Journal of Physiology (1990) **424**, *pp.* 445–457
With 5 figures
Printed in Great Britain

EFFECTS OF INTRACELLULAR pH ON CALCIUM-ACTIVATED POTASSIUM CHANNELS IN RABBIT TRACHEAL SMOOTH MUSCLE

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(*Received 16 February 1989*)

SUMMARY

1. The effects of intracellular pH (pH_i) on calcium-activated potassium channels (Ca^{2+}-activated K^+ channels) were studied in membrane patches of smooth muscle freshly dispersed from the rabbit trachea. Single-channel currents were recorded with an 'inside-out' patch clamp technique, mainly at 0 mV, with the external (electrode) medium containing 130 mM-K^+ and the internal (bath) medium 6 mM-K^+.

2. With an internal Ca^{2+} concentration ($[Ca^{2+}]_i$) of 1 μM, the fraction of time during which the channel was in an open state (the open probability, P_0) was more than 0·8 at pH_i 7·4. The channel activity nearly disappeared at pH_i 7·0. The $[Ca^{2+}]_i$–P_0 relationship was shifted to higher $[Ca^{2+}]_i$ by acidosis, the shift being approximately an 8-fold increase for a fall in pH_i of 0·5 units.

3. The membrane potential and current intensity (*V–I*) relationship of single channels between +30 and −50 mV was shifted in a hyperpolarizing direction by intracellular acidosis. The shift was roughly 10 mV for 1 pH unit at 1 μM $[Ca^{2+}]_i$. At pH_i 7·4, $[Ca^{2+}]_i$ 1 μM, the V–P_0 relationship was shifted in a depolarizing direction by acidification. When $[Ca^{2+}]_i$ was increased to 10 μM, V–P_0 relationship became less sensitive to V as well as pH_i changes.

4. When P_0 was high, the probability density function of open and closed time distributions could be fitted by two exponentials. When P_0 was decreased to less than 0·3, either by reducing $[Ca^{2+}]_i$ or by lowering pH_i, another component having long closed times appeared. At similar P_0 values, the time constant of open time distribution was smaller with lower pH_i.

5. It is concluded that the main effect of an increase in intracellular hydrogen ions is to decrease the open probability of the Ca^{2+}-activated K^+ channel, by reducing the sensitivity to Ca^{2+} and also shortening the open state.

INTRODUCTION

The presence of potassium (K^+) channels which are activated by intracellular calcium (Ca^{2+}-activated K^+ channels) has been reported for many different tissues using the patch clamp technique (Marty, 1981; Barrett, Magleby & Pallotta, 1982; Wong, Lecar & Adler, 1982; Maruyama, Petersen, Flanagan & Petersen, 1983), including smooth muscles (Benham, Bolton, Lang & Takewaki, 1986; Inoue, Okabe, Kitamura & Kuriyama, 1986; McCann & Welsh, 1986). These channels have a large conductance (100–270 pS), but are very selective to K^+. Since activation of this channel would cause membrane hyperpolarization, the channel may play an important role in controlling electrical excitability.

Cytoplasmic pH (pH_i) is known to influence ionic currents of the plasma membrane in various cells. Intracellular acidification delays inactivation of the Na^+ current in frog skeletal muscle (Nonner, Spalding & Hille, 1980). In cardiac muscle, Ca^{2+} currents are inhibited by lowering pH_i (Vogel & Sperelakis, 1977; Irisawa & Sato, 1986; Kaibara & Kameyama, 1988). Injection of acidic solution shortens the action potential and depresses the plateau by decreasing the slow inward current and decreasing the outward current in guinea-pig ventricle (Kurachi, 1982). The delayed rectifying K^+ current in the squid axon (Wanke, Carbone & Testa, 1979), the crayfish slow muscle fibre (Moody, 1980) and also the inward rectifying K^+ current in the oocyte of the starfish (Moody & Hagiwara, 1982) are blocked by reducing pH_i. K^+ currents in human lymphocytes have been shown to be increased by intracellular alkalinization (Deutsch & Lee, 1989). In the rat carotid body cell, it has been shown with the whole-cell clamp method that Ca^{2+}-sensitive K^+ currents are inhibited by lowered extracellular pH (Peers, 1989). This may be due to block of the Ca^+-activated K^+ channel by intracellular acidification, because this has been demonstrated in rat pancreatic B-cells (Cook, Ikeuchi & Fujimoto, 1984), epithelium of choroid plexus from the lateral ventricle of *Necturus maculosus* and of *Rana esculenta* (Christensen & Zeuthen, 1987), and human red blood cells (Stampe & Vestergaard-Bogind, 1985) with the patch clamp method. In the present experiments, we have further investigated effects of pH_i on the Ca^{2+}-activated K^+ channel in the rabbit tracheal smooth muscle. The tone of airway smooth muscle is known to decrease when pH_i is made alkaline (Wray, 1988). This effect may be partly exerted through the modification of Ca^{2+}-activated K^+ channel activity.

METHODS

Rabbits (2–3 kg) of either sex were used. After anaesthetizing the rabbit with sodium pentobarbitone (50 mg/kg), the trachea was removed and the animal was killed by bleeding. The smooth muscle was then dissected out from a posterior part of the trachea. Single smooth muscle cells were obtained by enzymatic dissociation, using a technique similar to the method described by Madison, Tom-Moy & Brown (1984). The solution for cell dispersion contained collagenase (350 U/ml), elastase (5 U/ml), trypsin inhibitor (2 mg/ml), bovine albumin (50 mg/ml), and no Ca^{2+}. All organic compounds used were obtained from Sigma. The muscle, minced into small pieces, was incubated in this solution with mechanical agitation for 30 min at 35 °C. The cell suspension was then filtered with gauze and centrifuged for 1 min at 1000 r.p.m.

Patch pipettes were filled with solution containing (mM): KCl, 130; $CaCl_2$, 1; HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulphonic acid, pH adjusted with NaOH to 7·4), 10. The bath solution facing the cytoplasmic surface of the plasma membrane contained: KCl, 6; NaCl, 118; HEPES, 10; glucose, 11·8; and the required concentration of Ca^{2+} ($[Ca^{2+}]_i$). $[Ca^{2+}]_i$ was adjusted by adding the correct amount of Ca^{2+} in the presence of 5 mM-EGTA (ethyleneglycol-bis-(β-aminoethyl-ether)*N,N'*-tetraacetic acid), changes being 0·2 pCa ($-\log [Ca^{2+}]$) steps, according to the calculation by Fabiato (1981). The pH of the bath solution was adjusted with NaOH. We prefered a reversed K^+ concentration gradient (130 mM outside and 6 mM inside), because with the normal K^+ gradient the voltage–current relationship of single channels was found to be more complex, and obtaining a membrane patch with a single channel was difficult in preliminary experiments. At this concentration gradient (130/6, mM), inward currents of a reasonable amplitude (about 4 pA) could be observed during channel opening without shifting the membrane potential from 0 mV.

The method of recording single-channel currents was similar to that described by Hamill, Marty, Neher, Sakmann & Sigworth (1981). The pipettes had a resistance of 15–25 MΩ when filled with the pipette solution. After a proper electrode seal was obtained by applying weak negative pressure to the inside of the pipette, a patch of membrane was excised from the cell. All experiments were carried out at room temperature (22–24 °C).

Channel currents were recorded with an amplifier (Axopatch 1-B). Data were stored on a video-cassette using a PCM converter system (Sony 501ES) after passing a low-pass filter having a characteristic frequency of 1 kHz (2 kHz in the later experiments) and analysed as described by Colquhoun & Sigworth (1983), with a computor using the pClamp program (version 5·03, Axon Instruments, Inc.). The digitizing rate for computor analysis was 10 kHz. Channel opening and closing was determined by setting the threshold at the half-value of current amplitude and more than 300 events were accumulated for analyses.

RESULTS

Effects of intracellular pH on channel activated with Ca^{2+}

When the external K^+ concentration was 130 mM, the internal solution contained 6 mM-K^+ and 1 μM-Ca^{2+}, and the membrane potential was held at 0 mV, inward currents of about 4 pA could be recorded from a membrane patch of rabbit tracheal smooth muscle (Fig. 1*A*), as previously reported for other smooth muscles (Benham *et al.* 1986; McCann & Welsh, 1986). This current was considered to reflect the activity of Ca^{2+}-activated K^+ channels, based on its Ca^{2+} sensitivity and conductance, as will be described. In most of patches (86/94), current records indicated the presence of more than one channel, but we selected patches which contained only a single channel for the quantitative analyses. Existence of a single channel was judged from current recordings when the channel was fully activated by the internal Ca^{2+}.

Figure 1*A* shows effects of intracellular pH (pH_i) on single-channel currents. At pH_i 7·4, the channel was mostly in an open state at 1 μM $[Ca^{2+}]_i$ and the fraction of the time during which the channel was open, the open probability (P_o), was 0·88. When the pH_i was raised to 7·8, the P_o was only slightly increased to 0·95, but when the pH_i was lowered to 7·0, the channel activity stopped nearly completely. In Fig. 1*B*, the P_o was plotted against pH_i. In the presence of 1 μM $[Ca^{2+}]_i$ P_o decreased to $0·007 \pm 0·005$ at pH_i 7·2 (the mean ± S.D., $n = 3$, Table 1). However, when the $[Ca^{2+}]_i$ was increased to 10 μM, the channel activity became less sensitive to pH_i, so that stronger acidification (pH_i 6·6 or less) was necessary to reduce P_o significantly.

The P_o was strongly affected by the $[Ca^{2+}]_i$, as is well known for Ca^{2+}-activated K^+ channels. The effect of pH_i on the relationship between P_o and $[Ca^{2+}]_i$ is shown in Fig. 2. The experiments were carried out under the same conditions as used for Fig. 1. At pH_i 7·4, the channel started to open at 0·25–0·4 μM $[Ca^{2+}]_i$ and became open most of the time when $[Ca^{2+}]_i$ was only slightly increased to 0·6–1·0 μM. When the pH_i was increased to 7·8 or 8·0, the $[Ca^{2+}]_i$–P_o curve was clearly shifted toward lower $[Ca^{2+}]_i$ values and even at 0·2 μM the channel was mostly in a fully open state. On the other hand, when the pH_i was lowered to less than 7·0 the sensitivity of the channel to Ca^{2+} decreased, so that clear channel activity was only observed at higher than 2 μM $[Ca^{2+}]_i$. At pH_i 6·6, $[Ca^{2+}]_i$ of higher than 10 μM was necessary to obtain the

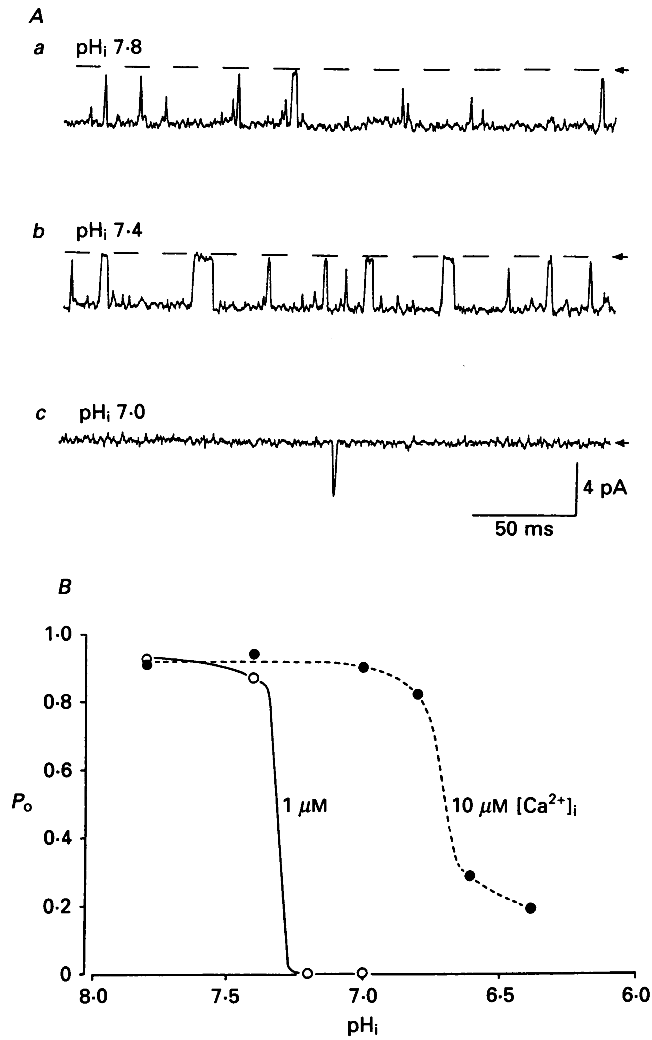

Fig. 1. *A*: effects of internal pH on currents recorded from a single Ca^{2+}-activated K^+ channel in an inside-out patch of rabbit tracheal smooth muscle cell. K^+ concentrations were 130 mM in the electrode and 6 mM in the bath, and the membrane potential was held at 0 mV. The pH of bathing solution was changed from 7·4 (*b*) to 7·8 (*a*) and then to 7·0 (*c*), each for 3 min. Current levels indicated by arrows correspond to the closed state of the channel. *B*: relationship between the open probability (P_o) and internal pH (pH_i) at 1 and 10 μM $[Ca^{2+}]_i$. Average of three channels (included in Table 1) for 1 μM and of two for 10 μM $[Ca^{2+}]_i$.

maximum P_o. The slope of the increase in P_o with increasing $[Ca^{2+}]_i$ became less steep as the pH_i was lowered, particularly at lower than pH 7·0.

In Fig. 2*B*, a relationship between pH_i and logarithmic Ca^{2+} concentrations necessary for 50% P_o was plotted based on the results shown in Fig. 2*A*. This was linear: there was an 8-fold increase of $[Ca^{2+}]_i$ for a fall in pH_i of 0·5 units.

The sensitivity of the channel to Ca^{2+} differed in different channels to some extent, but the effect of pH_i on the shift of $[Ca^{2+}]_i$–P_o relationship was essentially the same in six channels examined. The $[Ca^{2+}]_i$–P_o relationship was also studied using patch electrodes filled with solution of pH 6·8 or 8·0. However, when pH_i was kept constant

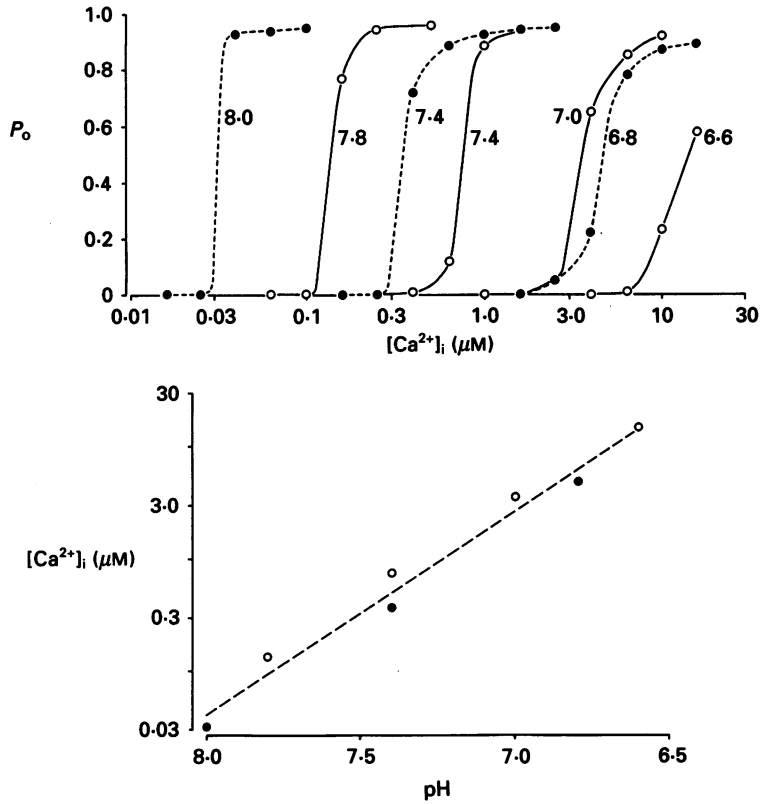

Fig. 2. *A*: effects of internal pH (indicated besides curves) on relationship between the open probability (P_o) and $[Ca^{2+}]_i$ in two different channels (●------●, data from five different channels accumulating data for 15 s; ○——○, data from the same channel, accumulating more than 300 events). The experimental conditions were the same as Fig. 1. The solution was changed first from 7·4 to alkaline and then to acidic solution, and at a constant pH from high to low Ca^{2+} solution. *B*: relationship between pH and $[Ca^{2+}]_i$ necessary for 50% activation of channel estimated from *A* (● and ○ are as in *A*).

at 7·4, no significant difference was found in the relationship with different external pH, indicating that the channel activity is modified by hydrogen ions (H^+) from the cytoplasmic side.

Effects of pH on voltage–current relationship of the single channel

When both sides of the membrane were exposed to 130 mM-K^+, the voltage–current (*V–I*) relationship was linear and from this relationship the conductance of the channel was calculated to be 184 ± 17 pS ($n = 4$), which was close to the values found in rabbit jejunum and guinea-pig mesenteric artery (Benham *et al.* 1986), but was smaller (about 70%) than those in canine tracheal muscle (McCann & Welsh, 1986) in similar symmetrical K^+ media. As shown in Fig. 3, under conditions where the outside solution contained 130 mM-K^+ and the inside 6 mM-K^+, the curve showed inward-going rectification, in accord with the constant field equation (Hodgkin & Katz, 1949), as found for other smooth muscles by Benham *et al.* (1986).

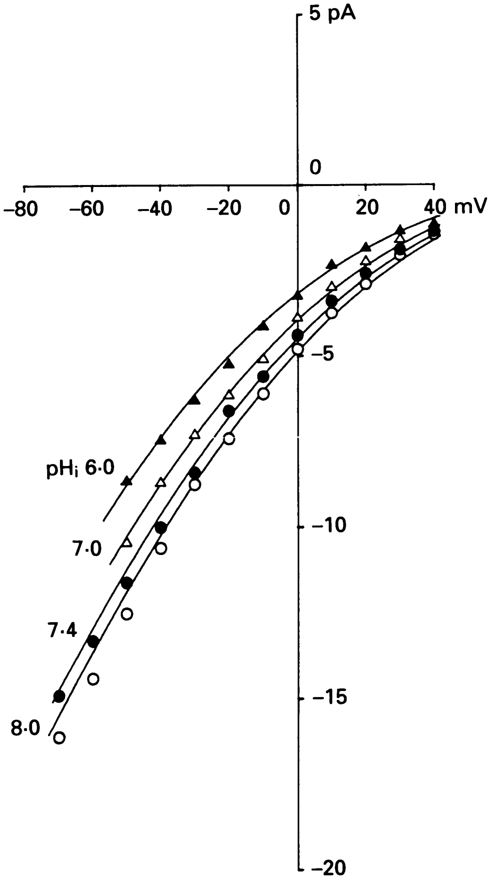

Fig. 3. Effects of internal pH (pH_i) on the voltage–current relationship of a single Ca^{2+}-activated K^+ channel in the presence of 1 μM $[Ca^{2+}]_i$. The same experimental conditions were used as in Fig. 1, except for changes in the membrane potential. Curves were drawn using a following equation (Adrian, 1969):

$$I_K = P_K \frac{F^2(V+\Psi)\,[[K^+]_i \exp(VF/RT) - [K^+]_o]}{RT\{\exp[(V+\Psi)F/RT] - 1\}}$$

where $[K^+]_o = 130$ mM, $[K^+]_i = 6$ mM, the permeability constant for K^+ (P_K) $= 4{\cdot}2 \times 10^{-13}$ cm^3/s, and the membrane surface charge (Ψ) was +5, +9, +15, and +25 mV for pH_i 8·0, 7·4, 7·0 and 6·0, respectively. Other symbols have their usual meanings.

In the membrane potential range studied (+40 to −70 mV) current intensity was reduced and the *V–I* curve was shifted in a hyperpolarizing direction by lowering pH_i. The shift of the curve was approximately 10 mV per 1 pH unit. Owing to the disappearance of channel activity in acidic solution during strong hyperpolarization of the membrane, *V–I* curves could not be obtained beyond −60 mV at pH_i 7·0 and 6·0.

The single-channel conductance calculated from the linear part of the curve was slightly reduced by lowering the pH_i. In the presence of 1 μM $[Ca^{2+}]_i$, the maximum

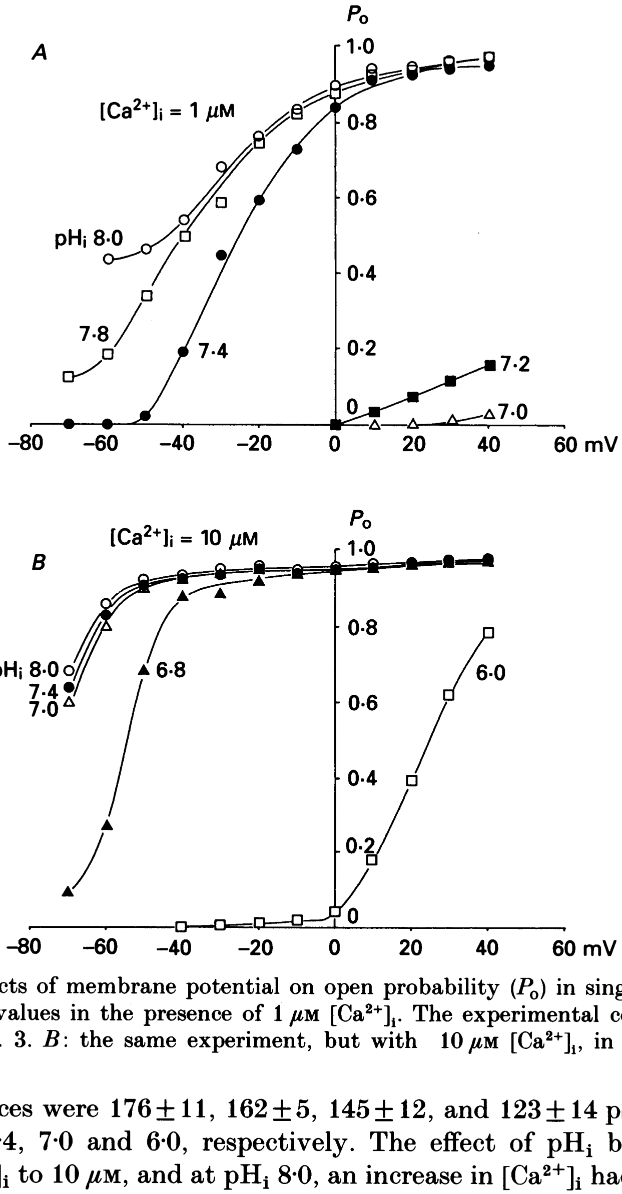

Fig. 4. *A*: effects of membrane potential on open probability (P_o) in single channels at different pH_i values in the presence of 1 μM $[Ca^{2+}]_i$. The experimental conditions were similar to Fig. 3. *B*: the same experiment, but with 10 μM $[Ca^{2+}]_i$, in another single channel.

slope conductances were 176 ± 11, 162 ± 5, 145 ± 12, and 123 ± 14 pS ($n = 3$) at pH_i values of 8·0, 7·4, 7·0 and 6·0, respectively. The effect of pH_i became less with increasing $[Ca^{2+}]_i$ to 10 μM, and at pH_i 8·0, an increase in $[Ca^{2+}]_i$ had a similar effect to acidification, reducing the conductance to 164 ± 5 pS at 10 μM.

Effects of pH on voltage–P_o relationships

The P_o of Ca^{2+}-activated K^+ channels is also known to be dependent on the membrane potential (Barrett *et al.* 1982; Benham *et al.* 1986). The effect of membrane potential on P_o was studied in the presence of 1 and 10 μM $[Ca^{2+}]_i$ at different pH_i values (Fig. 4*A* and *B*). At pH_i 7·4 and 1 μM $[Ca^{2+}]_i$, the P_o was roughly linearly increased by depolarizing the membrane in the range between −50 and 0 mV. When the inside became alkaline the P_o was less dependent on the membrane

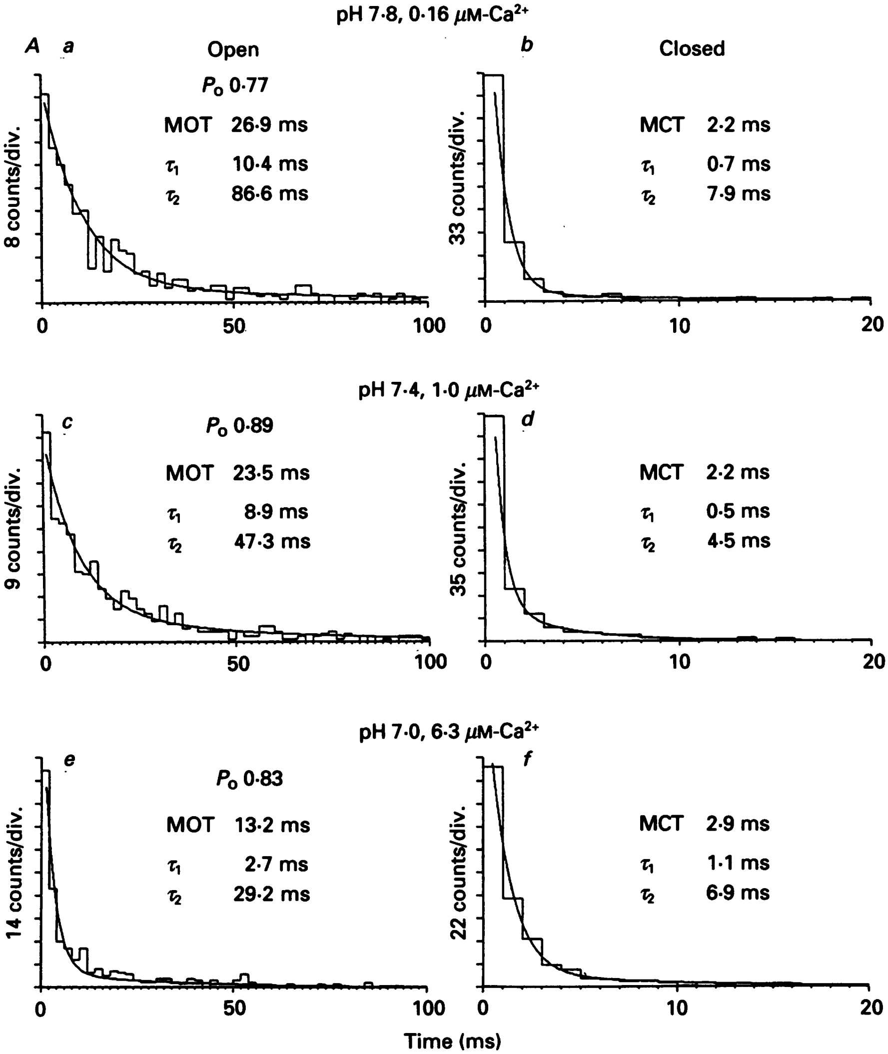

Fig. 5. For legend see facing page.

potential, so that at pH_i 8 the P_o was still about 0·6 at −60 mV, at which potential the channel was completely closed at pH 7·4. When the pH_i was lowered to 7·2, the P_o was markedly decreased, and it was only 0·16 even at +40 mV.

When the $[Ca^{2+}]_i$ was increased to 10 μM, the P_o was increased and became independent of the membrane potential between −40 and +40 mV, in a pH_i range between 7·0 and 8·0 (Fig. 4*B*). The P_o was slightly decreased by strong hyperpolarization to −70 mV. When the pH_i was lowered, the P_o and membrane potential at pH_i 6·8 became roughly similar to that obtained at 1 μM $[Ca^{2+}]_i$,

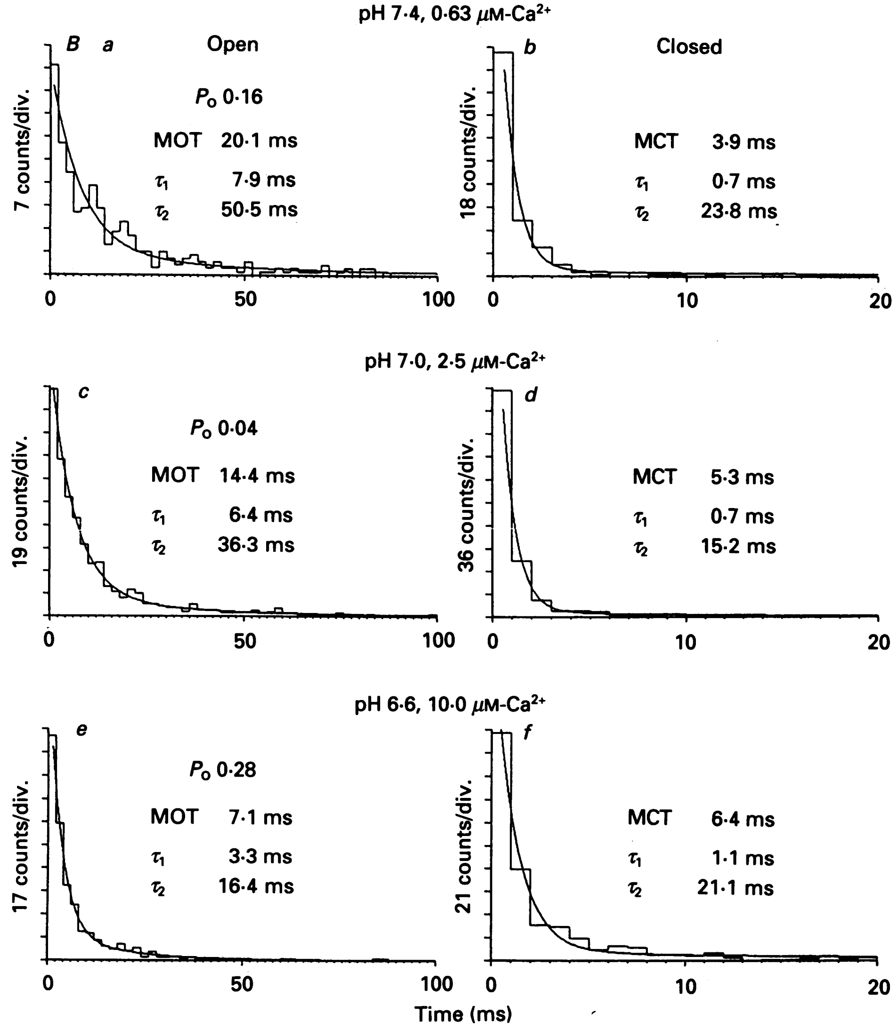

Fig. 5. Effects of lowering pH from 7·8 to 7·0 (*A*), and from 7·4 to 6·6 (*B*) on the probability density function of open and closed time distribution obtained from the same single channel. The experimental conditions were the same as in Fig, 2, but the open probability (P_0) was maintained high (*A*) or low in (*B*) by adjusting with $[Ca^{2+}]_i$, as indicated. Each solution was superfused at least 4 min. MOT and MCT: the mean open and closed times, respectively. τ_1 and τ_2: the time constants of two exponential distribution of open and closed times. Open time distribution was analysed between 0 and 200 ms with a 2 ms bin and closed time distribution was between 0 and 100 ms with a 1 ms bin, after accumulating more than 300 events.

pH_i 7·8. At pH_i 6·0, strong depolarization (+20 to +40 mV) was necessary to increase the P_o.

Effect of pH on channel kinetics

When histograms of duration of a channel being open (the open time) or closed (the closed time) were analysed between 0 and 200 ms for open state (with a bin width of 2 ms) and between 0 and 100 ms for closed state (with a bin width of 1 ms), it was generally found that the probability density function (PDF) of both open and closed times could be expressed by the sum of two exponentials, as shown in Fig. 5*A* and *B*, which were obtained from the same channel. In Fig. 5*A*, the PDF of open and closed times was examined for high P_o (0·77–0·89) at three different pH_i values. The P_o was kept more or less the same when pH_i was lowered from 7·8 to 7·4 and to 7·0, by simultaneously increasing the $[Ca^{2+}]_i$ from 0·16 to 1 μM, and to 6·3 μM, as shown

TABLE 1. Channel kinetics at different pH_i values in the presence of 1 μM-Ca^{2+} (pCa 6·0) obtained from three different channels

Channel		A	B	C
pH 7·4				
P_o		0·872	0·872	0·856
Open:	MOT (ms)	20·3	24·6	26·0
	τ_1	12·9	10·9	13·8
	τ_2	41·3	26·5	41·7
Closed:	MCT (ms)	1·1	1·6	1·9
	τ_1	0·6	0·5	0·7
	τ_2	7·2	5·0	3·7
pH 7·3				
P_o		—	—	0·22
Open:	MOT (ms)	—	—	12·1
	τ_1	—	—	7·1
	τ_2	—	—	20·4
Closed:	MCT (ms)	—	—	4·1
	τ_1	—	—	0·9
	τ_2	—	—	17·1
	(MCT, ms)	—	—	(127·8)
	(τ_3)	—	—	(265·8)
pH 7·2				
P_o		0·003	0·004	0·014
Open:	MOT (ms)	1·8	1·9	8·3
	τ_1	1·0	0·2	3·1
	τ_2	2·5	2·2	12·8
Closed:	MCT (ms)	4·0	4·6	4·1
	τ_1	0·6	0·6	0·7
	τ_2	4·3	5·8	6·1
	(MCT, ms)	(605·7)	(534·0)	(798·8)
	(τ_3)	(677·0)	(680·0)	(866·4)

P_o, the open probability; MOT and MCT, the mean open and closed times. The probability density function could be fitted by two exponentials having time constants of τ_1 and τ_2, between 0 and 200 ms with a bin of 2 ms for open and between 0 and 100 ms with a bin of 1 ms for closed time distribution, except for the slowest component (τ_3) of the closed time distribution observed at low P_o (between 20 and 4000 ms with a bin width of 20 ms).

in Fig. 2. The most clear change with decreased pH_i was shortening of time constants of open time distribution. However, the relative areas of the two components of open time distribution remained nearly the same. The distribution of closed time was not significantly affected by pH_i changes.

At low P_o (0·04–0·28), the same tendency of pH effect was found (Fig. 5*B*). At these low P_o values, however, closed times of much longer than 100 ms appeared, in addition to short closed times. The PDF of closed time analysed between 20 and 4000 ms with a bin width of 20 ms could be fitted with a single exponential having time constants of 292, 227, and 126 ms, at pH 7·4, 7·0, and 6·6, respectively. A full series of pH_i and $[Ca^{2+}]_i$ alterations, as shown in Fig. 5 could be studied successfully only in two channels, but qualitatively similar effects of pH_i within a limited range were always observed in all channels examined ($n = 6$).

When the P_o was decreased by reducing $[Ca^{2+}]_i$ at a constant pH, distribution of open times was not much modified, but the time constant of the slower component of closed times (τ_2) was increased (compare Fig. 5*A* and *B* at pH 7·4 and 7·0). This increase is probably due to the appearance of an additional very slow component of the closed time PDF affecting the apparent value of τ_2.

Parameters of channel activity obtained from three channels at different pH values in the presence of 1 μM $[Ca^{2+}]_i$ are shown in Table 1. At pH 7·4 and pCa 6·0, channel activities were similar to those shown in Fig. 5 (the middle). When the P_o became very small at pH 7·2, the parameters of open time were markedly decreased, but those of closed time remained roughly the same, except for the appearance of a very slow component. In channel C, the P_o was decreased from 0·856 to 0·220 by lowering pH from 7·4 to 7·3. Although the decrease in P_o was accompanied by shortening of the time constants of the PDF of open time, the τ_2 of closed time was increased, accompanied by an appearance of a very slow component of closed time distribution, as observed in the channel shown in Fig. 5.

DISCUSSION

Changes in the pH_i produce two main effects on Ca^{2+}-activated K^+ channels in the rabbit tracheal smooth muscle; one changing the single-channel conductance and the other modifying the channel activity. Compared with the effect on the channel activity, the effect on the conductance is less significant. *V–I* curves obtained at different pH_i values in the presence of 1 μM $[Ca^{2+}]_i$ can be fitted well by the equation in which a term for surface charge is incorporated into the constant-field equation (Adrian, 1969), by assuming that lowering pH_i reduces the internal negative charge of the plasma membrane. In the presence of 10 μM $[Ca^{2+}]_i$, the pH_i effect on *V–I* curves is decreased, probably because Ca^{2+} has already reduced the surface charge.

When $[Ca^{2+}]_i$ is 1 μM, the range of pH_i in which the membrane potential has a marked effect on the open probability is between 7 and 8. On the other hand, when $[Ca^{2+}]_i$ is increased to 10 μM, this range of pH_i shifts to between 6 and 7. Thus, the effect of the intracellular hydrogen concentration (H^+) on the potential dependence of the channel is qualitatively counteracted by $[Ca^{2+}]_i$. This agrees with reports on Ca^{2+}-activated K^+ channels in the rat pancreatic B-cell (Cook *et al.* 1984) and the choroid plexus from the lateral ventricle of *Necturus maculosus* and *Rana esculenta*

(Christensen & Zeuthen, 1987), although these channels have much lower affinity to internal Ca^{2+}, compared with those in smooth muscle.

Since an increase in $[Ca^{2+}]_i$ counteracts the reduction of the open probability caused by acidification, it may be that H^+ and Ca^{2+} compete with each other at the site of channel gate. However, the $[Ca^{2+}]_i$–P_0 curve is not simply shifted to the right in parallel by increasing $[H^+]_i$, its steepness is also reduced, particularly below pH_i 7·0, suggesting that alteration of channel kinetics is involved, in addition to competition between Ca^{2+} and H^+.

The gating mechanism of the Ca^{2+}-activating K^+-channel is controlled not only by $[Ca^{2+}]_i$ and membrane potential, but also by $[H^+]_i$, as found in pancreatic B-cells (Cook *et al.* 1984) and epithelial cells (Christensen & Zeuthen, 1987). The main effect of lowering pH_i is to decrease the Ca^{2+} sensitivity of the channel. Thus, the pH effect on the closed time distribution is likely to be exerted by altering Ca^{2+} binding at the gating site of the channels. In addition to this, the observation that the time constant of the open time distribution is always shorter at higher $[H^+]_i$ when compared at similar P_0 suggests that intracellular H^+ shortens the open state of the channel.

The functional role of the Ca^{2+}-activated K^+ channel in the tracheal muscle is still not clear. In the present experiments, in which the K^+ concentration gradient was the opposite of physiological gradient and the membrane was clamped at 0 mV, $[Ca^{2+}]_i$ necessary to activate this channel at pH 7 was about 1 μM at room temperature. This experimental condition, however, may have reduced the affinity of the channel for Ca^{2+}. It is possible that under physiological conditions the Ca^{2+}-activated K^+ channel contributes to the membrane conductance and that intracellular alkalosis activates the channel, resulting in membrane hyperpolarization and inhibition of the membrane excitation. This may explain a decrease in tracheal tone by intracellular alkalinization (Wray, 1988).

We are grateful to Professor Edith Bülbring and Dr Alison F. Brading, Oxford, for improving the manuscript.

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