents were seen at any time during the pulse as described above. Stepping back to zero potential resulted in a recovery of channel opening. Several other experiments gave similar time courses of recovery of the probability of channel opening following steps to potentials negative to −40 mV. The increase in channel opening frequency upon stepping from negative to zero potential presumably causes, in the whole cell, activation of this population of slow potassium channels and of the slow, non-inactivating potassium current which they pass.

Fig. 7. *A*, effect of potential on the steady-state probability of a channel being open. Time averaged currents were used to calculate probability ($P = I/Ni$) in an isolated patch clamped to various potentials. Bathing solution contained 126 mM-potassium. *B*, transient changes in channel opening upon step changes in potential. An isolated patch (bathing solution contained 126 mM-potassium) was stepped for 5 s from zero potential to −20 or −40 mV as shown in top trace. *a*, a single record showing the effect of a step from zero potential to −40 mV. Before hyperpolarization, four channels were open. During the step in potential the channels close and then re-open slowly after returning to zero potential. Notice that at −40 mV the gain has been increased 2·5 times to make the unit currents of similar amplitude. Filtered at 160 Hz. *b*, calculation from ten consecutive records of the average number of channels open confirmed the behaviour of channels in the single record shown in *a*. Upon hyperpolarization the average number of channels open declined from its steady state-value of about 2·1. Initially the calculated steady-state probability of a channel being open was 0·3 (seven active channels in patch assumed). Stepping to −40 mV reduced this probability by about 75% to a new steady level. Stepping to −20 mV, after an initial increase in probability, caused a slower fall in probability than at −40 mV. Returning to zero potential resulted in the probability of channel opening returning to its previous steady-state level over a period of about 10 s.

DISCUSSION

The cell membrane of longitudinal smooth muscle cells of rabbit jejunum apparently possesses a potential-sensitive, potassium-selective ion channel with slow kinetics which is non-inactivating and which is not sensitive to variations in the concentration of calcium within the cells. Rabbit intestinal smooth muscle at 37 °C shows regular slow depolarization–repolarization cycles or 'slow-wave' activity. These slow waves in published records are shown as depolarizations of up to 20 mV in size lasting about 2 s and occurring every 5 s or so. At the peak of the slow wave, membrane conductance is increased (e.g. Gonella, 1965; Mills & Taylor, 1971; El-Sharkawy & Daniel, 1976). The mechanism of these slow waves is believed to involve an initial inward sodium current (Holman, 1982; Tomita, 1982). Bearing in mind the difference in temperature, it seems possible that the slow K^+ channels observed in the present study may play a role in these slow waves. We found that substantial activation of these channels would be expected in the range of potential positive to −40 mV. They are the predominant channels operating in this range: their fractional open time and conductance are appreciable and as the membrane potential of a normal cell depolarized it would be expected to be subjected, after some delay due to their slow kinetics, to a substantial repolarizing electromotive force generated by the potential-dependent opening of these non-inactivating channels. This would be expected to repolarize the membrane. Thus, the observed slow waves would be generated by the reciprocal activation of sodium channels, resulting in the depolarizing phase, and of slow potassium channels, resulting in the repolarizing phase. Presumably also, any procedure producing maintained depolarization of the cell, such as the application of stimulant drugs or high-potassium solutions would result in the opening of these slow potassium channels and contribute to the substantial increase in potassium efflux which is observed (e.g. guinea-pig longitudinal muscle, Bolton & Clark, 1981; Bolton, Clark, Kitamura & Lang, 1981).

The dominant effect of these slow potassium channels will not be apparent around the resting membrane potential as these channels open only briefly and occasionally at this potential. Thus, other channels will be able to alter membrane potential in this range. Likewise, the slow kinetics of these potassium channels will mean that brief excursions of the membrane potential, such as action potentials, should be possible without their activation to any significant extent. If their kinetics were fast, then action potentials would presumably not be possible since other channels we have seen are either less frequent or carry less current.

These slow potassium channels have several characteristics which are similar to those of other channels reported in other types of excitable cell membrane. Individual channels showed burst-type kinetics and sub-levels of conductance. Burst-type behaviour has been a frequently reported characteristic of ionic channels whether they are associated with receptors (Sakmann *et al.* 1980; Colquhoun & Sakmann, 1981; Cull-Candy & Parker, 1982) or not (Conti & Neher, 1980; Lux *et al.* 1981; Pallotta *et al.* 1981). This gives rise to a distribution of closed times which is not a single exponential. Multiple conductance states of the same channel also do not seem to be restricted to receptor-operated channels (Hamill & Sakmann, 1981; Labarca & Miller, 1981; Trautmann, 1982; Barrett, Magleby & Pallotta, 1982; this work).

Sometimes the openings of channels from the same population in a patch have been suggested to fit binomial or Poisson statistics and hence to be indicative of independent behaviour of individual channels (Neher *et al.* 1978; Barrett *et al.* 1982). Some of our results fitted this idea well enough, but others showed significant discrepancies, in particular, channels in a patch showed evidence of cyclical variations in opening frequency which may have distorted the underlying binomial distribution. Others have detected similar discrepancies (Schindler & Quast, 1980; Gration, Lambert, Ramsey & Usherwood, 1981) which sometimes appear as fluctuations in average channel lifetime (Cull-Candy, Miledi & Parker, 1981).

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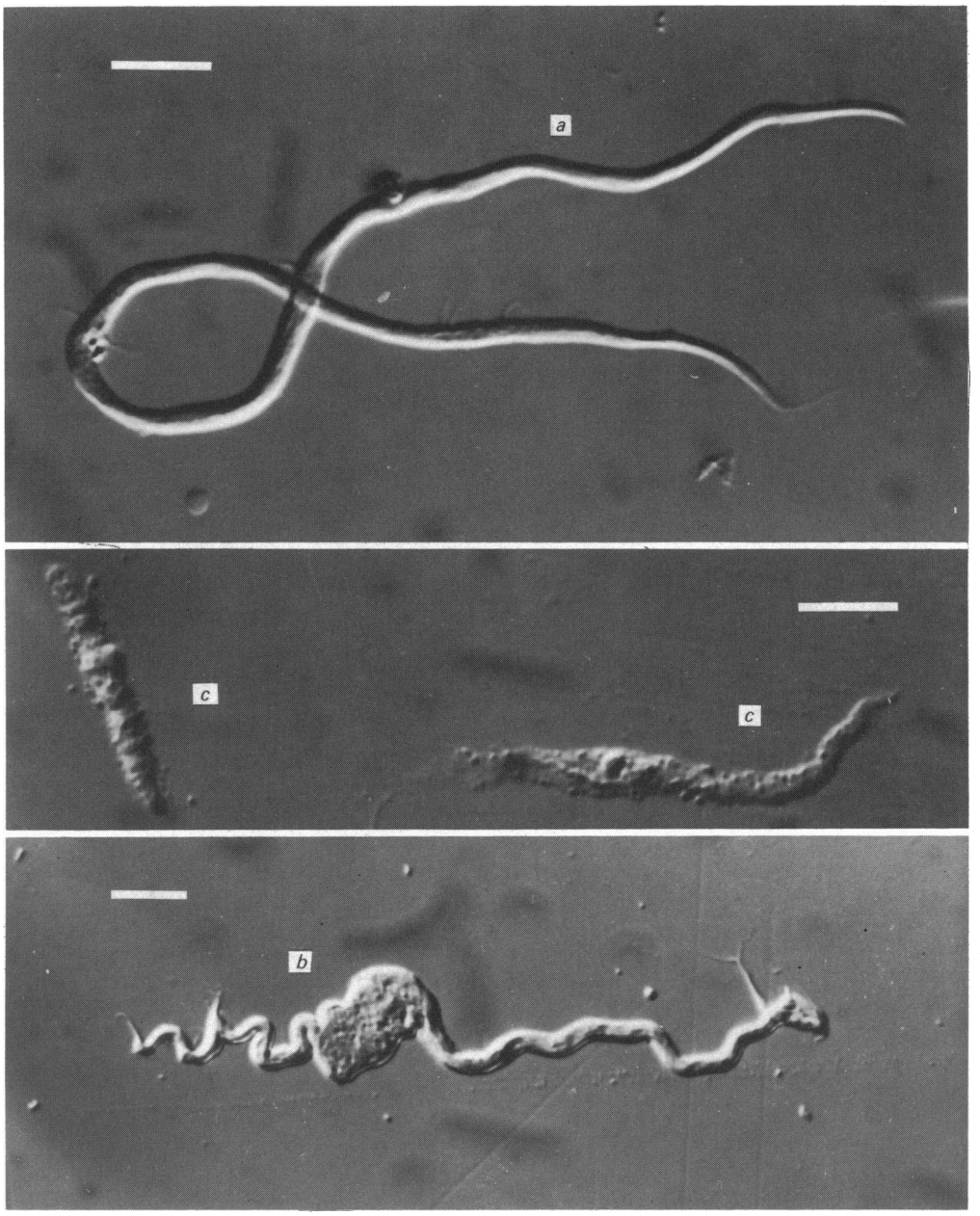
EXPLANATION OF PLATES 1 AND 2

Smooth muscle cells of longitudinal muscle layer of rabbit jejunum separated by collagenase treatment. Relaxed elongated cells are shown at *a*. They have highly refractile ('clean') cell membranes with few features except that small processes can be seen terminally, or the cell bifurcates. Similar small processes can be seen along their length. Cells in different stages of contraction are shown at *b*. Slightly contracted and relaxed cells were used for patch clamping. At *c* are shown lysed cells which also were sometimes formed if the cell was burst by the patch pipette. The inset in Pl. 2 shows a patch pipette (in air) at the same magnification. The calibration bars in all panels are 20 μm. The cells are in normal calcium-containing PSS.



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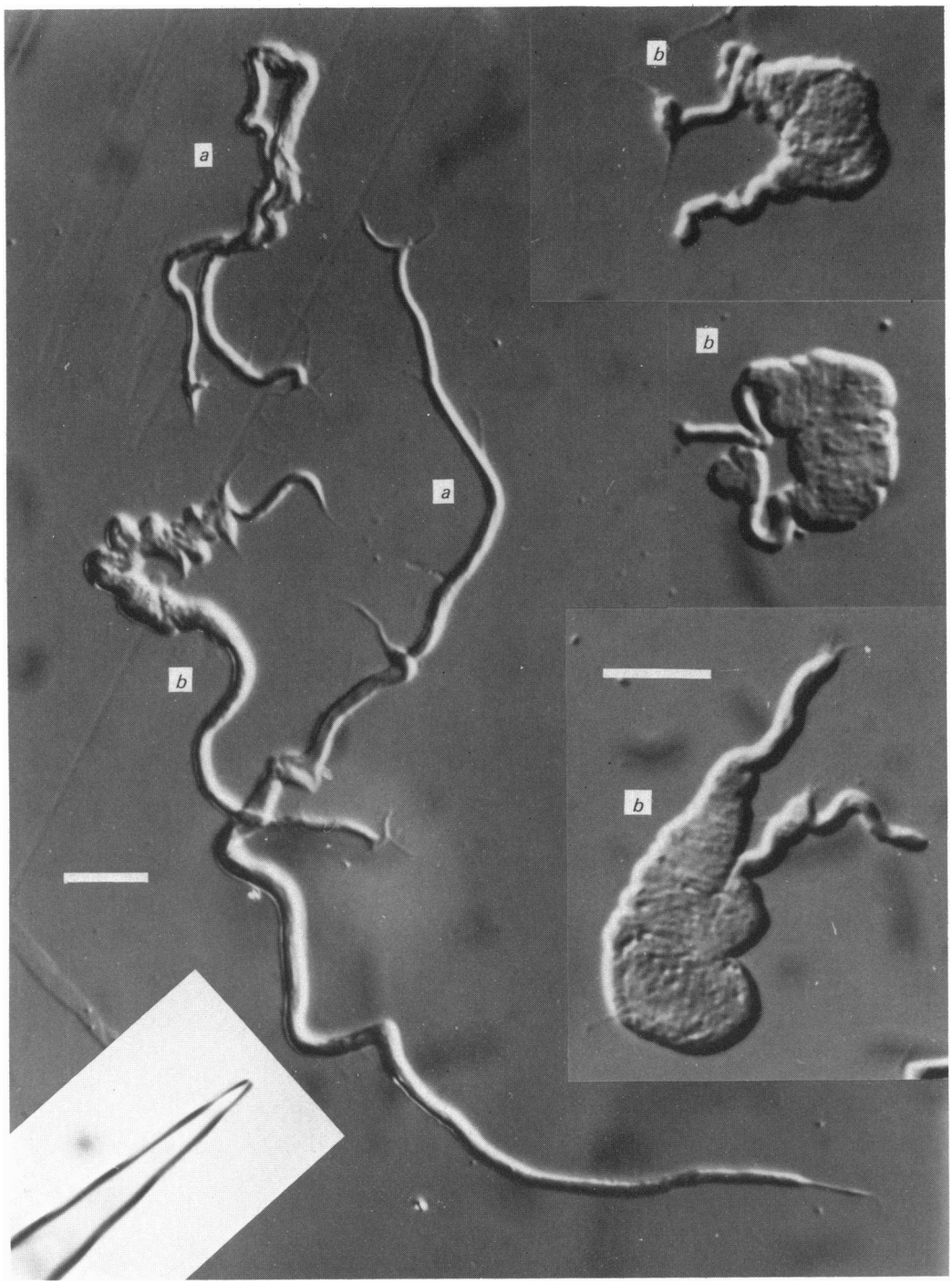

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