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MODULATION OF K^+ CURRENTS IN HUMAN LYMPHOCYTES BY pH

BY CAROL DEUTSCH AND SHERWIN C. LEE*

From the Department of Physiology, University of Pennsylvania, Philadelphia, PA 19104-6085, USA

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SUMMARY

1. Using whole-cell patch-clamp techniques, we found that the voltage-dependent K^+ conductance in human peripheral blood T lymphocytes is enhanced threefold at alkaline intracellular pH (pH_i) compared to acid pH_i. This pH dependence can be described by a model having two strongly co-operative proton binding sites with pK_a 7·15. A similar pH_i sensitivity exists for K^+ conductance in mitogen-activated cells.

2. The reversal potential, threshold voltage for activation of the K^+ conductance, and voltage dependence of steady-state inactivation are not affected by pH_i. Activation and inactivation kinetics are also unchanged.

3. Single-channel measurements made in whole-cell patch-clamp mode indicate that the effect of intracellular pH on the amplitudes of single-channel events parallels, but does not wholly account for, the effect of pH_i on the macroscopic currents.

4. Lowering extracellular pH (pH_o) shifts the threshold for activation of the K^+ current to a more depolarized voltage, consistent with a surface charge screening effect. Apparent changes in peak current and activation kinetics at acid pH_o can be accounted for by this voltage shift. An additional slowing of inactivation kinetics at low pH_o does occur.

5. The relevance of the pH sensitivity of the voltage-gated K^+ conductance to lymphocyte mitogenesis and volume regulation is discussed.

INTRODUCTION

The voltage-gated potassium channel in human peripheral blood T lymphocytes is sensitive to pH. Functionally, these channels mediate the volume-regulatory response to anisotonicity (Lee, Price, Prystowsky & Deutsch, 1988). This K^+ channel has also been implicated in mitogen- and growth-factor-stimulated proliferation of human and murine T cells (Chandy, DeCoursey, Cahalan, McLaughlin & Gupta, 1984; DeCoursey, Chandy, Gupta & Cahalan, 1984; Deutsch, Krause & Lee, 1986; Lee, Price & Deutsch, 1986*a*; Lee, Sabath, Deutsch & Prystowsky, 1986*b*). In particular, this conductance increases during cell-cycle progression (Matteson &

* Authors' names are in alphabetical order.

Deutsch, 1984; Deutsch *et al.* 1986; Lee *et al.* 1986*b*; DeCoursey, Chandy, Gupta & Cahalan, 1987), and potassium channel blockers inhibit DNA (Chandy *et al.* 1984; DeCoursey *et al.* 1984; Deutsch *et al.* 1986; Lee *et al.* 1986*a*) and specific protein synthesis (Chandy *et al.* 1984; Sabath, Monos, Lee, Deutsch & Prystowsky, 1986).

Many investigators have postulated that intracellular [H^+] regulates proliferation in biological systems (Zetterberg & Engström, 1981; Pouysségur, Sardet, Franchi, L'Allemain & Paris, 1984; Taylor & Hodson, 1984), including lymphocytes (Gerson, Kiefer & Eufe, 1982). Changes in intracellular pH have been observed in lymphocytes and thymocytes in both the short-term and long-term response to mitogens (Gerson *et al.* 1982; Deutsch, Taylor & Price, 1984; Hesketh, Moore, Morris, Taylor, Rodgers, Smith & Metcalfe, 1985; Gelfand, Cheung & Grinstein, 1988). We have suggested that mitogenesis and volume regulation have overlapping pathways (Lee *et al.* 1986*b*), and shown that (1) the voltage-gated K^+ conductance mediates the volume-regulatory response (Lee *et al.* 1988), and (2) pH modulates the volume-regulatory response (Deutsch & Lee, 1989). In order to elucidate the mechanism of pH modulation of volume regulation, and possibly its role in mitogenesis, we studied the effect of pH on whole-cell macroscopic and single-channel conductance.

Preliminary results of this study have been published (Lee, Krause & Deutsch, 1985; Lee & Deutsch, 1988).

METHODS

Preparation of human peripheral blood lymphocytes

Heparinized human venous blood was collected from healthy donors. Mononuclear cells were separated by gradient centrifugation using a modifed Ficoll–Hypaque technique as previously described (Deutsch *et al.* 1986). Cell number was measured by a Coulter counter, and lymphocyte viability was determined by Trypan Blue exclusion. Viability was routinely 95% or greater.

Cultures for study of activated cells were prepared as previously described (Deutsch *et al.* 1986) and stimulated with 0·1 μg/ml phorbol myristate acetate (PMA). DNA synthesis was assessed by measuring the incorporation of [^{3}H]thymidine. PMA induced about a thirtyfold increase, at 72 h, in incorporated radioactivity compared with unstimulated controls. Stimulation at 24 h was also reflected in an increased cell capacitance (2·5–4·0 pF), which is typical of activated cells (Deutsch *et al.* 1986).

T lymphocytes were selectively attached to dishes for patch-clamp studies using the monoclonal antibody OKT11 (Ortho Pharmaceutical, Raritan, NJ, USA), as described previously (Matteson & Deutsch, 1984; Deutsch *et al.* 1986).

Electrophysiological recording

We employed the whole-cell patch-clamp method of Hamill, Marty, Neher, Sakmann & Sigworth (1981) as applied to lymphocytes (Matteson & Deutsch, 1984; Deutsch *et al.* 1986), using a Model EPC-7 patch clamp (List-Medical-Electronic, FRG). Electrodes were made of Kimax-51 capillary tubing (Kimble 34500) and coated with Sylgard 184 (Dow Corning).

Conditions of the experiments and procedures for data collection were as described previously (Deutsch *et al.* 1986). Whole-cell data were collected and analysed with a small computer (LSI 11/23, Digital Equipment Corporation, Maynard, MA, USA). Raw data were corrected for linear leak and capacitance. Voltage-clamp holding potential was normally −70 mV. Cell capacitance, a measure of cell surface area, was determined by integrating the area under uncompensated capacitative spikes produced by repetitive 1 mV steps, as described in Deutsch *et al.* (1986). Series-resistance compensation was not used because it did not appreciably improve these recordings (as discussed, Deutsch *et al.* 1986).

Single-channel data were recorded with a digitizing tape system and analysed with the assistance of the computer. Single-channel data were low-pass filtered with an 8-pole Bessel filter (902LPF,

Frequency Devices, Haverhill, MA, USA). We recorded single-channel currents in whole-cell mode by depolarizing to 0 mV for a long time. Under these conditions almost all the K^+ channels are inactivated. In good preparations, the residual K^+ channel activity is low enough so that distinctive single-channel events can be recorded (Cahalan, Chandy, DeCoursey & Gupta, 1985; Krause, Lee & Deutsch, 1988).

The bath solutions are described in Table 1. Normal bath solution had a pH of 7·3. Both HEPES and MES (10 mM) were used to buffer the extracellular medium, depending on the pH. At pH values where either or both could be used, they gave the same result. At very low pH_o ($< 5·5$) some cells developed a leak conductance. This was especially true in a few cells where we used 10 mM-citrate buffer. We did not investigate this leak conductance further.

Solution osmolarity was measured with a freezing point depression osmometer (Precision Systems Osmette, Framingham, MA, USA). The bath was grounded via an agar bridge (saline at pH 7·3, no glucose, 3% (w/v) agar), which was in contact with a small reservoir containing pipette solution. Electrical connections were made with Ag–AgCl wire. All experiments were done at room temperature.

Pipette solutions to set intracellular pH

The following considerations influenced the design of the pipette solutions described in Table 2.

Our approach was to use relatively large amounts of pH buffers near their pK_as. Measuring pH_i directly, Byerly & Moody (1986) found that 100 mM buffer was needed to control pH_i in snail neurones with a suction electrode technique. Using a functional test, Wanke, Carbone & Testa (1979) found that 45 mM buffer was sufficient to change pH_i in perfused squid axon. In our experiments there was a consistent effect of pH_i on conductance with 25 mM buffer in the pipette solution. We found little difference between 25 and 50 mM buffer, but used the higher amount in almost all cases. With this high buffer concentration, the pH of the cell interior equilibrated with that of the pipette solution quickly: within the 20–30 s that elapsed between the initiation of whole-cell dialysis and the first test voltage step, cells attained a stable peak current that proved to be characteristic of each pH_i.

To ensure that there were no differences due to buffer-specific effects, we evaluated whole-cell currents using 10 mM-HEPES *versus* 50 mM-HEPES, pH 7·2; 50 mM-HEPES *versus* 50 mM-phosphate, pH 7·2; 50 mM-HEPES *versus* 10 mM-HEPES, 50 mM-MES, pH 7·2; 50 mM-HEPES *versus* 25 mM-HEPES, 25 mM-MES, pH 6·85; 50 mM-MES *versus* 30 mM-citrate, pH 5·6; 50 mM-TAPS *versus* 50 mM-glycylglycine, pH 8·2. No differences were observed in these comparisons. Only the results obtained with 50 mM-tris-(hydroxymethyl)aminomethane (Tris, pH 8·2) differed from those of its pH-matched control: a significantly lower average current was observed.

Another consideration was to maintain low levels of intracellular free Ca^{2+} while varying pH_i. There is no direct evidence to suggest that the lymphocyte K^+ channel is Ca^{2+}-activated; rather, high intracellular Ca^{2+} has been associated with decreased K^+ current (Bregestovski, Redkozubov & Alexeev, 1986). To avoid changes in the K^+ current that might be associated with increased $[Ca^{2+}]_i$, solutions at low pH, where EGTA is a poor Ca^{2+} chelator, had little or no added Ca^{2+}. Calculated free Ca^{2+} for all solutions of $pH > 6·2$ was less than 40 nM. For the solutions of lower pH, assuming a Ca^{2+} contaminant of 10 μM, the worst case (solution 1) would have had slightly more than 1 μM-free Ca^{2+}. Calculated free Mg^{2+} was in the range of 1·2–1·4 mM for solutions 1–9.

Finally, we tried both F^- and Cl^- as the primary anion in the pipette solution. Human T cells dialysed with an all-Cl^- solution are less stable than those dialysed with an all-F^- solution (Cahalan *et al.* 1985; Krause *et al.* 1988). However, good data could be obtained with Cl^- in some cases. Again, the use of F^- *versus* Cl^- made no difference to the pH_i sensitivity of the K^+ conductance.

Reagents

Media were made up from reagent-grade chemicals whenever possible. PMA was from C.C.R. Inc. (Eden Prairie, MN, USA). KF was from Aldrich (Milwaukee, WI, USA). FCCP (carbonyl cyanide *p*-trifluoromethoxy-phenylhydrazone) was from DuPont (Wilmington, DE, USA). pH buffers HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid), MES (2-(*N*-morpholino)ethanesulphonic acid), TAPS (*N*-tris-(hydroxymethyl)methyl-3-aminopropane-sulphonic acid), glycylglycine and citric acid were from Sigma (St Louis, MO, USA). Trypan Blue, antibiotics, vitamins and amino acids for culture media were from GIBCO (Grand Island, NY, USA).

TABLE 1. Bath solutions

External pH	NaCl	KCl	$CaCl_2$	$MgCl_2$	MES	HEPES	Citrate	Glucose	NaOH
5·49	144	5	2·5	1	10	—	—	5·5	2·4
5·60	137	5	2·5	1	—	—	10	5·5	30
5·95	144	5	2·5	1	10	—	—	5·5	5
6·73	141	5	2·5	1	10	—	—	5·5	8·2
6·85	145	5	2·5	1	—	10	—	5·5	3·5
7·30	140	5	2·5	1	—	10	—	5·5	4·5
8·20	130–140	5	2·5	1	—	10–20	—	5·5	9·5–19

TABLE 2. Pipette solutions

Solution	Internal pH	KCl	KF	$CaCl_2$	$MgCl_2$	K_2EGTA	MES	HEPES	TAPS	Citrate	KOH
1	5·17	60	—	0	1·36	11	—	—	—	50	104
2	5·55	128	—	0·01	1·42	11	50	—	—	—	3
3	5·70	130	—	0	1·38	11	50	—	—	—	6
4	6·24	119	—	0·01	1·42	11	50	—	—	—	13·6
5	6·85	112	—	0·5	1·7	11	25	25	—	—	16
6	7·23	105	—	1	2	11	—	50	—	—	20
7	7·50	97	—	4	2·4	11	—	30	20	—	26·8
8	7·68	95	—	4·7	2·5	11	—	25	25	—	28·5
9	8·20	85	—	8·4	3	11	—	—	50	—	37
10	7·20	—	130	1	2	11	—	10	—	—	4
11	7·20	—	110	1	2	11	—	50	—	—	17
12	7·20	—	135	0	1·5	5	3	3	3	—	3·6

RESULTS

K^+ current as a function of pH_i

Human peripheral blood T cells were dialysed in whole-cell patch-clamp mode with solutions containing buffers at pH values near their respective pK_as. Cells were held at −70 mV and stepped to various depolarized voltages to elicit voltage-gated outward K^+ current. Figure 1*A* shows that the activation kinetics are very similar at pH_i 6·2, 7·2 and 8·2. Similarly, inactivation kinetics in the same cells (Fig. 1*B*) do not vary as a function of pH_i. However, what clearly did change as a function of pH_i was average peak current.

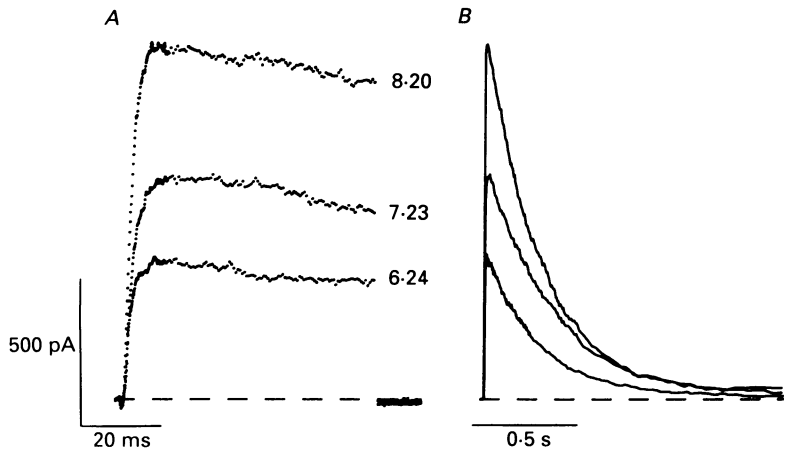

Fig. 1. Potassium current kinetics as a function of intracellular pH. Each trace in *A* is the current induced in a cell in response to a 60 ms voltage step from the holding potential of −70 mV to +50 mV. Intracellular pH was set with pipette solutions 4 (pH 6·24, 50 mM-MES), 6 (pH 7·23, 50 mM-HEPES) and 9 (pH 8·20, 50 mM-TAPS) from Table 2. In *B* the same three cells were stepped to +50 mV for 1·4 s to observe inactivation. The sampling rates were 10 kHz (*A*) and 0·5 kHz (*B*), and the data were filtered at 3 kHz with the 3-pole Bessel filter in the List EPC-7. Temperature was 25 °C. Bath pH was 7·30.

Single-donor studies were carried out to minimize scatter due to inter-donor variability. For our most extensive data set (Fig. 2) we used a donor (C.G.) whose cells gave very reproducible results with all-Cl^- pipette solutions. The results from these cells display a correlation between cell capacitance (a measure of cell surface area) and peak current in individual cells (Fig. 2 inset). Such a correlation has not been noted in previous work (Cahalan *et al.* 1985; Deutsch *et al.* 1986), but is apparent here probably because the cells were from a single donor. The somewhat poor correlation coefficient for the least-squares-fit line undoubtedly reflects not only the difficulty of measuring these small capacitances (Deutsch *et al.* 1986) but also the distribution of current in cells of a given size. Hence, we felt it was appropriate to normalize mean peak current to cell capacitance. The K^+ conductance increased approximately threefold when pH_i was increased from 5·2 to 8·2, most of the change occurring between 6·5 to 7·5. The dotted curve in Fig. 2 is a calculated, Henderson–Hasselbach titration curve for a model with two strongly co-operative

sites with pK_a 7·15. This pK_a and a Hill coefficient of 2 gave the best fit to the data, but the fit is sensitive to the choice of the titration end-points, which we estimated to be pH_i 5·5 and 8·2. There is a suggestion of a second titratable site at low pH (< 5·5).

The peak current–voltage relationships at pH_i 8·20, 7·23 and 6·24 are shown in Fig. 3. The threshold voltage for activation of the K^+ conductance was the same in all cases, typically about −40 to −45 mV, indicating that there is no significant pH-sensitive surface potential at the cytoplasmic face of the lymphocyte plasma membrane. Once full activation of the current was achieved (at about −20 mV), current increased ohmically. There were no deviations from linearity in the *I–V* relations to suggest that H^+ participates in a voltage-dependent block of the K^+ channel. The reversal potential, typically approximately −70 mV, was unaltered by pipette pH. Since H^+ concentrations are very much less than those of K^+, the H^+/K^+ permeability ratio would have to be at least 10000 to produce a 1 mV change in calculated reversal potential for pH_i 7·2 *versus* pH_i 6·2.

There was also no effect of pH_i on steady-state inactivation (data not shown). The outward current elicited by a step to +50 mV from a holding potential of −70 mV was always ⩾ 90% of that found when the holding potential was −90 mV; significant decrease of the maximum current did not occur until the holding potential approached the threshold for activation (i.e. −55 to −50 mV), as reported previously (Deutsch *et al.* 1986).

K^+ current as a function of extracellular pH

To determine if this conductance were sensitive to changes in bath pH, cells were patch-clamped in whole-cell mode using a 50 mM-HEPES internal solution, and the external pH varied from 5 to 8·2.

The conductance was relatively insensitive to bath pH in the range 6·6–8·2. However, at pH values below this, peak current was decreased. At any given voltage, maximum current was less and activation and inactivation kinetics were slower at low pH_o than they were at neutral pH_o. Most of this was due to a shift in the current–voltage relationship, not to a change in the slope conductance, as shown in Fig. 4*A*. In this example, a cell was obtained in the whole-cell configuration at pH_o 5·5, and then pH_o was sequentially changed to 7·44, 6·68 and 5·84. The threshold for activation of current shifted from approximately −50 mV at pH 7·4 to approximately −15 mV at pH 5·5. Activation and inactivation kinetics were slowed as pH_o was lowered (Fig. 4*B*). These effects were reversible, although when pH_o was lowered, the full development of the positive threshold shift required a number of minutes, whereas the negative shift that occurred when pH_o was raised was very quick.

The threshold shift can be explained by screening of membrane surface charge, which can be titrated by H^+ (Hille, 1968; Gilbert & Ehrenstein, 1970). The effect on activation (lower set of symbols, Fig. 4*B*) appears to be due entirely to the shift in threshold; if the data for activation at pH_o 5·5 are shifted this amount (35 mV) to the left, they superimpose on the data for activation at pH_o 7·44. However, correlation with the shift in threshold was only partly true for inactivation. Inactivation kinetics (upper set of symbols, Fig. 4*B*) have little voltage dependence for large depolarizations (Cahalan *et al.* 1985; Deutsch *et al.* 1986), and the voltage at which this limiting time constant was attained was shifted to more positive potentials at

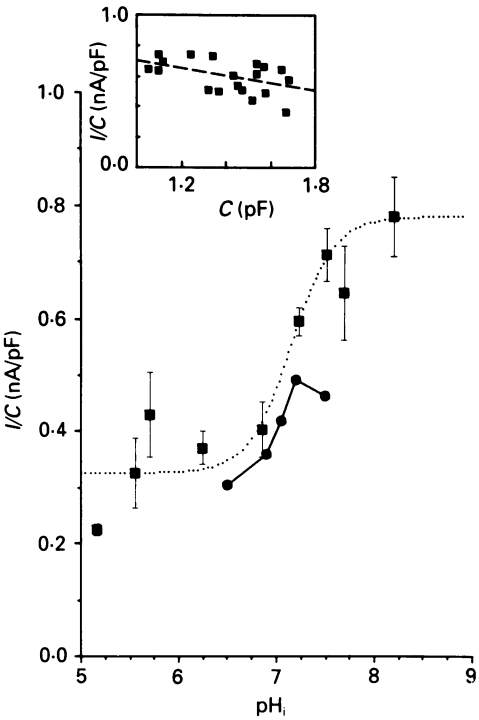

Fig. 2. The effect of intracellular pH on whole-cell current. Mean specific current (whole-cell peak current at +50 mV divided by cell capacitance) is plotted as a function of pH_i for two representative data sets. Each data set (a total of four) was independently obtained over a period of a few weeks using cells of an individual donor, and cells were generally used within 30 h of isolation (see Methods). For the points in the upper curve (■), data were obtained using the pipette solutions described in Table 2: 50 mM-buffer, all Cl^- (solutions 1–9). At least five cells were sampled at each pH above 6; below 6, points represent three cells (pH 5·17), two cells (pH 5·55) and four cells (pH 5·70) (donor: C.G., seventy cells total). Error bars represent standard error of the mean. For the points in the lower curve (●), we concentrated on the pH_i range where K^+ current amplitude is most sensitive. These data were obtained with pipette solutions very similar to those in Table 2. Each point is the mean of at least three cells (donor: S.F., forty-six cells total). Essentially identical modulation of K^+ current with pH_i was found in the two other single-donor studies (not shown), even though one set of data was obtained using pipette solutions constructed with 25 mM buffer, all Cl^-, and the other with 50 mM buffer, all F^-. These data are plotted as specific current because we found a correlation between cell capacitance and peak current, and this representation reduced the scatter. The inset is current/capacitance *versus* capacitance for the data of the upper curve at pH_i 7·23. The linear least-squares fit to these data (dashed line) is $I/C = 0{\cdot}96 - 0{\cdot}26C$, $r^2 = 0{\cdot}25$ (nineteen cells). A similar relation was obtained for the data of the lower curve. The pK_a of 7·15 was determined as the slope of a least-squares line fitted to linearized data, assuming the values at pH_i 5·55 and 8·20 were the correct asymptotic extremes of the titration (analysis after Albert & Serjeant, 1971). The dotted curve is our best theoretical fit of the data. It was obtained with a two-site Henderson–Hasselbalch equation, with the Hill assumption of strong co-operativity, so that the singly protonated form could be ignored. The equation is

$$I/C = (I/C_{max}K_a^2 + I/C_{min}[H^+]^2)(K_a^2 + [H^+]^2)^{-1},$$

where $I/C_{max} = I/C$ at pH 8·20, $I/C_{min} = I/C$ at pH 5·55, and $pK_a = 7{\cdot}15$. This is essentially a Hill plot with a coefficient of 2.

low pH_o. However, the limiting time constant was still slower at low pH_o ($\sim$ 165 ms at 5·50) than at neutral pH_o ($\sim$ 90 ms at 7·44).

The shifts in threshold voltage and peak current at +50 mV were nearly linear with pH_o in the acid range, as shown in Fig. 4*C*. The linear least-squares fit gives a change of 7·3 mV per e-fold change in $[H^+]$. This would imply a fixed charge separation on

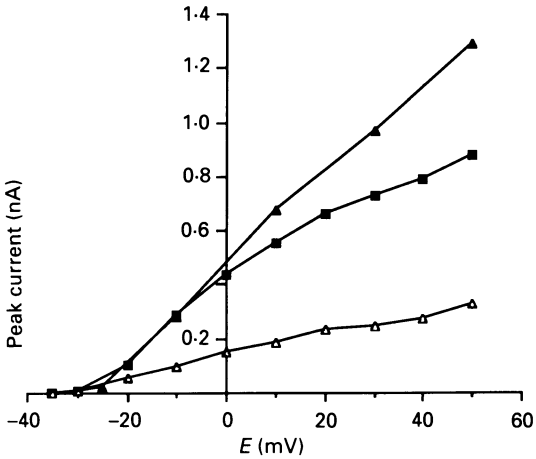

Fig. 3. Peak current–voltage relationships as a function of pipette pH. Each symbol represents the data for an individual cell at the indicated pH_i (using solutions 4, 6 and 9). Cells were stepped from −70 mV to the indicated membrane potential for 60 ms. The membrane potential was held at −70 mV for 45–60 s between voltage steps to allow sufficient time for recovery from inactivation. Threshold voltages were the same regardless of pH_i. Bath solution was pH 7·30. ▲, pH 8·20; ■, pH 7·23; △, pH 6·24.

the surface of about 2 nm (from Fig. 1 of Gilbert & Ehrenstein, 1970). We did not lower pH_o sufficiently to determine the pK_a of the H^+-titratable groups, but our data are similar to and would be consistent with the pK_a of 4·5–5·0 found for frog node (Hille, 1968; Gilbert & Ehrenstein, 1970).

Varying pH_i while in whole-cell configuration

We attempted to carry out pH titrations of the K^+ conductance from the inside of a single cell. The rationale for these studies was twofold: first, changes in the same cell would yield a more sensitive profile of the pH dependence of the conductance. Second, we had to eliminate the possibility that breaking into the cell with pipette solutions of different pH altered the number of available channels and/or their ability to be activated.

We first attempted to shift intracellular pH in a lymphocyte in the whole-cell configuration by using a low-buffer-capacity pipette solution (solution 12, Table 2). We then varied the pH of the bathing solution, reasoning that pH_i would track pH_o according to the pH profile previously reported by this laboratory (Deutsch *et al.* 1984). This procedure failed to alter conductance.

In order to effect more definitively a change in intracellular pH, we used the proton ionophore FCCP to equilibrate intra- and extracellular pH for a given change in

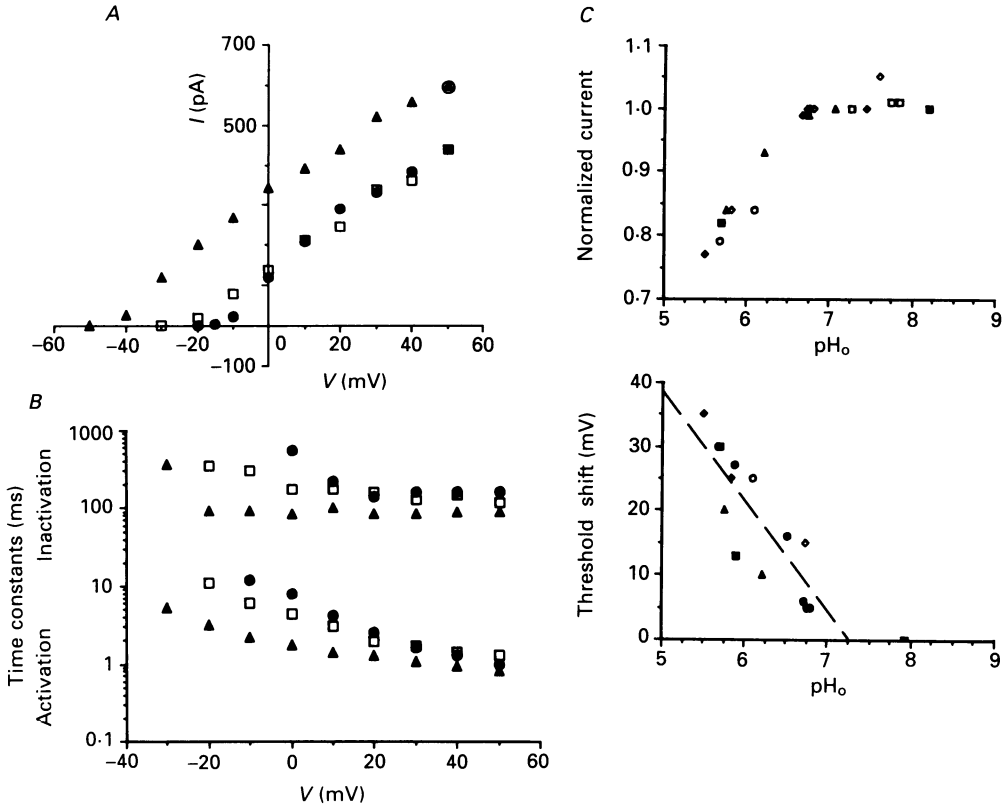

Fig. 4. The effect of extracellular pH on whole-cell current. *A*, peak current–voltage relationships for a single T lymphocyte at extracellular pH values: ●, 5·50; □, 5·84; ▲, 7·44. The larger open circle surrounding the filled triangle at +50 mV is a single measurement at pH 6·68. Data were obtained with solution 11 (Table 2) in the pipette. *B*, time constants for activation and inactivation as a function of pH_o and voltage for the data in *A*. Data were fitted and constants determined as described previously (Deutsch *et al.* 1986). Note logarithmic scale. *C*, normalized peak current at +50 mV and threshold shift for activation of current as a function of extracellular pH. Each different symbol represents data from a different cell, six in the upper panel and seven in the lower one. Current was normalized to the value at pH_o 7·3, and the threshold shift was measured with respect to the threshold at pH_o 7·3. The dashed line in the lower panel is a least-squares fit to the data according to the equation: threshold shift (in mV) = $124{\cdot}0 - 17{\cdot}1(pH_o)$, r^2 = 0·87. Data were obtained with solutions 11 or 12 (Table 2) for the pipette solution.

extracellular pH. For a single cell, when the bathing medium of a patch-clamped quiescent T cell was changed sequentially from point A to point E (Fig. 5*A*), in the presence of FCCP (25 μM), the conductance changed, reproducibly and reversibly, as a function of pH_o. There is a greater than twofold difference between the acid and alkaline end-points. Inasmuch as whole-cell conductance varies about 15% in this range of pH_o when the ionophore is not present (Fig. 4*C*), these results agree with those obtained for a direct change of pipette pH from $pH_i \sim 5{\cdot}2$ to $pH_i \sim 6{\cdot}7$ (Fig. 2).

In Fig. 5*B*, the peak current–voltage relation for a single cell at low pH_o in the

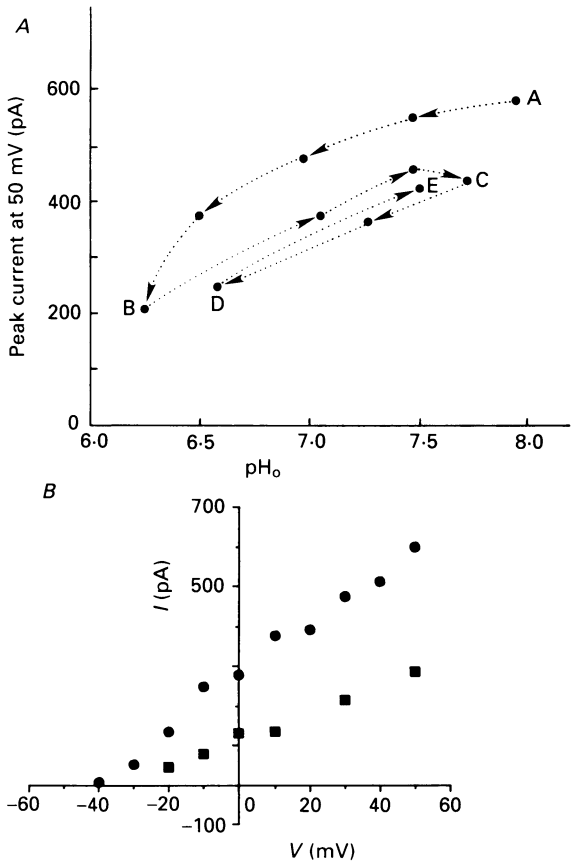

Fig. 5. Manipulation of intracellular pH with FCCP and the effect on whole-cell current. *A*, peak current at +50 mV *versus* pH of the bathing medium in a FCCP-treated quiescent T lympho ,yte. The bath was changed sequentially from point A to point E, and always contained 25 μM-FCCP. (The FCCP was added from a 17 mM stock in ethanol.) Although this is a high concentration of FCCP, cells exposed to this level of ionophore at room temperature for 2 h or more still excluded Trypan Blue. The only electrical effect of FCCP (and an indicator of its interaction with the patch-clamped cell) was to produce a small increment in leak current, especially at low pH_o. *B*, peak current–voltage relationships for a cell (pipette pH = 7·20, solution 12) in the absence (●, pH 6·32) and presence (■, pH 6·43) of 25 μM-FCCP at acid pH. Data were collected as described in Fig. 3. Threshold voltages were the same in the presence and absence of FCCP; however, the slope conductance was not.

presence (■) and absence (●) of FCCP is shown. There is a marked change in slope, but no shift in threshold voltage, similar to that observed when pH_i was changed directly (Fig. 3). Likewise, FCCP was used to assess the pH sensitivity of K^+ conductance in single lymphocytes, which had been mitogenically activated for 24 h in culture. These cells showed a similar pH_i-dependent decrease in K^+ conductance when treated with FCCP and low pH_o (data not shown).

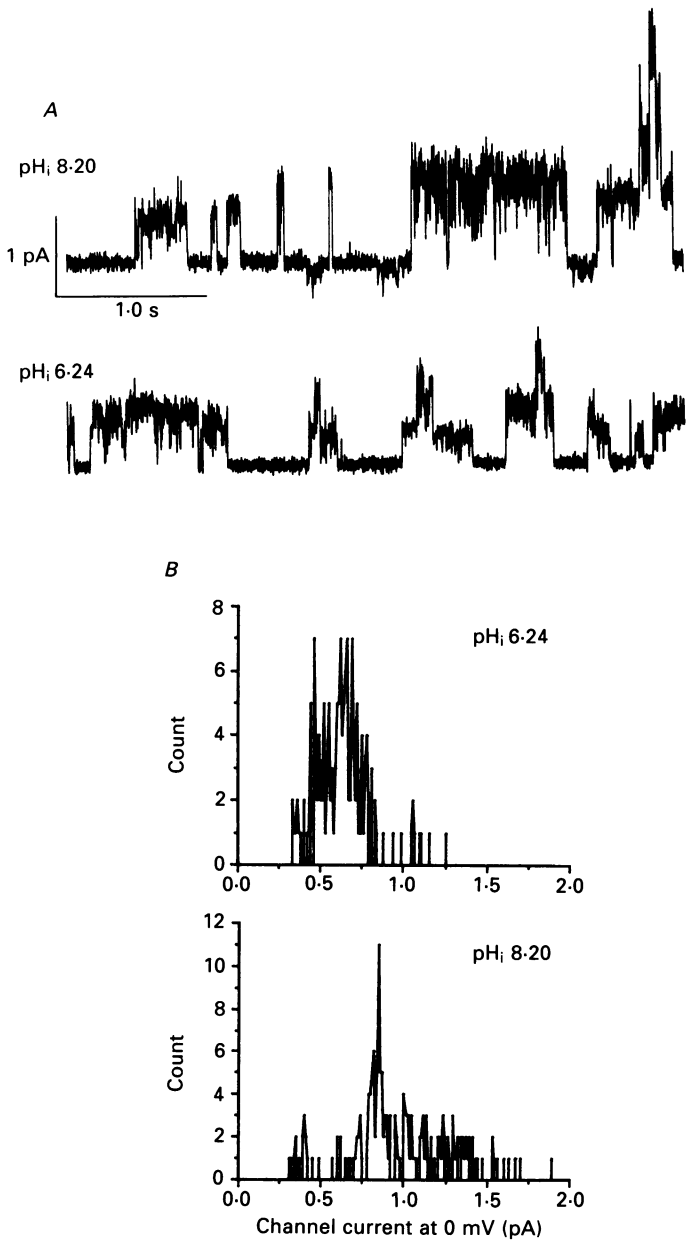

Fig. 6. Single-channel currents. *A*, single-channel events were recorded in the whole-cell configuration with the membrane potential held at 0 mV. Outward current channels (upward deflection) were recorded at pH$_i$ 8·20 and 6·24. Sampling rate was 10 kHz, and the data were filtered at 1 kHz. Solutions and conditions were as described in Fig. 1. *B*, current amplitude histograms of single-channel records obtained at 0 mV as in panel *A*. The data at pH$_i$ 6·24 have a mean of 0·63 pA and a median of 0·62 pA, $n = 155$ events. At pH$_i$ 8·20, mean current was 0·97 pA, median 0·90 pA, $n = 177$.

Single-channel currents

To elucidate the mechanism by which pH_i modulates K^+ conductance, we studied single-channel currents using the appropriate buffer solutions (4, 9) in the pipette. At pH_i 7·2, unitary currents have conductances of 9 and 16 pS and appear to correspond to the macroscopic voltage-gated K^+ current (Cahalan *et al.* 1985). Examples of such unitary currents at acidic and alkaline pH_i are shown in Fig. 6*A*, and corresponding current amplitude histograms in Fig. 6*B*. At pH_i 6·24, we observed single-channel conductances of approximately 7 and 10 pS. At pH_i 8·20 the most prevalent channel had a conductance of about 12 pS; larger channel conductances were seen, but no clear determination of size could be made.

DISCUSSION

Sensitivity of the K^+ channel to pH

Whole-cell K^+ current in human peripheral blood T cells is enhanced at alkaline intracellular pH and inhibited at acid pH. pH_i affects the magnitude of K^+ conductance with little or no effect on kinetics or voltage sensitivity. Similarly, low intracellular pH has been shown to block K^+ currents in excitable tissues (Moody, 1984; Byerly & Moody, 1986). For example, in the squid axon, inhibition of the voltage-gated K^+ current by low-pH buffers could be modelled by a single-proton binding site with a pK_a of 6·9 (Wanke *et al.* 1979). Our results in lymphocytes are similar except that the conductance *versus* pH_i curve is best fitted assuming two strongly co-operative sites with a pK_a equal to 7·15. We found no evidence for a direct voltage-dependent block by protons.

In general, whole-cell K^+ current in response to a voltage step is given by the equation $n \times p(V, t) \times g_K \times E_K(V)$, where n is the number of channels, p is their voltage- and time-dependent probability of being open, g_K is the mean single-channel conductance, and $E_K(V)$ is the electrochemical driving force. The difference in average peak current between pH_i 8·2, 7·2 and 6·2 could result from any combination of (1) a change in the number of channels available to open; (2) a change in the probability of the channels being open as a function of voltage and/or time after the voltage step; or (3) a change in the mean single-channel conductance. Our measurements of the relative magnitudes of the single-channel currents at pH_i 6·2 and pH_i 8·2 parallel the relative magnitudes of the whole-cell currents. Assuming that the histograms in Fig. 6*B* accurately represent the channels in the cell, we would expect whole-cell current to change according to the ratio of mean channel amplitude at pH_i 6·24 to mean channel amplitude at pH_i 8·20. This predicts a 54% increase, which is inadequate to account for the approximately 125% increase in whole-cell current (Fig. 2).

It is likely that no net change in $p(V, t)$ occurs, because activation and inactivation kinetics, steady-state inactivation, threshold for activation and shape of the I–V relations were all the same for whole-cell currents at pH_i 6·2 and 8·2. Although this does not preclude an effect of pH_i on channel open time or frequency of opening, it is likely that the reason for the greater effect of pH_i on whole-cell conductance is that pH_i affects the number of channels that open, as well as the single-channel

conductance. Such a mechanism has been demonstrated for the pH sensitivity of Ca^{2+}-activated K^+ channels in pancreatic β-cells (Cook, Ikeuchi & Fujimoto, 1984). One caveat is that the source of our single-channel data was residual, intermittent channel activity in cells held at 0 mV. Although these unitary events are similar to those observed during a voltage ramp (Cahalan *et al.* 1985), and are sensitive to the same pharmacological blockers as is the macroscopic current, the channels active during a maintained depolarization could be slightly different from the population of K^+ channels that give rise to the transient, voltage-gated currents.

In contrast to intracellular pH, extracellular pH had no effect at pH > 6·7. At $pH_o < \sim 6{\cdot}7$ there was a decrease in K^+ current for a given applied voltage, consistent with shielding of the membrane surface charge by H^+, as described for frog node (Hille, 1968), and by Ca^{2+}, as described for the lymphocyte (Fukushima, Hagiwara & Henkart, 1984; Cahalan *et al.* 1985). The slowing of activation at low pH_o is due to the shift of the activation threshold to more positive voltages. The effect of pH_o on inactivation, however, suggests some sort of specific interaction with the channel in addition to the surface charge screening effect.

The relatively small effect of pH_o on K^+ conductance enabled us to use pH_o to manipulate pH_i in an individual cell in whole-cell patch clamp. To increase the permeability of the membrane to H^+, we added large concentrations of the protonophore FCCP, allowing us to set predictably the intracellular pH. Actual pH_i would be due to a steady state between H^+ influx mediated by FCCP and H^+ buffering by the pipette solution. Since our normal holding potential was −70 mV, pH_i would be about 1 pH unit more acid than pH_o at equilibrium. Under these conditions K^+ conductance at acid pH_o in the presence of FCCP (Fig. 5) was consistent with the K^+ conductance observed when an acid pH_i was set directly (Fig. 2). Moreover, the reversibility of this effect (Fig. 5*A*) constitutes a clear demonstration of the pH_i sensitivity of the K^+ conductance.

Biological significance

The pH sensitivity of the voltage-gated K^+ conductance is also observed in the intact lymphocyte. The evidence comes from our studies of lymphocyte volume regulation. We know (1) that this K^+ conductance underlies the volume-regulatory decrease in response to hypotonically induced swelling (Lee *et al.* 1986*b*, 1988); (2) that pH_o 8·5 enhances and pH_o 5·5 inhibits this volume-regulatory response (Deutsch & Lee, 1989); and (3) that pH_i is ~ 7·9 and ~ 6·2 at pH_o values of 8·5 and 5·5, respectively (Deutsch *et al.* 1984). The enhancement of K^+ conductance at a pipette solution pH of 7·9 *versus* its inhibition at pH 6·2 can explain the comparable effects of pH_o 8·5 and 5·5 on the volume-regulatory response.

Quiescent human peripheral blood lymphocytes are pH-homeostatic, maintaining a pH_i of 7·1 (Deutsch *et al.* 1984). However, when cells are stimulated with mitogen a markedly different relationship of intra- to extracellular pH obtains. Although the kinetics for this conversion are different for different mitogenic stimuli, all mitogen-stimulated cells eventually (within 24 h of mitogen addition) maintain little or no pH gradient (Deutsch *et al.* 1984; Deutsch & Price, 1985). Thus, for any given external pH, the internal pH will be a function of the stage of the cell cycle: in activated cells, it will be considerably more acid below pH_o 7·1, and more alkaline above pH_o 7·1,

than in quiescent cells. We have found that the K^+ conductance of both quiescent and phorbol-ester-stimulated cells is sensitive to internal pH in the range through which lymphocyte pH changes in response to growth stimuli (Fig. 2). It is therefore possible that K^+ conductance is inhibited by acid pH *in situ* and that the requirement of the cells to maintain an essential level of net K^+ conductance leads to a compensatory up-regulation of channels. This could account for the increased current found in stimulated (cycling) cells (Matteson & Deutsch, 1984; Lee *et al.* 1986*b*; Deutsch *et al.* 1986; DeCoursey *et al.* 1987) when measured at pH_i 7·2.

It is well documented that membrane electrical properties modulate cell function, and that pH_i can mediate physiological control mechanisms. For instance, acid blocks the slow inward current in ventricular cells, which may serve as a protective response to ischaemia (Kurachi, 1982); and in pancreatic β-cells metabolic acid production may be a physiological regulator of the Ca^{2+}-activated K^+ conductance involved in secretagogue-induced insulin production (Cook *et al.* 1984). Similarly, internal acidification could function to decrease K^+ conductance and terminate cycle progression. Musgrove, Seaman & Hedley (1987) have shown that cytoplasmic acidification coincided with the onset of the transition from exponential growth to quiescence in human and mouse T cell leukemia and melanoma lines.

Is the K^+ conductance a requirement for growth? And, if so, is its modulation by cellular pH a means of physiological regulation? We have hypothesized that the K^+ conductance is necessary for volume regulation, and that the volume-regulatory response and the mitogenic response have overlapping pathways (Lee *et al.* 1988). Furthermore, if the ability of a cell to regulate its volume is necessary for cycle progression, then pH modulation of the K^+ conductance may be a feedback mechanism for growth control.

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