J. Physiol. (1986), **372**, *pp.* 113–127
With 10 *text-figures*
Printed in Great Britain

CALCIUM-ACTIVATED POTASSIUM CHANNELS IN CANINE AIRWAY SMOOTH MUSCLE

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(*Received 8 February 1985*)

SUMMARY

1. Airway smooth muscle cells from canine trachealis muscle were dispersed by treatment with collagenase and elastase. Cells were identified as smooth muscle by their binding of anti-smooth muscle γ-isoactin monoclonal antibodies and by their contraction in response to acetylcholine.

2. The patch-clamp technique was used to study single channel currents in cell-attached and isolated patches of membrane. The most common single channel currents had a conductance of 266 ± 12 pS (mean $\pm$ S.D., $n = 7$) in symmetrical 135 mM-K solutions.

3. The reversal potential of the channel was unaltered by large chemical gradients for Cl, Na and Ca and was determined exclusively by the chemical K gradient. Thus, the channel is highly selective for K.

4. In both cell-attached and isolated patches of membrane, depolarization increased the frequency of channel opening and the duration of the open state.

5. In isolated patches of membrane, increasing [Ca] on the cytoplasmic side of the membrane from 10^{-8} to 10^{-6} M increased both the frequency of channel opening and the duration of the open state.

6. Tetraethylammonium, tetramethylammonium, or Cs (10 mM) on the cytoplasmic side of the membrane caused a voltage-dependent decrease in conductance of the open channel while having no obvious effect on channel kinetics. These blocks were completely reversible. Ba (10 mM) on the cytoplasmic side of the membrane slightly decreased inward currents and completely blocked outward currents through the channel.

7. External Ba (10 mM) caused a voltage-dependent decrease in inward current. External tetraethylammonium (10 mM) completely blocked single channel currents.

INTRODUCTION

Airway smooth muscle controls airway calibre, thereby determining the distribution of ventilation to the alveoli. In diseases such as asthma, contraction of airway smooth muscle increases airway resistance. Despite its importance in both normal physiology

and pathophysiology, little is known about the cell membrane transport processes that regulate the membrane voltage and thus the contractile state of the muscle.

Previous studies indicate that action potentials and slow wave activity are not present in airway smooth muscle. Examination of the cell membrane current–voltage relationships shows that the membrane rectifies strongly with high conductance at outward currents (Kirkpatrick, 1981). The purpose of this study was to investigate the cell membrane transport processes that are responsible for these properties. We isolated single airway smooth muscle cells and used the patch-clamp technique described by Hamill, Marty, Neher, Sakmann & Sigworth (1981) to examine the behaviour of single ion channels. In our preliminary studies we found that K channels were the most frequently observed channels, as well as being the largest. In this report, we describe evidence for a Ca-activated K channel in airway smooth muscle.

Analysis of single channel currents can be divided into two categories: studies of channel kinetics and studies of the properties of the open channel. Our first goal was to quantify the single channel current–voltage relationship under a diverse set of ionic conditions. Examining the current–voltage relationship of the open channel allowed us to measure single channel conductance, single channel ion selectivity, and the blocking of single channel currents. Secondly, we have described some observations regarding channel kinetics.

METHODS

Cell isolation

Mongrel dogs (20–25 kg) of either sex were anaesthetized with pentobarbitone (25 mg/kg). To reduce the number of red blood cells in the final cell suspension, we perfused the tracheal circulation. The tracheal circulation was isolated by ligation of both carotid arteries above the insertion of the superior thyroid artery and by ligation of the subclavian and proximal carotid arteries. Both common carotids were then cannulated and perfused with 2 l phosphate-buffered Ca-free and Mg-free Ringer solutions. The first 100 ml Ringer solution contained 60 u./ml heparin. Blood and perfusate were removed by suction through a catheter inserted in the superior vena cava. The heart was stopped by injection of 25 ml 3 M-KCl.

After perfusion, the trachea was removed and the membranous tissue surrounding the trachea was stripped away. The trachealis muscle was then removed from the posterior surface of the trachea.

Smooth muscle cells were enzymically dispersed, using techniques similar to those described in a preliminary communication by Madison, Tom-Moy & Brown (1984). Approximately one-half of the trachealis muscle was placed in 20 ml of an enzyme solution containing collagenase (Worthington, CLSPA, 100 u./ml) and elastase (Worthington, ESFF, 5 u./ml). The enzyme solution was prepared in medium 199 Earle's Base (in mg/l: $CaCl_2$, 200; $Fe(NO_3)_3 . 9H_2O$, 0·72; KCl, 400; $MgSO_4$, 97·7; NaCl, 6800; $NaHCO_3$, 2200; $NaH_2PO_4 . H_2O$, 140) supplemented with bovine serum albumin (10 mg/ml), dextrose (1·8 mg/ml), penicillin (100 u./ml) and streptomycin (100 μg/ml). The tissue was minced into small pieces and allowed to incubate in the enzyme solution at 37 °C with mechanical agitation for 2 h. The cell suspension was then filtered through a double layer of gauze and spun for 15 min at 200 *g*. The supernate was removed and the cells were plated at $2·5 \times 10^4$ cells/cm^2 in 35 mm poly-L-lysine-coated Petri dishes. The cell media consisted of: medium 199 Earle's base supplemented with 10% v/v fetal calf serum, non-essential amino acid (1 ml/100 ml, KC Biological, U.S.A.), penicillin (100 u./ml) and streptomycin (100 μg/ml). The cells were incubated for 3–72 h at 37 °C and 6% CO_2 before use.

Cell identification

40% of the cells had the elongated appearance expected for smooth muscle. To further identify them as smooth muscle cells, we stained the cells for smooth muscle isoactin. The cells were fixed for 20 min in phosphate-buffered saline solution containing 3·7% v/v formaldehyde. The cell

membrane was then permeabilized by treating with cold methanol (−20 °C) for 10 min. The cells were then dehydrated by washing with a series of five phosphate-buffered solutions containing 95 %, 70 %, 50 %, 30 % and 0 % methanol, respectively. The final wash was poured off and the cells were incubated for 90 min in a humidified chamber with one drop of a solution containing 10 μg/ml mouse monoclonal antibody against smooth muscle γ-isoactin (kindly provided by Dr J. Lessard, Division of Basic Sciences, Children's Hospital Research Foundation, Cincinnati, OH, U.S.A.). The first incubation was followed by a second 90 min incubation in one drop of solution containing fluorescein-labelled rabbit antimouse IgG. The cells were viewed with epifluorescence. The elongated cells that we identified as smooth muscle fluoresced brightly. Approximately one-fourth of the cells did not fluoresce. Non-fluorescent cells did not have the morphological characteristics of smooth muscle. All patch-clamp studies were done on cells with the characteristic smooth muscle cell appearance. As further evidence of the cells' identity, we observed that 5×10^{-6} M-acetylcholine produced contraction of the elongated cells.

Patch-clamping technique

Our technique for construction of pipettes and seal formation is similar to that described by Hamill *et al.* (1981). Patch-clamp pipettes were prepared from 100 μl haematocrit micropipettes (Rochester Scientific Co., Inc., U.S.A) on a modified Industrial Science Associates (U.S.A.) micropipette puller. The tip of the pipette was then fire-polished under 320 × magnification. Pipettes had a resistance of 4–8 MΩ when filled with 135 mM-KCl.

Cells in a Petri dish were placed on the stage of an inverted microscope (Leitz Diavert, F.R.G.) mounted on a vibration–isolation table (model XJ-A, Newport Corp., U.S.A.). The micropipette was advanced to the cell surface using a three-dimensional hydraulic micromanipulator (model MO-103, Narishige Scientific Instrument Laboratories, Japan) under direct observation (320 × magnification). Seal formation was potentiated by application of light suction to the pipette interior, although seals formed spontaneously on rare occasions. Typical seal resistance with HEPES-buffered Ringer solution in the pipette was 20–40 GΩ, but with 135 mM-KCl in the pipette, seal resistances were lower (3–5 GΩ).

We used a 'Yale model' voltage clamp for voltage clamping and current amplification. Currents were low pass filtered (500–700 Hz) on an eight-pole Bessel filter (Frequency Devices, Inc., U.S.A.), and recorded on a strip chart recorder (Model 220, Gould Inc., U.S.A.). The 90 % rise time of the strip chart recorder was 3 ms. (Results were analysed by hand.)

Our terminology for describing patch-clamp conformations is the same as that used by Hamill *et al.* (1981). After advancing the pipette on to the cell surface and applying light suction, a cell-attached patch was achieved. Withdrawing the pipette tip from the cell left a small patch of membrane or a small vesicle sealed at the tip of the pipette. Vesicle formation resulted in distorted currents of reduced amplitude. Passing the pipette across the water–air interface for several seconds apparently ruptured the external portion of the vesicle or made it leaky, resulting in currents of normal amplitude which originated from a patch of membrane oriented such that the cytoplasmic side of the membrane faced the bathing solution. This is termed an inside-out patch.

'Internal' refers to the cytoplasmic side of the membrane, and 'external' to the outside of the membrane. Voltages were referenced to the cell exterior, so that a negative voltage is hyperpolarizing, and a positive voltage is depolarizing. Currents were referenced to the membrane patch. Outward current corresponds to cations flowing out of the cell and is labelled positive; inward current is labelled negative. In the current tracings, outward current is indicated by an upward deflexion.

We show representative examples of current tracings and current–voltage relationships, but all experiments were repeated at least 3–5 times. The results were always quantitatively and qualitatively similar. The representative examples were the result of seventy-one patches of membrane used to compile the data for this study.

Solutions

The Ringer solution used to bathe the cells during seal formation and cell-attached recording contained (in mM): Na, 140; K, 5; Ca, 1·2; Mg, 1·2; Cl, 145. All solutions were HEPES (10 mM) buffered to pH 7·4 in room air. Na and K concentrations were measured by flame photometry (Instrumentation Lab. Inc., U.S.A.). Ca concentrations less than or equal to 10^{-6} M were achieved by buffering 1 mM-formal [Ca] with ethyleneglycol-bis-(β-aminoethylether)*N*,*N*′-tetraacetic acid (EGTA), assuming a dissociation constant of 10^{-7} (Marty, 1981). Ca-free solutions were prepared by adding 10 mM-EGTA to nominally Ca-free solution.

All solutions were filtered through 0·22 μm Millex-GS filters (Millipore Corp., U.S.A.) just prior to use. Solution changes during recordings were accomplished by washing out the 3 ml of bathing solution with 30 ml of the new solution. All experiments were done at room temperature (21–23 °C).

Reagents

We used the following compounds: bovine serum albumin (Sigma Chemical Co., U.S.A.), ethyleneglycol-bis-(β-aminoethylether)*N,N'*-tetraacetic acid (EGTA) (Sigma), fetal calf serum (Cancer Center Tissue Culture Lab (CCTCL), University of Iowa), HEPES (Sigma), Medium 199, Earle's Base (CCTCL), non-essential amino acid (KC Biological, U.S.A.), penicillin and streptomycin (CCTCL), tetraethylammonium chloride (TEA) (Kodak, U.S.A.), tetramethylammonium chloride (TMA) (Fisher Scientific Co., U.S.A.).

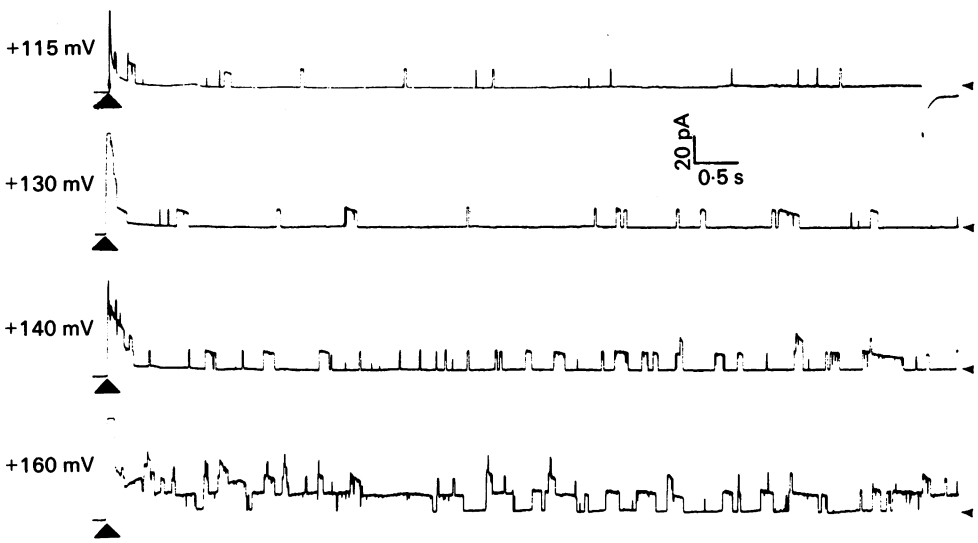

Fig. 1. Recording from a cell-attached patch. The bathing and pipette solutions both contained 140 mM-Na, 5 mM-K, 1·2 mM-Mg, 1·2 mM-Ca, 144·8 mM-Cl and 10 mM-HEPES. The large arrowheads mark the onset of a step change in voltage from the cell resting potential to the indicated voltage. The small arrowheads indicate the current level when no channels are open. Seal resistance was 10 GΩ.

RESULTS

Cell-attached patches. Fig. 1 is a representative example of the effect of voltage on channel activity in the cell-attached conformation. Depolarizing voltage steps of 115, 130, 140 and 160 mV elicited outward current steps. Single channel amplitudes were relatively independent of voltage in this range, suggesting saturation of channel current. When the membrane was depolarized to potentials less than 90 mV, channel openings were rare. Depolarizing voltage steps to greater than 150 mV reduced single channel amplitudes, thus displaying a negative slope conductance. Examination of Fig. 1 demonstrates that there is more than one channel in the patch, and at several points when the patch is depolarized by 160 mV three channels are open simultaneously. This was the typical finding; we rarely found only one channel in a patch, even when using pipettes with small tips (> 8 MΩ).

Fig. 1 also shows that at +130 mV and +140 mV the unitary steps do not appear as square waves but have an exponential decrease (time constant ~ 300 ms) in amplitude during prolonged openings. This phenomenon can be explained by the fact that the clamping voltage is the voltage between bath and pipette interior. Therefore, when a large K channel opens in the patch under the pipette, the flow of K out of the cell hyperpolarizes the cell, thus decreasing the electrical driving force for K exit from the cell. This phenomenon was not observed in excised patches in which the absolute voltage across the membrane is clamped. Similar findings have been reported by Fenwick, Marty & Neher (1982).

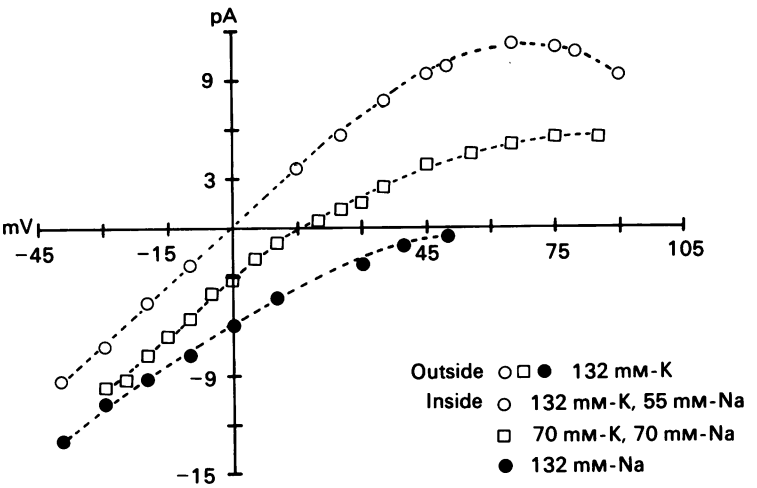

Fig. 2. Effect of K and Na gradients on the current–voltage relationship. Na and K concentrations were as indicated. ○, symmetrical K solutions (chemical K gradient, 0 mV) with a large chemical Na gradient (< −160 mV). □, chemical K gradient of +16 mV with a chemical Na gradient of < −120 mV. ●, current–voltage relationship under NaCl/KCl, bi-ionic conditions.

Comparing the four records in Fig. 1, it is also apparent that both the frequency of channel opening and the duration of the open state increase with membrane depolarization. Thus, the channel displays voltage gating in cell-attached patches.

Single channel conductance. The current–voltage relationship of seven different patches of membrane excised inside-out with 135 mM-K on both sides of the membrane is well defined by a line passing through the origin with a slope of 266 ± 12 pS (mean ± S.D., $n = 7$). Variations in the internal Ca concentration between 10^{-6} and 10^{-10} M had no effect on conductance, nor did the use of EGTA-buffered *versus* nominally Ca-free solutions.

Channel selectivity. To determine channel selectivity for K *versus* Na, Cl and Ca, we examined the effect of ion substitutions on the current–voltage relationships of single channels. Fig. 2 shows the current–voltage relationship under three different conditions in which we varied the K and Na concentrations and gradient across excised inside-out patches. In the plot that passes through the origin (○) no K gradient was present, but a substantial Na gradient was present (55 mM inside/0·1 mM outside). The fact that the plot passes through the origin, despite the large chemical

gradient for Na, indicates a high selectivity for K over Na. The rectification at depolarizing voltages suggests that, although Na has a low permeability, it does behave as a voltage-dependent blocker of outward current. Comparison of this plot with Figs. 3 and 7 demonstrates, that in the absence of Na, rectification was not observed. □ in Fig. 2 show that single channel currents reversed at the K reversal potential (+16 mV) calculated from the Nernst equation, despite a substantial Na gradient (70 mM outside/0·45 mM inside). ● show that single channel currents did not reverse even at +50 mV under bi-ionic NaCl/KCl conditions. At voltages more positive than +50 mV no single channel currents were observed indicating that the amplitude of the open channel was too small to measure. These results indicate that the channel is highly selective for K over Na.

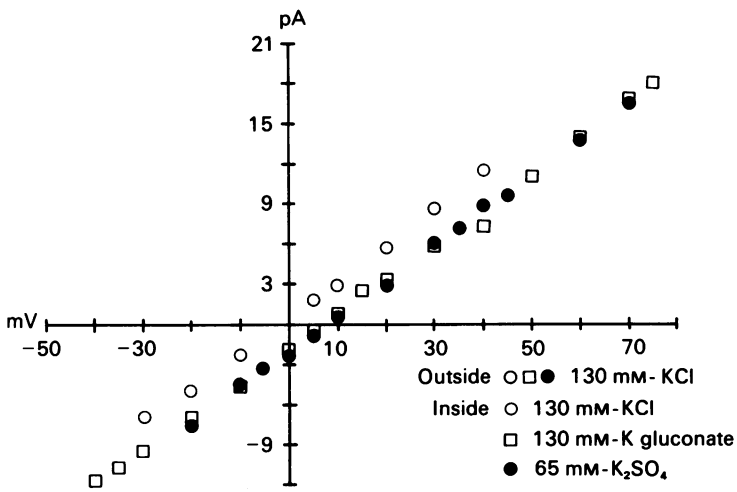

Fig. 3. Effect of anion replacement on the current–voltage relationship. The K concentration was 130 mM symmetrically. The external Cl concentration was 125 mM throughout. ○, inside Cl, 130 mM. □, inside gluconate, 130 mM. ●, inside SO_4, 65 mM.

Fig. 3 shows the current–voltage relationship of an excised, inside-out patch during anion substitutions. The anion on the internal side of the membrane was replaced while the K concentration was kept constant at 130 mM on both sides of the membrane. The Cl concentration on the outside was 130 mM throughout. If the channel showed some permeability to Cl, then replacement of internal Cl would shift the reversal potential to more negative values. However, the reversal potential shifted to a more positive value. This shift in reversal potential after Cl replacement can be explained entirely by changes in the K activity of K gluconate or K_2SO_4 solutions as compared to KCl solutions. When we used the activity of K in the 130 mM-K gluconate and 65 mM-K_2SO_4 solutions (K gluconate measured with a K-selective electrode, and K_2SO_4 activity coefficients extrapolated from literature values (Robinson & Stokes, 1955)), the expected reversal potentials for replacement of KCl with K_2SO_4 and K gluconate were 9·8 and 10 mV, respectively. Thus, the channel has extremely high selectivity for K over Cl. For the remainder of the experiments Cl was the anion unless otherwise indicated.

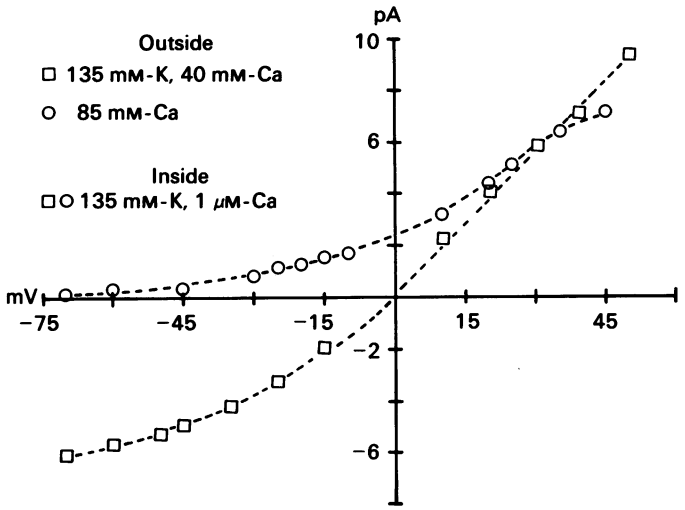

Fig. 4. Effect of Ca gradients on the current–voltage relationship. The inside solution contained 135 mM-K, 10^{-6} M-Ca and 132 mM-Cl. □, outside solution contained 135 mM-K (chemical K gradient, 0 mV) and 40 mM-Ca (chemical Ca gradient, > 230 mV). ○, outside solution contained 85 mM-Ca and no K. The lines were fit by eye.

Fig. 4 shows the current–voltage relationship of single channel currents in excised inside-out patches in the presence of large Ca gradients. The current–voltage relationship passes through the origin with symmetrical K solutions and a large Ca chemical gradient (40 mM outside/10^{-6} M inside) (□). The rectification suggests that 40 mM-Ca on the membrane exterior causes a voltage-dependent block of single channel currents. Under bi-ionic conditions (○), we observed outward current (carried by K), but not inward (Ca) current. Outward current was still seen at −70 mV. At hyperpolarizing voltages greater than this, no channel openings were seen. The failure to observe channel activity at extreme hyperpolarization could mean that the channel did not open, or more likely, that the amplitude of the open channel was too small to measure. Both experiments indicate that the channel has an extremely low permeability to Ca.

Ca and voltage activation. We examined the relationship between the internal Ca concentration and channel activity to determine if the activity of this channel is regulated by the Ca concentration on the cytoplasmic surface of the membrane. We also examined the effect of voltage on channel activity in excised patches. Fig. 5 shows a representative record of single channel currents under ten different conditions. By comparing recordings in the same column, it is apparent that both the frequency of channel opening and the duration of the open state increased as the patch was depolarized from +20 to +40 mV at a constant Ca concentration. Single channel amplitudes also increased as the driving force for outward K current increased. These findings are consistent with the voltage gating observed in cell-attached patches (Fig. 1). By comparing records in the same row, the effect of Ca on the cytoplasmic side of the membrane becomes apparent. As the internal Ca concentration increased, both the probability of channel opening and the duration of the open state increased. Thus,

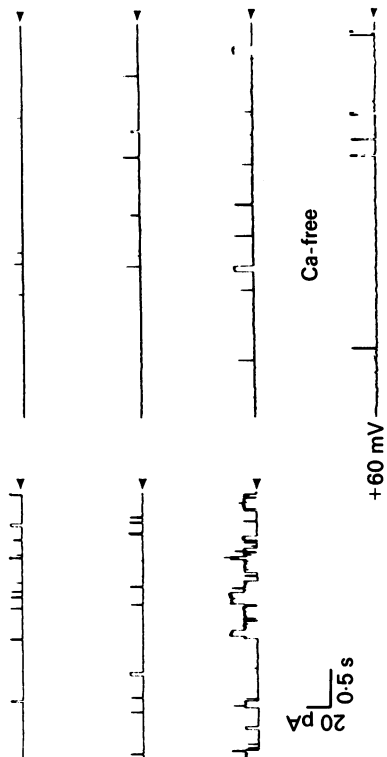

Fig. 5. Effect of internal Ca concentration and voltage on channel activity in excised inside-out patches. These single channel records were obtained from the same patch as shown in Fig. 1, after excising it from the cell. (Pipette solution same as given in Fig. 1.) The internal solution contained 130 mM-KCl with Ca concentrations as indicated. The effect of voltage on channel kinetics can be observed by comparing tracings in the same column. The effect of Ca concentration on channel kinetics can be observed by comparing tracings in the same row. The arrowheads mark the current level when no channels are open.

an increase in Ca concentration and membrane depolarization had similar effects on channel kinetics. The tracing in the lower right-hand corner of Fig. 5 shows outward currents at +60 mV when the cytoplasmic side of the membrane was bathed with a nominally Ca-free solution containing 10 mM-EGTA. The presence of channel activity, even in a Ca-free ([Ca] < 10^{-10} M) solution, indicates that the channel can be activated even at very low internal [Ca]. Increasing external [Ca] from 10^{-6} M up to 1 mM had no further detectable effect on the probability of the channel opening or the duration of the open states (not shown).

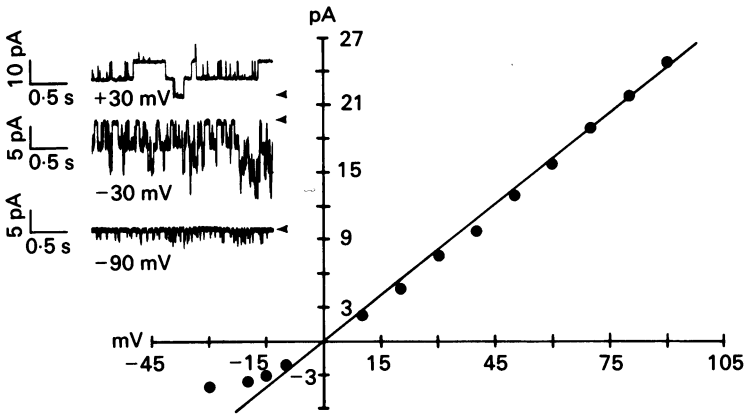

Fig. 6. Effect of external Ba on the current–voltage relationship. The line represents the single channel conductance in the absence of blockers (observed in previous patches) and is included for comparison. External solution (pipette solution): 10 mM-Ba, 130 mM-K, 1 mM-Ca. Internal solution: 130 mM-K, nominally Ca-free. The inset shows single channel recordings at three different membrane potentials. The inset and all subsequent insets were taken from the same experiment as that used to obtain the current–voltage relation. The arrowheads mark the current level when no channels are open.

K channel blockers. Current flow through several different types of K channels can be blocked by a variety of cationic molecules. To further investigate the conductive properties of this K channel we examined the effect of Ba, TEA, TMA and Cs. Fig. 6 shows the current–voltage relationship of an excised inside-out patch with 10 mM-external Ba. There was no K gradient. The continuous line represents the current–voltage relationship taken from previous patches with symmetrical K solution in the absence of Ba. External Ba had little effect on outward current but the amplitude of inward currents was reduced. The rectification of inward currents indicates that the Ba block is voltage sensitive. Note the difference in current amplitude at +30 mV and −30 mV (inset in Fig. 6); in the absence of Ba, these amplitudes would be equal but opposite. The recording at −90 mV (inset in Fig. 6) shows that inward current is still observed, but it is difficult to determine the amplitude because the current traces lack defined unitary steps. It would appear, however, that single channel amplitudes decrease between −30 and −90 mV, thus demonstrating a negative slope conductance and yielding further evidence for the voltage sensitivity of Ba block.

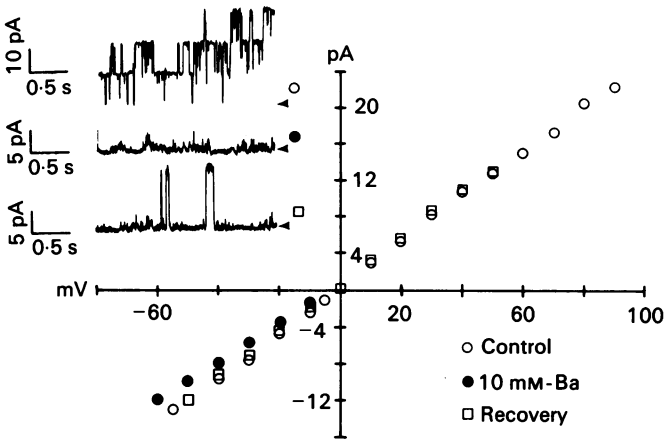

Fig. 7. Effect of internal Ba on the current–voltage relationship. External solution: 130 mM-K, 1 mM-Ca. Internal solution: 130 mM-K, nominally Ca-free. The current–voltage relation was examined before adding Ba (○), in the presence of 10 mM-Ba added to the internal solution (●), and after Ba was washed out (□). The inset illustrates single channel currents at +50 mV.

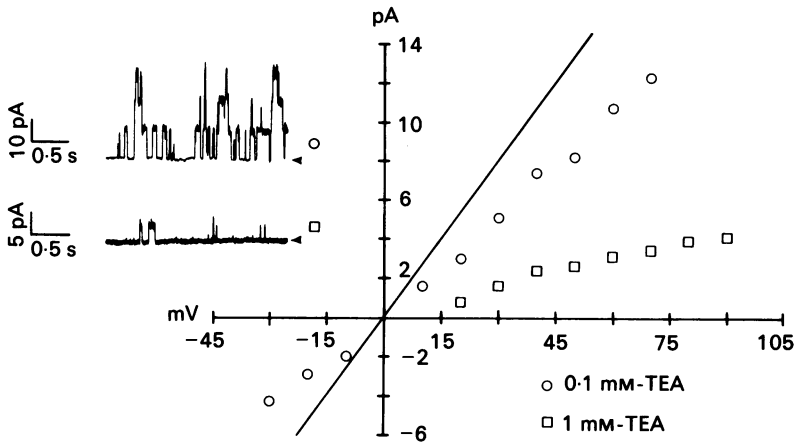

Fig. 8. Effect of external tetraethylammonium (TEA) on the current–voltage relationship. The line represents single channel conductance in the absence of blockers and is included for comparison. External solution: 135 mM-K, 1 mM-Ca. Internal solution: 135 mM-K, 10^{-6} M-Ca. ○, 0·1 mM-external TEA. □, 1 mM-external TEA. The insets show single channel currents at +60 mV with 0·1 mM-TEA (top), and 1 mM-TEA (bottom).

The effect of internal Ba is shown in Fig. 7. Internal Ba (10 mM) reduced the amplitude of inward current. However, upon depolarization, well defined steps of outward current were not observed. Ba seemed to reverse the effect of voltage on the channel. In the presence of Ba channel opening probability decreased with depolarization. At depolarizing voltages greater than 60 mV, flickering channel activity was observed. Comparison of the top and middle tracing in the inset illustrates the lack of outward current in the presence of internal Ba. Comparing the top and bottom inset illustrates that although removal of Ba allowed single channel

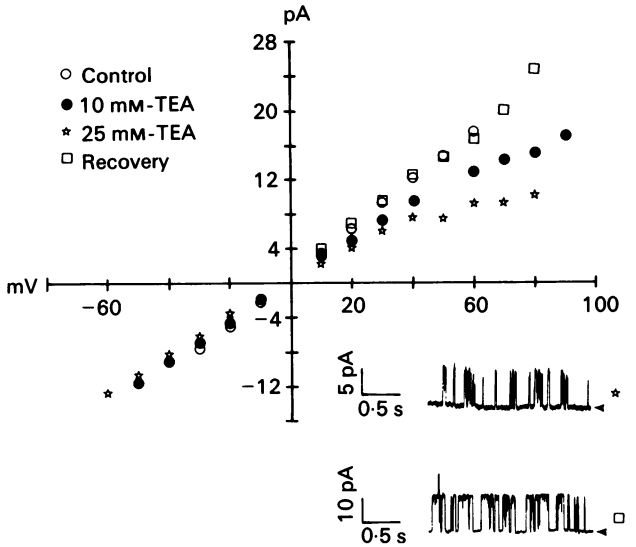

Fig. 9. Effect of internal tetraethylammonium (TEA) on the current–voltage relationship. External solution: 135 mM-K, 1 mM-Ca. Internal solution: 135 mM-K, 10^{-6} M-Ca. The current–voltage relation was examined before adding TEA (○), with 10 mM-internal TEA (●), with 25 mM-internal TEA (☆), and after TEA was washed out (□). The insets show single channel currents at +50 mV with 25 mM-internal TEA (top), and after washing TEA out of the bathing solution (bottom).

amplitude to return to the original level, the frequency of channel opening was reduced.

We next examined the effect of TEA from the external and internal surfaces of the membrane. Fig. 8 shows that 1 mM-external TEA reduced single channel conductance by over 75 % ($n = 4$). A reduction in single channel conductance was also observed with 0·1 mM-external TEA. In five patches with 10 mM-TEA in the pipette (external solution) no channel activity was observed. For comparison, in the absence of TEA, K channels were observed in more than 90 % of all patches. This observation indicates that the K channels are completely blocked by 10 mM-external TEA.

Internal TEA also blocked K current as shown in Fig. 9. The current–voltage relationships were examined before and after addition of 10 and 25 mM-TEA to the internal solution, and during a 'recovery' period after TEA was washed out. Rectification at depolarizing voltages indicates that TEA block was voltage dependent. TEA block was completely reversible.

We also examined the effect of a TEA analogue to see whether the channel discriminated between blocks by different quaternary ammonium ions. Fig. 10 shows the effect of internal TMA. TMA on the cytoplasmic side blocked outward K currents in a voltage-dependent manner much like TEA. However, at +50 mV, 25 mM-TEA reduced single channel amplitude by 50 % ($n = 3$) while at the same voltage and concentration TMA reduced single channel amplitudes by 65 % ($n = 4$). Thus, internal TMA was more potent than TEA. TMA showed no obvious effect on channel kinetics and the reduction in amplitude was reversible.

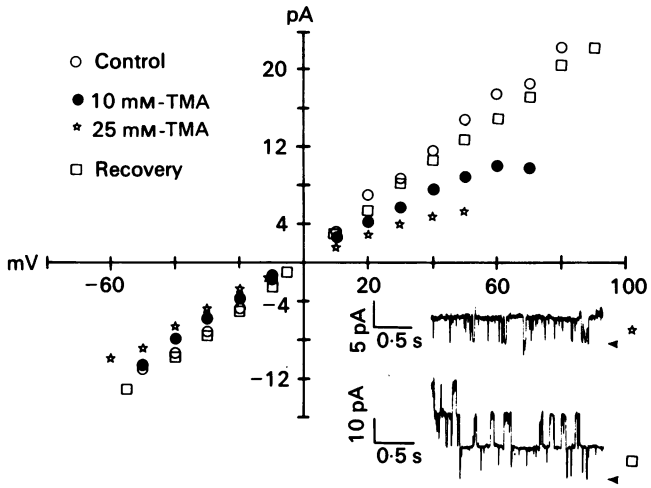

Fig. 10. Effect of internal tetramethylammonium (TMA) on the current–voltage relationship. External solution: 130 mM-K, 1 mM-Ca. Internal solution: 130 mM-K, nominally Ca-free. The current–voltage relationship was examined before addition of TMA (○), with 10 mM-internal TMA (●), with 25 mM-internal TMA (☆), and after TMA was washed out (□). The insets show single channel currents at +50 mV with 25 mM-internal TMA (top), and after washing TMA out (bottom).

Finally, we examined the effect of Cs on the cytoplasmic surface of the membrane (data not shown). Cs block from the cell interior was very similar to TMA block, TEA block, and Na block in that all four reduced the amplitude of outward currents in a voltage-dependent manner, had no obvious effect on channel kinetics, and were completely reversible.

DISCUSSION

We have shown that canine airway smooth muscle contains a Ca-activated K channel. This K channel is by far the predominant channel in the isolated airway smooth muscle cells. The channel was observed in 90% of all patches and even with small pipettes, multiple channels were observed. The channel has a conductance of 266 ± 12 pS (mean $\pm$ S.D., $n = 7$) with 135 mM-K on both sides of the membranes. Despite the large conductance of this channel it shows a high degree of selectivity; the channel showed no measurable permeability to Na, Ca, or Cl. This combination of high conductance and high selectivity has been reported in the Ca-activated K channels of rat neurone and rat muscle (Latorre & Miller, 1983). The smooth muscle of rabbit jejunum (Benham, Boltan, Lang & Takewaki, 1985) and toad stomach muscularis (Singer & Walsh, 1984) has also been reported to contain a large Ca-activated K channel.

The channel was voltage gated in both the cell-attached and excised-patch conformations, showing an increased frequency of opening and increased time spent in the open state with patch depolarization. Voltage gating of large (> 130 pS in 150 mM-KCl) Ca-activated K channels has been reported in rabbit transverse tubule

(Latorre, Vergara & Hidalgo, 1982), chromaffin cells (Marty, 1981), rat pituitary (Wong, Lecar & Adler, 1982), frog neurone (Adams, Constanti, Brown & Clark, 1982), mammalian salivary glands (Maruyama, Gallacher & Peterson, 1983), rat myoballs (Methfessel & Boheim, 1982) and rat muscle (Pallotta, Magleby & Barrett, 1981).

In agreement with our results, it has been reported that internal application of Cs, Na and TEA block current through Ca-activated K channels in bovine chromaffin cells (Yellen, 1984). Block of the channel by internal TEA is presumed to result from TEA binding to a site near the wide, cytoplasmic mouth of the channel (Lattore & Miller, 1983). In smooth muscle from jejunum and guinea-pig small mesenteric artery, Benham and co-workers (Benham *et al.* 1985) have reported voltage-insensitive block of Ca-activated K channels. However, that conclusion was based on an examination in the voltage range of ± 40 mV. In that range we also find minimal voltage dependence (Fig. 9); only with higher voltages did the voltage dependence of block become apparent. Moreover, in other cells variability in the voltage dependence of internal TEA block has been reported (Coronado & Miller, 1982; Vergara, Moczydlowski & Lattore, 1984; Yellen, 1984). In addition to block by internal TEA, we found a voltage-dependent block by internal TMA and Ba. Ba was the only internal blocker of outward K current that was more effective than TMA.

We observed (Fig. 8) that the K-selective ion channel is very sensitive to external TEA. This is consistent with findings in other Ca-activated K channels in different preparations (transverse tubule (Latorre *et al.* 1982) and the frog neurone (Adams *et al.* 1982)). It has also been reported that Ba is an effective blocker at both faces of the Ca-activated K channel isolated from rabbit muscle (Vergara & Latorre, 1983). The Ba block was reported to only affect the open channel probability. Similarly, we observed an irreversible voltage-dependent decrease in the open state probability in the presence of 10 mM-internal Ba, and in addition found that external Ba (10 mM) slightly decreased single channel conductance at inward currents.

Airway smooth muscle cell membrane rectifies strongly having high conductance at outward currents and low conductance at inward currents (Kirkpatrick, 1975). It is virtually impossible to elicit action potentials in airway smooth muscle cells bathed in normal Ringer solution by passing outward current through a stimulating electrode. The lack of electrical activity is due to the strong rectifying nature of the membrane: outward current will not depolarize the membrane to the threshold for regenerative electrical activity because it decreases membrane resistance. However, TEA at low concentrations (1 mM) blocks Rb efflux in high K-depolarized smooth muscle (Imaizumi & Watanabe, 1981) and at higher concentrations (30 mM) TEA increases membrane resistance eliminating membrane rectification. TEA (30 mM) also depolarizes the membrane 10 mV and induces spontaneous rhythmic variations in membrane potential (Kroeger & Stephens, 1975). It has been suggested that airway smooth muscle has two K conductances (Imaizumi & Watanabe, 1981). The resting K conductance can be blocked by 30 mM-TEA and the voltage-sensitive K conductance which can be blocked by low doses of TEA (1 mM). Both conductances must be blocked for TEA to induce contraction.

Our finding of a voltage-gated Ca-activated K channel can readily account for the voltage-sensitive K conductance which is blocked by 1 mM-TEA. Our data indicates

this K channel would open at physiological voltages if cytosolic Ca is increased into the range between 10^{-7} and 10^{-6} M. The associated increase in membrane conductance would tend to stabilize the membrane potential, shifting it towards the Nernst potential for K. Considering the Ca activation and voltage gating of this channel we speculate that the physiological role is in repolarization of the cell membrane after depolarization and concomitant increases in [Ca] associated with contraction.

We gratefully acknowledge the technical assistance of Timothy Ruppert and Phil Karp and the secretarial assistance of Mrs Deanna Ollendick, Ms Mary Uhl and Mrs Ann Ziskovsky. Special thanks to Dr Bruce Bean for his patient and knowledgeable advice, without which this study would have been difficult to complete. This work was supported by grants from the National Heart, Lung and Blood Institute (HL-14388 and HL-35804) and the Council for Tobacco Research. John D. McCann is an American Heart Association student fellow. Michael J. Welsh is an Established Investigator of the American Heart Association.

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