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CONTROL OF RESTING MEMBRANE POTENTIAL BY DELAYED RECTIFIER POTASSIUM CURRENTS IN FERRET AIRWAY SMOOTH MUSCLE CELLS

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SUMMARY

1. In order to determine the physiological role of specific potassium currents in airway smooth muscle, potassium currents were measured in freshly dissociated ferret trachealis cells using the nystatin-permeabilized, whole-cell method, at 35 °C.

2. The magnitude of the outward currents was markedly increased as bath temperature was increased from 22 to 35 °C. This increase was primarily due to the increase in maximum potassium conductance ($g_{K,max}$), although there was also a small leftward shift in the relationship between g_K and voltage at higher temperatures. The maximum conductance and the kinetics of current activation and inactivation were also temperature dependent. At 35 °C, gating of the current was steeply voltage dependent between −40 and 0 mV. Current activation was well fitted by fourth-order kinetics; the mean time constants of activation (30 mV clamp step) were 1·09 ± 0·17 and 1·96 ± 0·27 ms at 35 and 22 °C, respectively.

3. Outward currents using the nystatin method were qualitatively similar to delayed rectifier currents recorded in dialysed cells with high calcium buffering capacity solutions. 4-Aminopyridine (4-AP; 2 mM), a specific blocker of delayed rectifier potassium channels in this tissue, inhibited over 80 % of the outward current evoked by voltage-clamp steps to between −10 and + 20 mV ($n = 6$). Less than 5 % of the outward current was blocked over the same voltage range by charybdotoxin (100 nM; $n = 15$), a specific antagonist of large-conductance, calcium-activated potassium channels in this tissue.

4. The degree to which delayed rectifier and calcium-activated potassium conductances control resting membrane potential was examined in current-clamp experiments. The resting membrane potential of current clamped cells was −33·6 ± 1·0 mV ($n = 62$). Application of 4-AP (2 mM) resulted in a 14·4 ± 1·0 mV depolarization ($n = 8$) and an increase in input resistance. Charybdotoxin (100 nM) had no effect on resting membrane potential ($n = 6$).

5. Force measurements were made in isolated strips of trachealis muscle to determine the effect of pharmacological blockade of individual potassium conductances on resting tone. In the presence of tetrodotoxin (1 μM) and atropine

(1 μM), 4-AP increased baseline tension in a dose-dependent manner, with an EC_{50} of 1·8 mM ($n = 13$); application of 5 mM 4-AP increased tone to 86·8 ± 8·1 % of that produced by 1 μM methacholine, and this tone was almost completely inhibited by nifedipine (1 μM). Conversely, antagonism of calcium-activated potassium (K^+_{Ca}) channels with charybdotoxin (100 nM) resulted in only a slight increase in resting tone (4·9 ± 3·0 % of methacholine response).

6. We conclude that delayed rectifier potassium channels play a critical role in maintaining resting membrane potential in ferret trachealis smooth muscle. Calcium-activated potassium channels appear to comprise a smaller component of the resting potassium conductance, and accordingly are less important in defining the passive behaviour of this muscle.

INTRODUCTION

Previous studies have suggested that potassium currents are important for the maintenance of normal electrical and contractile stability of airway smooth muscle (ASM). Thus agents that block potassium channels produce phasic or tonic contractions and initiate spontaneous electrical activity (Kirkpatrick, 1975; Kroeger & Stephens, 1975; Small, 1982; Kannan, Jager, Daniel & Garfield, 1983; Ahmed, Foster & Small, 1985). Whole-cell and single-channel patch-clamp experiments have identified two prominent K^+ channels in ASM cells (Kotlikoff, 1990; Hisada, Kurachi & Sugimoto, 1990; Muraki, Imaizumi, Kawai & Watanabe, 1990; Boyle, Tomasic & Kotlikoff, 1992), which can be separated pharmacologically (Boyle *et al.* 1992). Large conductance, calcium activated potassium (K^+_{Ca}) channels in smooth muscle are blocked by charybdotoxin (CHTX) and iberiotoxin, peptidyl toxins purified from scorpion venom (Smith, Phillips & Miller, 1986; Kume & Kotlikoff, 1991; Green, Foster & Small, 1991; Boyle *et al.* 1992; Savaria, Lanoue, Cadieux & Rousseau, 1992), and by low concentrations of tetraethylammonium (TEA) ions (Langton, Nelson, Huang & Standen, 1991; Boyle *et al.* 1992), and are activated by rises in cytosolic calcium following exposure to agonists (Singer & Walsh, 1980; Benham & Bolton, 1986; Kotlikoff, 1990; Saunders & Farley, 1992; Janssen & Sims, 1992). Low-conductance, delayed rectifier (K^+_{DR}) potassium channels are insensitive to charybdotoxin and changes in cytosolic calcium concentration, and are blocked by 4-aminopyridine (4-AP) and 3,4- diaminopyridine (3,4-DAP), which do not block K^+_{Ca} channels (Boyle *et al.* 1992; Saunders & Farley, 1992). Additionally, agents that activate ATP-sensitive potassium channels (K^+_{ATP}) are capable of relaxing airway smooth muscle (Berry, Elliott, Foster, Green, Murray & Small, 1991), although definitive current measurements in this tissue have not been reported.

Although recent studies have shown that K^+_{Ca} channels are co-ordinately regulated by agents that alter ASM tone (Kume, Takai, Tokuno & Tomita, 1989; Kume & Kotlikoff, 1991), the degree to which K^+_{Ca} and K^+_{DR} channels underlie resting electrical behaviour, determine membrane potential, and prevent spontaneous depolarizations is uncertain. Since the open-state probability of K^+_{Ca} channels (and attendant whole-cell conductance) is influenced by the level of cytosolic calcium and calcium buffering, whole-cell experiments that replace

cytosol with extracellular calcium buffers may not accurately reflect physiological channel activity (Kotlikoff, 1990; Hisada *et al.* 1990; Muraki *et al.* 1990; Green *et al.* 1991; Saunders & Farley, 1992;). The current study was undertaken to examine K^+ currents in individual ASM myocytes under physiological conditions. We were specifically interested in determining the degree of activity of K^+_{Ca} and K^+_{DR} channel activity at physiological potentials, and the role of these channels in determining resting membrane potential. The nystatin whole-cell method (Horn & Marty, 1988) was used to measure voltage-dependent currents under conditions of physiological cellular buffering of cytosolic calcium, and to retain cytosolic regulatory molecules that modulate channel activity. Since the level of activity of potassium channels at any given voltage is also markedly influenced by temperature (Beam & Donaldson, 1983), we quantitatively examined currents at 22 and 34–36 °C. Finally, the effect of blockade of specific currents on membrane potential of non-dialysed cells, and resting tone of *in vitro* muscle strips, was examined.

METHODS

Cell dissociation

Cell dissociation methods were similar to those described previously (Kotlikoff, 1988). Mixed breed ferrets of either sex were anaesthetized to a deep surgical plane with an intraperitoneal injection of pentobarbitone sodium and killed by exsanguination. The trachea was rapidly removed and placed in ice-cold modified Krebs–Ringer–Henseleit (KRH) solution containing (mM): 115 NaCl, 5 KCl, 1 KH_2PO_4, 1 $MgSO_4$, 25 *N*-2-hydroxyethylpiperazine-*N'*-2-ethane-sulphonic acid (Hepes), 15 glucose and 2 $CaCl_2$ and 10 mg/ml Phenol Red. The mucosa and serosa were dissected away from the trachealis muscle, and approximately 0·5 g wet tissue was then cut into small pieces, and transferred into 5 ml of oxygenated digestion medium (M199, Gibco) containing 1·7 mM EGTA, 300 U/ml collagenase D (Boehringer Mannheim, Germany), 8 U/ml elastase (Worthington) and 5 mg Type 1 soybean trypsin inhibitor (Sigma, USA). The slurry was incubated in a shaking water bath at 37 °C until the tissue fragments started to break up (20–30 min). The digestion medium was then diluted with medium M 199, the tissue pieces greatly agitated, the slurry filtered through 100 μm Nytex mesh, and resuspended in medium M 199 (Sigma). The cell suspension was stored at 4 °C, and cells were used within 8 h of isolation.

Solutions and reagents

The normal bath solution used was (mM): 125 NaCl, 5 KCl, 1 $MgSO_4$, 10 Hepes, 1·8 $CaCl_2$, pH 7·4 (NaOH). The pipette solution contained (mM): 80 potassium aspartate, 50 KCl, 5 $MgCl_2$, 3 EGTA, 1 $CaCl_2$, 10 Hepes, pH 7·3 (KOH). All external and internal solutions were filtered (0·2 μm Acrodisc, Gelman) before use. Nystatin (Sigma) was dissolved in DMSO (50 mg/ml), frozen in aliquots, and diluted to a final concentration of 300 or 200 units/ml in the pipette solution just prior to use. Charybdotoxin was obtained from Peptides International (KY, USA), or was a kind gift from Dr Greg Kaczorowski, Merck Research Laboratories (NJ, USA). Application of substances was done by bath addition of pH adjusted, concentrated solutions. All other substances were purchased from Sigma (USA).

Recording and analysis

Patch-clamp recordings were made using the nystatin perforated-patch method (Horn & Marty, 1988). Patch pipettes with resistances of 2·5–4·0 MΩ were pulled from borosilicate capillary glass (TW 150F-4, WPI). Voltage- and current-clamp protocols were generated, and currents amplified, filtered (3 kHz, −3 dB), digitized, and stored on an EPC-9 system (Heka Instruments, Germany). Junction potentials between pipette and bath were compensated before sealing. Membrane capacitance and series resistance were continuously monitored and compensated, using the internal circuit of the EPC-9 amplifier. Recording of the membrane potential and voltage-clamp experiments were only started with a series resistance below 40 MΩ (usually 10–15 min after seal formation). Experiments in which a sudden drop in series resistance occurred

during or after seal formation were excluded from this study. Leak subtraction was performed by subtraction of an appropriately scaled, ensemble-averaged current evoked by a 10 mV hyperpolarizing voltage step from −70 or −80 mV, after determining that no conductances were activated in this voltage range. Activation kinetics were determined by a least squares fit (Sigmaplot, Jandel Scientific) of the data to a Hodgkin and Huxley activation model (Hodgkin & Huxley, 1952) of the form:

$$I = I_{max}(1-e^{-t/\tau})^4,$$

where I_{max} is the maximum current, and τ is the activation time constant. Temperature was altered in the recording chamber using a peltier heating device controlled by a feedback power supply (Johnson Biomedical Instrument Foundation, University of Pennsylvania).

Tension recording

Ferret tracheae were dissected in oxygenated, Hepes-buffered balanced salt solution containing (mM): 137·5 NaCl, 5 KCl, 1 $MgCl_2$, 1·5 $CaCl_2$, 10 glucose, and 5 Hepes (pH 7·4). The tracheae were opened longitudinally and the mucosa was removed by fine dissection. Muscle strips (approximately 1 × 5 mm) were then transferred to oxygenated 10 ml muscle baths filled with the same solution. Strips were attached to force transducers (50-7905, Harvard Apparatus, USA) and the output recorded on a strip chart recorder (Gould, USA). The strips were equilibrated for 45 min and the length for maximal active force development (L_{max}) determined by increasing the length of the muscle strip by 1 mm increments until the maximal active contractile response to 0·1 μM methacholine was achieved. At the completion of each experiment, the length and weight of each muscle strip were determined and used to calculate the cross-sectional area of the muscle strips, assuming a tissue density of 1·05 g/cm^3 (Herlihy & Murphy, 1973). Isometric forces were normalized for cross-sectional area and reported in units of stress (× 10^4 N/m^2).

RESULTS

Permeabilized-patch outward currents

In order to examine outward currents under physiological conditions of cytosolic calcium buffering and temperature, voltage-dependent potassium currents were studied at near physiological temperatures (33–36 °C) with the nystatin perforated-patch technique. In forty-five experiments in which access resistances below 40 mΩ were achieved, outward currents with time-dependent activation and inactivation similar to previously reported delayed rectifier currents (Kotlikoff, 1990; Boyle *et al.* 1992) were observed. Figure 1*A* shows the time dependence and current–voltage relationships for outward currents evoked by voltage-clamp steps in a typical cell. Identical voltage-clamp protocols are shown for the same cell recorded at 22 and 35 °C to illustrate the effect of temperature on outward currents. Steps of varying duration demonstrate the time-dependent development of the current, which activated with some delay, and the slower inactivation of the current to a steady-state value, similar to previous reports of delayed rectifier currents in smooth muscle (Okabe, Kitamura & Kuriyama, 1987; Beech & Bolton, 1989; Kotlikoff, 1990; Boyle *et al.* 1992). Comparison of the currents at different temperatures indicates that at near physiological temperatures peak outward currents were of greater magnitude, and currents activated and inactivated with markedly faster kinetics. Moreover, in the physiological range of resting potential (−40 to −50 mV), more outward current is available, as demonstrated by the appearance of an appreciable macroscopic outward current at the lowest voltage clamp step (−40 mV) only at 35 °C. These temperature-related alterations in current were

observed at equivalent access resistances (R_S). The current–voltage relationships of the net outward currents in each condition demonstrate the increase in peak current and the apparent left shift in the current–voltage relationship at higher temperature (Fig. 1*B*). In order to examine quantitatively the effect of temperature

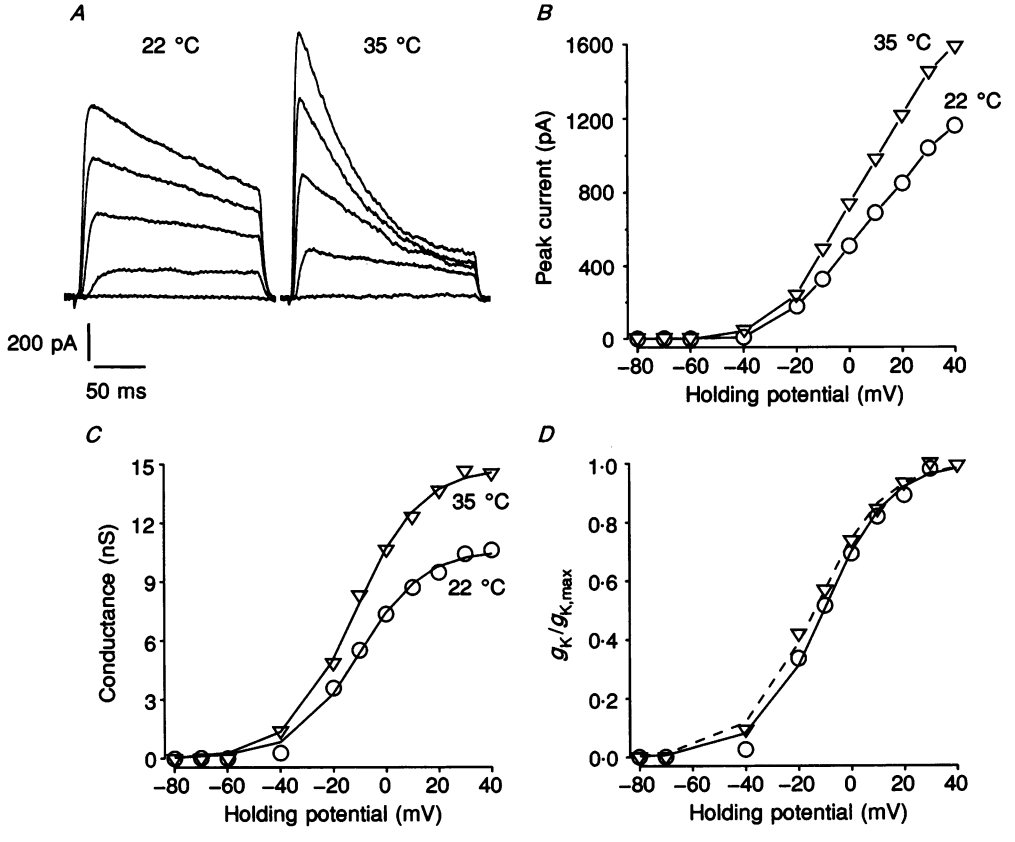

Fig. 1. Whole-cell potassium currents from ferret tracheal myocytes recorded using the nystatin technique. *A*, evoked currents following voltage-clamp steps from a holding potential of −80 mV to from −40 to 40 mV in 20 mV increments. Left, currents recorded at room temperature (22 °C); right, currents recorded at 35 °C. A prominent effect on activation and inactivation kinetics, and an increase in the current available at −40 mV can be seen. *B*, plot of peak current *versus* voltage. *C*, potassium conductance *versus* voltage. Conductance was calculated using tail current reversal potential measurements from five experiments ($E_R = -69{\cdot}3$ mV). *D*, fractional conductance *versus* voltage, demonstrating little shift in voltage dependence of conductance. Lines represent Boltzmann equation fits to the data (dashed line, 35 °C). Parameters for 22 and 35 °C were −10·2 and 14·0 mV for V_{50}, and 12·4 and 13·1 for k, respectively.

on current amplitude, the fractional conductance of the current was plotted using measurements of the current reversal potential from tail current experiments ($n = 5$). As shown in Fig. 1*C*, the major effect of temperature on current magnitude was an increase in the maximum conductance; in the experiment shown, peak

conductance increased by 39·6 %, whereas there was only a slight leftward shift (−3·8 mV) in the relationship between fractional conductance and membrane potential (Fig. 1*D*). In a series of seven similar experiments, maximal K^+ conductance ($g_{K,max}$) measured in the same cells increased 23·3 ± 4·93 % when

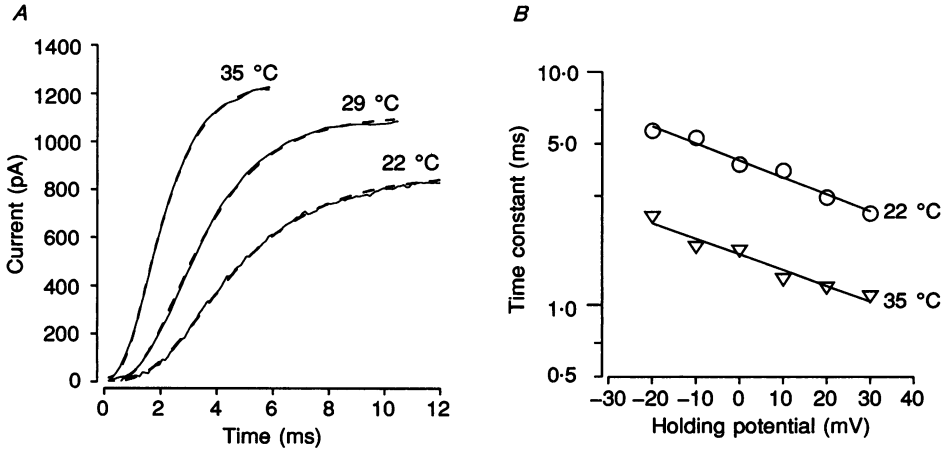

Fig. 2. Activation kinetics of potassium currents are temperature dependent. *A*, current traces evoked by a voltage-clamp step to 30 mV from −80 mV at different bath temperatures. Current activation was time dependent, with a substantial delay in current activation at the lower temperatures. Dashed lines show the fit of n^4 activation kinetics to the current data; time constants (τ) for the fits to 22, 29, and 35 °C lines shown are 2·46, 1·83, and 1·10 ms respectively. *B*, semilog plot of τ *versus* voltage for steps to varying potentials; for clarity, only experiments at the temperature extremes are plotted. The lines shown represent a linear regression of the data, predicting an e-fold change in τ for every 59 mV (22 °C) and 65 mV (35 °C).

temperature was increased from room temperature to 33–36 °C. This effect of temperature on maximal K^+ conductance corresponded to a Q_{10} (temperature coefficient) of 1·14, which is close to the increase in the conductance of K^+ ions in water between 0 and 25 °C (Hille, 1992). The increase in maximal conductance, where the number of open channels should be roughly equivalent at both temperatures, is thus consistent with a major effect determined by the increase in ion diffusivity. Since maximal conductance was determined from the peak current, the relative kinetics of current activation and inactivation, which affect the peak current value and are markedly temperature dependent (Fig. 1*A* and below), could also influence the increase in $g_{K,max}$ observed at 37 °C.

The effect of temperature on current activation and inactivation kinetics was also quantitatively evaluated. As shown in Fig. 2*A*, the rate of current activation was markedly increased at higher temperatures. Previous studies have reported that activation kinetics of delayed rectifier currents can be fitted by n^4 or n^2 processes (Beech & Bolton, 1989; Kotlikoff, 1990), and that this may relate to some contamination of the outward current by a calcium-activated potassium current (Beech & Bolton, 1989). In the present study, current activation was well fitted by n^4 kinetics, and the fit was improved at higher temperatures. These data are consistent with previous findings that kinetics of potassium currents are qualitatively altered

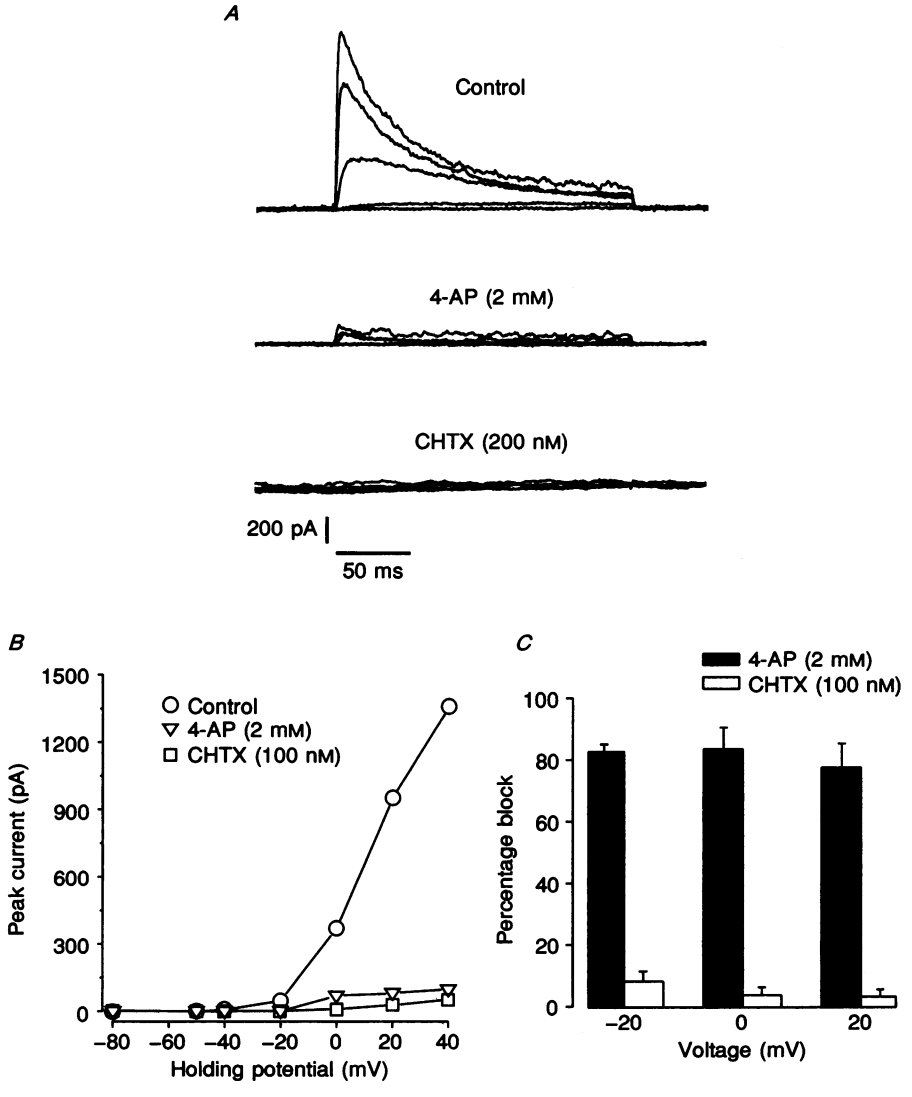

Fig. 3. Voltage-dependent potassium currents recorded under physiological conditions are predominantly delayed rectifier currents. *A*, evoked currents from a cell recorded at 35 °C at voltage-clamp steps from −40 to 40 mV in 20 mV increments, from −70 mV. Top, control recording; middle, following application of 2 mM 4-AP; bottom, following application of 200 nM charybdotoxin. *B*, peak current *versus* voltage plotted for the experiment shown in *A*. *C*, average percentage block of outward current from experiments in which only 2 mM 4-AP ($n = 6$), or 200 nM charybdotoxin ($n = 10$) was applied after control recordings. Data show the peak current as a percentage of control after full development of the block.

at lower temperatures, such that at lower temperatures the kinetics are less well described by a fourth-order process (Beam & Donaldson, 1983). The time constant of current activation, τ, was markedly temperature dependent. Figure 2*B* shows the effect of temperature and voltage on current activation kinetics for the

experiment shown in Fig. 2*A*. As can be seen from the similarities between the slopes of the logarithm of the rate constants at different temperatures, increasing the temperature from 22 to 35 °C did not alter the relationship between voltage and the rate constant. In the experiment shown τ changed e-fold every 59 mV at 22 °C, and every 65 mV at 35 °C. These values are quite similar to the previously reported value of an e-fold change every 56 mV for the delayed rectifier current in portal vein cells at room temperature (Beech & Bolton, 1989). In four experiments, τ measured at 30 mV clamp steps decreased from $1{\cdot}96 \pm 0{\cdot}27$ to $1{\cdot}09 \pm 0{\cdot}17$ between room temperature and the higher temperature measurement (34–36 °C), or a Q_{10} of approximately 1·6. A prominent effect of temperature on current inactivation was also observed. As previously reported, delayed rectifier currents inactivate with complex kinetics generally well fitted by a bi-exponential decay process to a non-zero current (Beech & Bolton, 1989; Kotlikoff, 1990). At higher temperatures currents inactivated much more rapidly (Fig. 1*A*), although inactivation was not complete, and a sustained, steady-state current was observed.

Pharmacological identification of outward currents

Outward currents activated and inactivated with kinetics quite similar to those reported for delayed rectifier currents in airway smooth muscle (Kotlikoff, 1990; Boyle *et al.* 1992), suggesting that under conditions of physiological calcium buffering, little of the voltage-dependent outward current was due to activation of calcium-activated potassium channels. 4-Aminopyridines, agents that selectively block delayed rectifier channels (Okabe *et al.* 1987; Beech & Bolton, 1989; Boyle *et al.* 1992), and charybdotoxin, a selective antagonist of the large-conductance, calcium-activated potassium channel (Boyle *et al.* 1992), were used to identify the outward currents. Figure 3*A* shows a typical experiment, in which application of 4-aminopyridine (2 mM) blocked over 90 % of the outward current. A small remaining noisy current, observed mainly at holding potentials positive to 0 mV, was blocked by 100 nM CHTX. The current–voltage relationships for this experiment are shown in Fig. 3*B*. The degree of blockade of outward current by 4-AP and CHTX was not dependent on the sequence of addition. 3,4-DAP showed similar blocking potency to 4-AP. Application of apamin (10–20 nM, $n = 3$) or dendrotoxin (10–50 nM, $n = 6$) had no effect on outward currents. Quantitative assessments of the proportion of 4-AP and CHTX-sensitive current were made in cells at potentials above −20 mV, where enough current was evoked to provide reliable estimates. As shown in Fig. 3*C*, in sixteen experiments in which block by a single agent was assessed at voltage-clamp steps between −20 and 20 mV, over 80 % of the current was blocked by 2 mM 4-AP, whereas the CHTX-sensitive (200 nM) current component was quite small. These results indicate that the outward current evoked by step depolarization is predominately due to the activation of delayed rectifier channels, and that K^+_{Ca} channels have a low open probability in resting cells recorded under physiological conditions.

Blockade of delayed rectifier potassium channels depolarizes airway myocytes

In order to obtain more information about the physiological importance of the outward current recorded under physiological conditions, especially the role of these currents in determining resting membrane potential, we performed current-

clamp experiments. In these experiments, the resting membrane potential was measured after full access was achieved, and hyperpolarizing current injections were imposed in order to assess membrane conductance before and after addition of specific potassium channel antagonists. In sixty-two cells, the mean value for

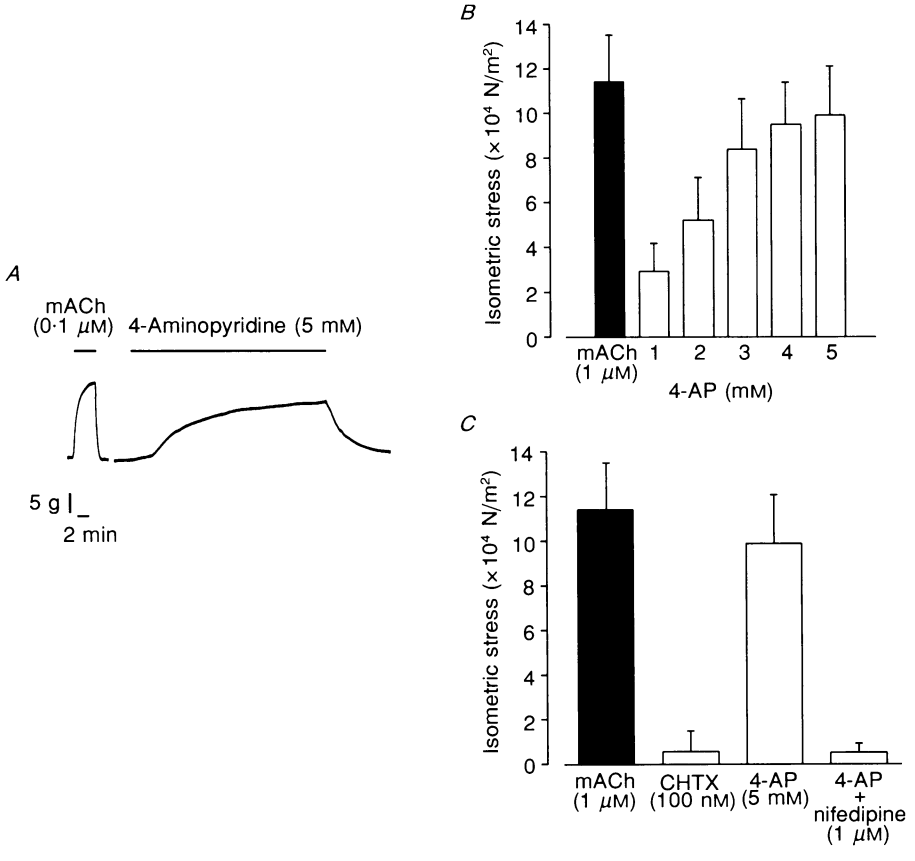

Fig. 4. 4-Aminopyridine but not charybdotoxin augments resting tension in ferret tracheal strips. *A*, a typical experiment showing the time course and extent of tension development relative to a previous contraction to 0·1 μM methacholine (mACh). *B*, dose dependence of maximum force production following application of 4-AP. Data shown are means and standard errors of the mean from thirteen strips. The calculated EC_{50} from the data shown was 1·8 mM. *C*, mean data from all experiments demonstrate that 4-AP (5 mM) markedly increases tension in resting muscle strips, whereas charybdotoxin (100 nM) had little effect. Experiments using higher doses of charybdotoxin did not result in greater force production (data not shown). Tension induced by 4-AP was almost completely blocked by 1 μM nifedipine.

resting potential was $-33{\cdot}6 \pm 1{\cdot}0$ mV. These values are somewhat lower than measurements in tissue strips (Kirkpatrick, 1975; Kroeger & Stephens, 1975), and may reflect alterations in potential associated with exchange of monovalent ions between the patch pipette and cytosol, pH shifts due to the lack of physiological buffering conditions, or alterations in resting conductance associated with cell disaggregation. Application of 2 mM 4-AP depolarized cells by $14{\cdot}4 \pm 1{\cdot}0$ mV (8

cells), whereas CHTX (200 nM, 6 cells) did not produce a measurable depolarization. In four of the eight experiments in which 4-AP depolarized current-clamped airway myocytes, prior application of 200 nM CHTX was without effect, These data indicate that K^+_{DR} channels (but not K^+_{Ca} channels) are open at resting membrane potentials and contribute to the transmembrane potential.

Blockade of delayed rectifier potassium channels augments resting tension

Based on the voltage-clamp and current-clamp data obtained in single cells, we reasoned that selective inhibition of potassium conductances should have predictable effects on resting tone in airway smooth muscle cells. To test these predictions, force measurements were made in ferret tracheal smooth muscle strips, under conditions of selective channel blockade. Isolated muscle strips were pretreated with tetrodotoxin (1 μM) and atropine (1 μM), and exposed either to 4-aminopyridine (1–5 mM) or CHTX (100 nM). Blockade of K^+_{DR} channels with 4-AP led to tonic force development (Fig. 4 *A*), whereas the application of CHTX had relatively little or no effect on tension. The effect of 4-AP was dose dependent, with an EC_{50} of 1·86 mM (Fig. 4 *B*). In thirteen strips in which roughly maximally effective concentrations of these agents were applied, 4-AP (5 mM) application resulted in tension generation that was 87 % of the response to 1 μM methacholine, whereas exposure to CHTX (100 nM) resulted in only 4·9 % force development (Fig. 4 *C*). The increase in tension associated with application of 4-AP was almost completely abolished by the application of 1 μM nifedipine. These data are consistent with the effect of selective potassium channel blockade on cell membrane potential.

DISCUSSION

Using patch-clamp techniques, several types of potassium channels have been identified in smooth muscle cells. Recordings from on-cell and excised patches initially identified a prominent, large-conductance, calcium-activated potassium channel (Walsh & Singer, 1983; Inoue, Kitamura & Kuriyama, 1985; Benham, Bolton, Lang & Takewaki, 1986; McCann & Welsh, 1986). In general, the open-state probability of these channels was found to be quite low at physiological membrane potentials and cytosolic Ca^{2+}, raising a question as to their physiological role. In whole-cell studies of dissociated airway myocytes, a prominent calcium-sensitive outward current has been described by several groups (Muraki *et al.* 1990; Kotlikoff, 1990; Green *et al.* 1991; Saunders & Farley, 1992), but the magnitude varied depending on the degree of cytosolic calcium buffering employed. Experiments using internal solutions with high calcium buffering capacity have demonstrated low-noise, delayed rectifier currents, which are insensitive to cytosolic calcium, relatively insensitive to TEA, but blocked by 4-aminopyridines (Beech & Bolton, 1989; Kotlikoff, 1990; Boyle *et al.* 1992). Single-channel measurements of low-conductance (5–8 pS, Beech & Bolton, 1989, or 13 pS, Boyle *et al.* 1992) delayed rectifier potassium channels with equivalent time-dependent activation and voltage- and time-dependent inactivation have been described. The present study was undertaken to determine the degree of K^+_{Ca} and K^+_{DR} channel activity in airway smooth muscle cells under physiological calcium buffering conditions, and to examine the degree to which these channels underlie resting potassium conductance.

The predominant outward current recorded in non-dialysed, ferret tracheal myocytes was a low-noise, aminopyridine-sensitive delayed rectifier current. A small charybdotoxin-sensitive current component was observed at test potentials above −20 mV, but this accounted for less than 10 % of the outward current over the voltage range from −20 to 40 mV. The threshold of activation of the delayed rectifier current was between −50 and −40 mV in forty-five cells measured at 35–37 °C, indicating that channel opening is likely in the physiological range of membrane potentials. This voltage threshold is more negative than previously reported (Kotlikoff, 1990), which probably reflects the more physiological conditions of the non-dialysed, whole-cell preparation, as well as the effect of temperature on the current. The peak current amplitude, as well as current activation and inactivation kinetics, were markedly influenced by temperature. The magnitude of the peak current was increased at higher temperatures in a manner consistent with an increase in single channel conductance, as well as a slight leftward shift in the voltage dependence of channel activation. The increase in current at physiological potentials appears to be due primarily to the effect of temperature on $g_{k,\max}$, as opposed to a prominent effect on channel gating (Fig. 1*C* and *D*). Thus at higher temperatures the relationship between fractional conductance and voltage was only slightly shifted to the left (less than 4 mV) relative to that observed at room temperature. The increase in outward current at very negative potentials may also result from an increase in activation kinetics in this range in which the current activates quite slowly (Fig. 1*A*). Kinetics of activation and inactivation were much more rapid at higher temperatures. Since most airway smooth muscle cells do not produce spike potentials, however, the steady-state current may be most relevant to overall tissue electrical behaviour.

Whereas previous studies have shown that the delayed rectifier current in smooth muscle inactivates over a time course of several seconds (Beech & Bolton, 1989; Kotlikoff, 1990; Boyle *et al.* 1992), the current study demonstrates much more rapid inactivation (as well as activation) under more physiological recording conditions. While we have continued to describe this current as a delayed rectifier current, it should be noted that the current inactivation kinetics bear substantial similarities to A-type potassium currents. The voltage dependence of activation and inactivation and the availability of the current at the resting potential, however, more closely resemble classical delayed rectifier currents (Rudy, 1988). Also, the current does not completely inactivate (Figs 1 and 3), and the likely functional role of this conductance in tonic responses of smooth muscle (see below) appears to be quite unlike that of A-type channels. The expression of mammalian genes homologous to the *Shaker* gene family has revealed a substantial structural similarity between A-type and delayed rectifier potassium channels (Jan & Jan, 1992) and a more precise characterization of the current will probably require the molecular characterization of these channels in smooth muscle.

The finding that the principal outward current in non-dialysed cells was a 4-aminopyridine-sensitive, delayed rectifier current was supported by current-clamp data. Application of 4-AP, but not CHTX resulted in a measurable cellular depolarization, as well as a decrease in membrane conductance. These data strongly suggest that the resting membrane potential is influenced by delayed rectifier, but not CHTX-sensitive K^+_{Ca} channels. Consistent with these findings in isolated cells,

exposure of muscle strips to 4-AP, but not CHTX resulted in a marked increase in tension. The EC_{50} of 4-AP for the development of tension in muscle strips (1·86 mM), was quite close to the measured IC_{50} of this compound for the delayed rectifier potassium current in pulmonary artery smooth muscle cells (approximately 0·3 mM; Okabe *et al.* 1987), and the maximum effect of 4-AP (approximately 5 mM; Fig. 4*A*) was roughly equivalent to the drug concentrations required to maximally inhibit the current (Okabe *et al.* 1987; Beech & Bolton, 1989; Boyle *et al.* 1992). By contrast, experiments selectively antagonizing the large-conductance, K^+_{Ca} channel with CHTX resulted in minimal inhibition of outward currents, no measurable depolarization or alteration in membrane conductance at resting membrane potentials, and very slight increases in tension in muscle strips. These data indicate that under conditions of physiological cytosolic calcium buffering and resting membrane potential, the open-state probability of K^+_{Ca} channels is quite low. It should be noted, however, that smooth muscle tissues may differ substantially in terms of the degree to which individual potassium channels regulate resting membrane potential and tonic force generation. In some, but not all, spontaneously active smooth muscle tissues, selective inhibition of large-conductance K^+_{Ca} channels with charybdotoxin and iberiotoxin produces a concentration-dependent increase in contractility (Suarez-Kurtz, Garcia & Kaczorowski, 1991), suggesting that in some smooth muscle tissues, K^+_{Ca} channels contribute appreciably to resting membrane conductance. These differences appear to correlate with the spiking activity of different smooth muscles and may reflect the absence of K^+_{DR} channels in some smooth muscle tissues (Suarez-Kurtz *et al.* 1991). Differential K^+ channel expression may also underlie the behaviour of guinea-pig trachealis, in which application of CHTX converts slow waves to spontaneous action potentials (Murray, Berry, Cook, Foster, Green & Small, 1991), although it does not result in force generation (Suarez-Kurtz *et al.* 1991). Our findings are also consistent with previous reports that concentrations of TEA well in excess of that needed to completely inhibit large-conductance K^+_{Ca} channels (Langton *et al.* 1991), but in the range in which partial block of K^+_{DR} is observed (Okabe *et al.* 1987; Kotlikoff, 1990), are required to abolish outward rectification or initiate phasic electrical activity (Kroeger & Stephens, 1975; Kirkpatrick, 1975; Imaizumi & Watanabe, 1981; Janssen & Daniel, 1991). We cannot exclude the participation of other conductances (e.g. ATP-sensitive potassium channels) in the determination of membrane potential. In summary, our results are consistent with the hypothesis that K^+_{DR} channels confer the properties of outward rectification, electrical stability, and graded electrical responses to agonist that is characteristic of airway smooth cells (Kirkpatrick, 1975; Kroeger & Stephens, 1975; Coburn, 1979).

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