A Highly Temperature-sensitive Proton Current in Mouse Bone Marrow-derived Mast Cells

Miyuki Kuno, Junko Kawawaki, and Fusao Nakamura

From the Department of Physiology, Osaka City University Medical School, Abeno-ku, Osaka 545, Japan

Abstract Proton (H⁺) conductive pathways are suggested to play roles in the regulation of intracellular pH. We characterized temperature-sensitive whole cell currents in mouse bone marrow-derived mast cells (BMMC), immature proliferating mast cells generated by in vitro culture. Heating from 24 to 36°C reversibly and repeatedly activated a voltage-dependent outward conductance with Q₁₀ of 9.9 ± 3.1 (mean ± SD) (n = 6). Either a decrease in intracellular pH or an increase in extracellular pH enhanced the amplitude and shifted the activation voltage to more negative potentials. With acidic intracellular solutions (pH 5.5), the outward current was detected in some cells at 24°C and Q₁₀ was 6.0 ± 2.6 (n = 9). The reversal potential was unaffected by changes in concentrations of major ionic constituents (K⁺, Cl⁻, and Na⁺), but depended on the pH gradient, suggesting that H⁺ (equivalents) is a major ion species carrying the current. The H⁺ current was featured by slow activation kinetics upon membrane depolarization, and the activation time course was accelerated by increases in depolarization, elevating temperature and extracellular alkalization. The current was recorded even when ATP was removed from the intracellular solution, but the mean amplitude was smaller than that in the presence of ATP. The H⁺ current was reversibly inhibited by Zn²⁺ but not by bafilomycin A₁, an inhibitor for a vacuolar type H⁺-ATPase. Macroscopic measurements of pH using a fluorescent dye (BCECF) revealed that a rapid recovery of intracellular pH from acid-load was attenuated by lowering temperature, addition of Zn²⁺, and depletion of extracellular K⁺, but not by bafilomycin A₁. These results suggest that the H⁺ conductive pathway contributes to intracellular pH homeostasis of BMMC and that the high activation energy may be involved in enhancement of the H⁺ conductance.

Key words: H⁺ conductive pathway • pH regulation • temperature • ATP • recovery from acid-load

Introduction

The importance of intracellular pH (pHᵢ) regulation is unquestionable in various cellular functions, and the pHᵢ regulatory mechanisms vary considerably among different types of cells (Hoffmann and Simonsen, 1989). Recently voltage-activated H⁺ currents were found in certain cell types of hematopoietic (DeCoursey and Cherny, 1993; Demaurex et al., 1993; Kapus et al., 1993; Holevinsky et al., 1994; Nordström et al., 1995) and nonhematopoietic origin (Thomas and Meech, 1982; Byerly et al., 1984; DeCoursey, 1991; Krause et al., 1993). The H⁺ conductive pathway is considered to contribute significantly to pHᵢ homeostasis, as well as ion exchangers, H⁺ pumps and ion cotransporters. However, permeation mechanisms of the H⁺ conductance remain to be proved, and the putative H⁺ channel is suggested to be different from the general water-filled ion channels (DeCoursey and Cherny, 1995).

Bone marrow-derived mast cells (BMMC) are immature, proliferating mast cells differentiated from multi-

Address correspondence to Miyuki Kuno, MD, Ph.D., Department of Physiology, Osaka City University Medical School, Abeno-ku, Osaka 545, Japan. Fax: 06-645-2015; E-mail: kunomyk@msic.med.osaka-u.ac.jp

1 Abbreviations used in this paper: BCECF, 2',7'-bis-(2-carboxyethyl)-5(6)carboxy-fluorescein; BMMC, bone marrow-derived mast cells; EᵢH⁺, equilibrium potential for H⁺; pHᵢ, intracellular pH; pHₑ, extracellular pH; pHᵢ₀, the pH of pipette solution; Vᵢ₀, reversal potential.
of BMMC during metabolic acidosis associated with cell growth, production of large amounts of chemical mediators, or performance of other cellular functions. A preliminary account has been made (Kuno et al., 1995b).

MATERIALS AND METHODS

Cells
Bone marrow cells were obtained from the femoral and tibial bones of male, 8–10-week-old mice (Balb/ca). The mice were killed by an overdose of ether. Pokeweed mitogen–stimulated spleen cell-conditioned medium (PWM-SCM) was prepared as described elsewhere (Nakahata et al., 1982). Briefly, mouse spleen cells were incubated at 37°C in a 95% air–5% CO₂ atmosphere with pokeweed mitogen (1:300 dilution) (GIBCO BRL Products, Gaithersburg, MD) in α-MEM (INC Pharmaceuticals Inc., Irvine, CA) supplemented with 10% FCS (INC Pharmaceuticals Inc.), 10⁻⁴ M 2-mercaptoethanol, streptomycin (0.1 mg/ml), penicillin (100 U/ml), and amphotericin B (0.25 μg/ml). After 5 d of incubation, the supernatant (PWM-SCM) was filtered with a millipore filter (0.22 μm) and stored at −80°C. Bone marrow cells were plated at 10⁶/ml in 35-mm petri dishes and were incubated at 37°C in a 95% air–5% CO₂ mixture. The culture medium contained α-MEM, 10⁻⁴ M 2-mercaptoethanol, 10% FCS, and 10% PWM-SCM. Half of the medium was changed every week. Cells were incubated at 37°C with 14–20 mM KOH. The pH of the pipette solutions was decreased to compensate for the osmolality, and the pH was adjusted to 5.5 with 120 mM Mes, K-glutamate or CsCl was reduced to 100 mM in 10 mM NaCl, 5 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES, 10 mM glucose, and 0.1% BSA (pH = 7.3). Preliminary account has been made (Kuno et al., 1995b), the cuvette and teflon-coated spin bar were rinsed by ethanol before use. The rate of the pH recovery was estimated by differentiating the pH recordings.

Substances
A condensed stock solution of Na₂ATP (500 mM) was prepared in 1 M Tris Cl, stored in a freezer, and added to the internal medium before use. Condensed stock solutions of 4,4’-diisothiocyanato-2,2’-stilbene disulphonate (DIDS) and ZnCl₂ were dissolved in distilled water. The free concentration of Zn²⁺ was not determined and the concentration described herein is only nominal, since Zn²⁺ forms complexes with anions. Bafilomycin A₁ was prepared in ethanol. The final concentration of ethanol was ≤0.5%. BCECF-AM, CHES and Mes were obtained from Dojindo Laboratories (Kumamoto, Japan), and other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

RESULTS

Elevation of Temperature Activates an Outward Current
Fig. 1 illustrates a representative time course of changes in the whole cell current when temperature of the recording chamber was altered (top). Upward and downward vertical lines in the bottom trace represent outward and inward currents evoked by voltage ramps (200 mV/s) applied at a holding potential of 0 mV.
(middle). Heating from 24 to 36°C reversibly and repeatedly activated outward currents both in the K⁺-rich (150 mM K⁺) and the standard Ringer (5 mM K⁺) solutions. Fig. 1, B and C, show superimposed current-voltage (I-V) relations obtained by the voltage ramps during heating in the K⁺-rich (B) and the standard Ringer (C) solutions.

Temperature-conductance relationships (see materials and methods) in three cells show that heating from 24 to 36°C gradually increased the outward conductance, although the magnitude varied greatly among cells (Fig. 2 A). The inward conductance of these cells remained unchanged or increased only slightly by the heating (Fig. 2 B). Fig. 2 C summarizes semilogarithmic plots of the outward conductance against temperature in six cells with a linear regression for all data. The conductance was normalized to that measured at 36°C. The factor by which the conductance changes per 10°C elevation (Q₁₀) was 9.9 ± 3.1 (n = 6).

Although the outward current described above was recorded at pHᵢ (pH of the pipette solution) 7.3, the current larger than 10 pA at the end of a 500-ms voltage pulse of +100 mV was evident only in 7 of 19 cells at 32°C. The current was seen more frequently at lower pHᵢ. At pHᵢ 5.5, 80 of 103 cells exhibited the outward current, although the percentage of cells with the current varied among different cell batches from 70 to 92%. The magnitude normalized by cell capacitance was generally much smaller at pHᵢ 7.3 (2.4 ± 7.3 pA/pF, n = 19) than that at pHᵢ 5.5 (9.6 ± 9.5 pA/pF, n = 103). At pHᵢ 5.5, the outward current was detected in some cells even at 24°C and was enhanced by heating with Q₁₀ of 6.0 ± 2.6 (n = 9). In later experiments, the heating-activated outward current was analyzed at pHᵢ 5.5 to optimize the detection of the current unless described otherwise.

Fig. 3 A shows a family of currents activated by depolarization pulses in +20-mV increments applied at −40 mV at 32°C. The outward current was characterized by slow activation kinetics at depolarization, differing from the rapidly activating, outwardly rectifying Cl⁻ current that we reported previously (Kuno et al., 1995a). Activation followed a single exponential time course (thin lines) after a small delay and was facilitated at more positive potentials. The activation time course was also greatly

![Figure 1](https://www.jgp.org/content/41/6/766/F1.large.jpg)

**Figure 1.** Effects of changes in temperature on whole cell current. (A) Temperature (top), voltage (middle), and current (bottom) recordings during changes in bath temperature between 24 and 36°C. Vertical lines in the bottom indicate currents associated with voltage ramps applied at 0 mV. The cell was suspended first in the K⁺-rich (150 mM) solution and then in the standard (5 mM K⁺) solution (pHᵢ = 7.3). (B and C) Current-voltage relations obtained by voltage ramps during heating in 150 and 5 mM K⁺ solutions. *Denotes data at 24°C. A–C were obtained from the same cell. The pipette contained K-glutamate (pHᵢ = 7.3).

![Figure 2](https://www.jgp.org/content/41/6/766/F2.large.jpg)

**Figure 2.** Temperature-conductance relationships. (A and B) Outward (A) and inward (B) conductances of three cells plotted against temperature. Conductances were estimated from I-V curves obtained by voltage ramps applied at 0 mV, between +50 and +100 mV for outward currents and between −110 and −80 mV for the inward currents. Curves are the second order polynomial fit for data. (C) Semilogarithmic plots of the outward conductance against temperature. Data were normalized as the partition of the amplitude at 36°C. Different symbols indicate data from different cells (n = 6). The external solution contained the standard Ringer and the internal solution contained K-glutamate (pHᵢ/ pHᵢ = 7.3/7.3). The line is a least square’s fit for all data.
Temperature-sensitive $H^+$ Current

Averaged I-V plots obtained from different cells at three $\text{pH}_p$ (5.5–7.3) are presented in Fig. 4A. The $\text{pH}_o$ was the same (7.3) in all cases. The currents were measured at the end of 500-ms voltage pulses. The acidic intracellular solutions were buffered either with 10 or 120 mM Mes. Little current was detectable at potentials more negative than 0 mV. Although there was cell-to-cell variability in the potential at which activation occurred, lowering $\text{pH}_p$ shifted the voltage dependency to a more negative direction. On the other hand, elevating $\text{pH}_o$ from 7.3 to 8.7 reversibly shifted the I-V curves to a more negative direction in all cells tested ($n=12$) (Fig. 4B). This shift by extracellular alkalization was observed even in cells recorded with the pipette containing CsCl (Fig. 4C). The time course of activation was accelerated by increasing $\text{pH}_o$. At +80 mV with $\text{pH}_p$ 5.5, the time constant at $\text{pH}_o$ 8.2–8.7 was $327 \pm 61$ ms ($n=5$), significantly faster than that at $\text{pH}_o$ 7.3 (625 ± 255 ms, $n=11$; $P<0.05$).

The outward currents appeared within 10–30 s after the rupture of the patch membrane to form the whole cell configuration with the acidic pipette solutions buffered by either 10 or 120 mM Mes. Thus BMMC seemed to be acidified quickly, probably because of the small cell size (radius: $5.1 \pm 0.7 \mu m$, $n=142$). The current amplitude at the end of a 500-ms voltage pulse of +100 mV at $\text{pH}_p$ 5.5 was $8.9 \pm 10.0$ pA/pF ($n=63$) with 10
mM Mes, not significantly different from that with 120 mM Mes (10.8 ± 8.7 pA/pF, n = 40). I-V relations obtained by 500-ms voltage pulses from -100 to +100 mV, and intermittent voltage ramps were not affected by the buffering power. However, when a 1-s depolarizing pulse (+100 mV) was repetitively applied at an interval of 3 s, a gradual rundown of the outward current was observed with 10 mM Mes (by 19.1 ± 18.6% per 10 pulses, n = 16) but not with 120 mM Mes (by -1.5 ± 11.0% per 10 pulses, n = 14). In addition, when the cells were exposed to an 8-s depolarization (+60 mV), the current amplitude gradually increased to a peak and then reduced to 87.0 ± 9.3% (n = 11) of the maximum at the end of the depolarization with 10 mM Mes and 95.2 ± 5.6% (n = 11) with 120 mM Mes. The decline was greater with the lower concentration of Mes (P < 0.05). Thus a high buffering power in pipette solutions was needed to maintain the current.

To determine ion species carrying the current, the currents were studied under various experimental conditions. The outward current was observed both when the extracellular Cl\(^-\) ([Cl\(^-\)\(_o\)]) was totally replaced by isethionate\(^-\) (Fig. 5 A) and when the intracellular K\(^+\) was totally replaced by Cs\(^+\) (Fig. 5 B). Tail currents at 0 mV after the depolarizations were directed outwardly (arrow) and were fit by single exponential curves as in-

**Figure 5.** Heating-activated outward current recorded in various internal and external solutions. Whole cell currents evoked by voltage pulses up to +100 mV (A) or +120 mV (B and C) in +10-mV increments at pH\(_o\) 5.5 (32°C). The holding potential was 0 mV. The internal solution was buffered with 120 mM Mes. Selected traces of tail currents (arrows) were magnified with superimposed single exponential fits (right). (A) The internal and external media contained K-glutamate and Na-isethionate, respectively. Tail currents induced by prepulse of +10, +30, +50, +70, and +100 mV are superimposed. (B) The internal and external media contained CsCl and Na-isethionate. Tail currents with prepulse of +10, +30, +40, +60, and +100 mV are superimposed. (C) The internal and external media contained K-glutamate and NaCl. Tail currents at +10, +70, +90, +100, and +120 mV are superimposed. 50 μM of DIDS was added into all external media. pH\(_o\): 7.3 (A and B) and 6.0 (C).

**Figure 6.** Reversal potential of heating-activated outward current. (A) Tail currents after a depolarization prepulse (+80 mV) at pH\(_o\) 5.5. The outward current evoked by the prepulse is truncated. (B) Relationships between pH\(_o\) and the reversal potential (V\(_{rev}\)) estimated from tail currents following a prepulse of either +80 or +100 mV (1 s). Open and closed circles represent mean and SD of data recorded with 10 and 120 mM Mes. The solid curves are fitted by eye and the dotted line, a linear regression for data at pH\(_o\) 6.0, 6.7, and 7.3 with 120 mM Mes. Figures attached with data indicate the number of cells. (C) V\(_{rev}\) plotted against the ratio between extracellular and intracellular Cl\(^-\) concentrations ([Cl\(^-\)\(_o\)]/[Cl\(^-\)\(_i\)]) on a semilogarithmic scale. Open and closed squares represent data recorded with K-glutamate and CsCl in the internal solution, respectively. B and C were obtained from the same data. The external medium was same (standard Ringer) except for pH\(_o\).
dicated by superimposed thin lines in the magnified record (right). In these recordings, the external medium contained a Cl\(^{-}\) channel blocker, DIDS (50 \(\mu\)M), which blocks the Cl\(^{-}\) current in BMMC (Kuno et al., 1995a). Addition of a K\(^{+}\) channel blocker, Ba\(^{2+}\) (1 mM) did not affect the current. When the pH\(_{o}\) was 6.0, the outward current was small in most cells, but sizable currents were observed in a few cells (Fig. 5 C). Tail currents in these cells were going inwardly at 0 mV, suggesting that lowering pH\(_{o}\) from 7.3 to 6.0 shifted the reversal potential (V\(_{rev}\)) to a more positive level.

The V\(_{rev}\) was estimated from the I-V plots for instantaneous tail currents recorded in cells with larger currents. The voltage protocols were made of test pulses after the 1-s prepulse (either +80 or +100 mV) (Fig. 6 A). The amplitudes of tail currents at the start of each test pulse were determined from the single exponential fit as shown in Fig. 5. Closed circles in Fig. 6 B represent the averaged V\(_{rev}\) at pH\(_{o}\) 5.5 recorded with 120 mM Mes in the pipette solutions. The V\(_{rev}\) became more negative in alkaline media. The dashed line represents a linear regression for data at pH\(_{o}\) 6.0–7.3 with 120 mM Mes, having a slope of 45.5 mV per \(\Delta\)pH\(_{o}\) of 1. Data at higher pH\(_{o}\) deviated from the regression line. Departures from the line were more prominent with 10 mM Mes (open circles). When the V\(_{rev}\) was replotted against the ratio between extracellular and intracellular Cl\(^{-}\) concentrations ([Cl\(^{-}\])o and [Cl\(^{-}\])i) on a semilogarithmic scale (Fig. 6 C), the V\(_{rev}\) did not depend on the Cl\(^{-}\) gradient. Open and closed squares represent data recorded with pipette containing K-glutamate and CsCl, respectively, showing that the V\(_{rev}\) did not depend on the K\(^{+}\) concentration. These results suggest that the heating-activated outward current is carried primarily by H\(^{+}\) (equivalents).

**Effects of Zinc, Bafilomycin A\(_{1}\), and ATP on the H\(^{+}\) Current**

A blocker for H\(^{+}\) conductances, ZnCl\(_{2}\) (0.25–0.5 mM), reversibly suppressed the outward current to 10 ± 10.3\% (\(n = 8\)) of the controls (Fig. 7). On the other hand, the current amplitude in the presence of bafilomycin A\(_{1}\) (100 nM), a potent and selective inhibitor for the H\(^{+}\)-ATPase (Bowman et al., 1988), was 47.1 ± 37.5 pA at the end of a 500-ms pulse of 100 mV (\(n = 20\)), not significantly different from the controls (56.2 ± 41.0 pA; \(n = 22\)). It seems that the H\(^{+}\) current is not mediated via an electrogenic vacuolar type H\(^{+}\)-ATPase.

As high sensitivity to temperature implicated that a high activation energy would be involved in the current activity, we examined the effects of intracellular ATP on the H\(^{+}\) current. Fig. 8 shows I-V plots from cells recorded with ATP-containing (A) and ATP-omitting (B) pipette solutions. The data were obtained from the same batch of cells, to avoid batch-to-batch variation. The H\(^{+}\) conductance recorded without ATP was 104 ± 54 pS/pF (\(n = 10\)), significantly smaller than that observed with ATP (216 ± 143 pS/pF; \(n = 10\) (\(P < 0.05\)). Thus, ATP might be involved in potentiation of the H\(^{+}\) current.

**Figure 7.** Blockade of H\(^{+}\) current by Zn\(^{2+}\). Whole cell currents evoked by voltage pulses from −80 to +100 mV in 20-mV steps applied at −40 mV at 32°C (pH\(_{p}\)/pH\(_{o}\) = 5.5/7.3). The outward current was reversibly inhibited by 0.25 mM ZnCl\(_{2}\). The internal solution contained K-glutamate and 120 mM Mes and the external solution, Na-isethionate.

**Figure 8.** Effects of intracellular ATP on H\(^{+}\) current. I-V plots normalized by the cell capacitance for each 10 cells recorded with ATP (1 mM)-containing (A) and ATP-omitting (B) intracellular solutions. The current amplitude was measured at the end of 500-ms voltage pulses applied at −40 mV at 32°C (pH\(_{p}\)/pH\(_{o}\) = 5.5/7.3). The pipette solution was buffered with 120 mM Mes.
Measurements of Macroscopic pH$_i$ Recovery in Acid-loaded Cells

To assess the role of the H$^+$ conductance in regulation of pH$_i$, pH$_i$ recovery from acid-load in populations of BMMC was measured using a fluorescent pH-sensitive dye, BCECF (Fig. 9). Washing cells preincubated with NH$_4$Cl by ammonium-free solutions induced intracellular acidification (Roos and Boron, 1981), followed by a pH$_i$ recovery in an Na$^+$-free K$^+$-rich solution at 36°C (Fig. 9, closed circles) (see materials and methods). Although the time course and the resultant pH level at ~20 min differed among preparations, the pH$_i$ recovery was characterized by an initial rapid phase within 5–10 min. The Na$^+$-independent pH$_i$ recovery was reduced at 24°C (Fig. 9 A). In an Na$^+$-free NMG$^+$-rich medium (Fig. 9 B), which would hyperpolarize cells, the pH$_i$ recovery was greatly inhibited, suggesting that depolarization is required for the rapid H$^+$ efflux. Addition of 0.5 mM ZnCl$_2$ attenuated the pH$_i$ recovery (Fig. 9 C), but bafilomycin A$_1$ (100 nM) did not (Fig. 9 D).

The inhibitory effect of low temperature and Zn$^{2+}$ seemed to be more prominent during the initial phase of pH$_i$ recovery. The rate of the pH$_i$ change per time (ΔpH/min), obtained by differentiating the pH$_i$ recordings, was attenuated by lowering temperature (open circles) and was greatly inhibited by 0.5 mM Zn$^{2+}$ (squares) (Fig. 10). The rate at 0 min was 0.049 ± 0.029 pH U/min (n = 7) at 24°C, 0.010 ± 0.010 pH U/min (n = 5) in the presence of Zn$^{2+}$, and 0.110 ± 0.068 pH U/min (n = 12) in their controls. These results support an idea that a temperature- and Zn$^{2+}$-sensitive H$^+$ conductive pathway is responsible for a rapid pH$_i$ regulation during intracellular acidification. The slow pH$_i$ recovery remaining in the presence of Zn$^{2+}$ may be mediated by unidentified H$^+$ efflux mechanisms other than the H$^+$ conductance.

Discussion

A Temperature-sensitive H$^+$ Conductance

We recently reported that an electrophysiological profile of BMMC was heterogeneous at room temperature, such that an inwardly rectifying K$^+$ current and an outwardly rectifying Cl$^-$ current were exhibited in subpopulations (Kuno et al., 1995a). The present study described a voltage-activated outward current which was negligible or small at room temperature but was remarkably augmented by heating up to 36°C. H$^+$ (equivalents) was suggested to be an ion species primarily responsible for the heating-activated current from several lines of evidence. First, either a decrease in pH$_p$ or an increase in pH$_c$, increased the current amplitude and shifted the activation voltage to more negative potential.
tials. Second, the current was observed even when the extracellular Cl\(^-\) was replaced by an impermeable anion, isethionate\(^-\), and when the intracellular K\(^+\) was replaced by Cs\(^+\). Third, the \(V_{\text{rev}}\) was dependent on the H\(^+\) gradient but was unaffected by the substitution of other major ion constituents (Cl\(^-\), K\(^+\), Na\(^+\)). Fourth, the current was not inhibited by K\(^+\) or Cl\(^-\) channel blockers (Ba\(^{2+}\) and DIDS) but by Zn\(^{2+}\), a blocker for voltage-activated H\(^+\) currents (Kapus et al., 1993).

The H\(^+\) current in BMMC shares major properties of that in other cell types (voltage- and time-dependent activation, outward rectification, sensitivity to both pHo and pH\(_i\), and a blockage by heavy metals) but is distinct in its high sensitivity to temperature. Although H\(^+\) currents of snail neurons have a stronger temperature sensitivity than K\(^+\) currents over a temperature range of 10–25°C (Byerly and Suen, 1989), sizable H\(^+\) currents were described in many mammalian cells at room temperature. It is possible that the H\(^+\) conductive pathway comprises a diverse family of related types whose characteristics may be tissue specific and that activation mechanisms of the H\(^+\) current in BMMC may differ from that in other cells. However, literature on the sensitivity of voltage-activated H\(^+\) currents to temperature is too scarce to draw this conclusion at this moment.

**Characteristics of the H\(^+\) Current in BMMC**

Although H\(^+\) (equivalents) was estimated to be a primary constituent for the heating-activated outward current in BMMC, the \(V_{\text{rev}}\) deviated from the \(E_{\text{pp}}\) predicted by the Nernst formula especially at high pH\(_{\text{pp}}\) even when the current was recorded with a high buffering power (120 mM Mes) in the pipette solutions. Prepulse protocols consisted of different durations (0.5–4 s), and the holding potentials (0 and \(-60\) mV) did not affect the result. Similar deviation of the \(V_{\text{rev}}\) was often described with H\(^+\) currents in other cell types (Mahaut-Smith, 1989; DeCoursey, 1991; Bernheim et al., 1993; Demaurex et al., 1993; Kapus et al., 1993), and some intrinsic buffering action or diffusion limitation suggested a deviation from the nominal pH\(_i\) value (DeCoursey, 1991; Kapus et al., 1993; Holevinsky et al., 1994). The deviation of the \(V_{\text{rev}}\) from the expected value was greater with 10 mM Mes, although the buffering power of the pipette solutions (10 or 120 mM Mes) did not significantly affect the current amplitude obtained by 500-ms step pulses or ramp pulses from \(-100\) to \(+100\) mV. This may result from depletion of the intracellular H\(^+\) concentration during repetitive prepulses. Decline of voltage-activated H\(^+\) currents during long-lasting depolarization has been reported (Barish and Baud, 1984; DeCoursey, 1991; Demaurex et al., 1993; Kapus et al., 1993) and is explained by depletion of H\(^+\) currents caused by preceding H\(^+\) efflux (Kapus et al., 1993). It seems that a high intracellular buffering power is needed to maintain the H\(^+\) current during long-lasting or repetitive depolarization.

So far, direct electrophysiological evidence that the H\(^+\) currents are mediated by channels is not available (Lukacs et al., 1993), as the current noise is small and single channel current is hardly seen even in isolated patches (DeCoursey and Cherny, 1995). Noise analysis estimated that the single channel conductance is \(<10\) S (Byerly and Suen, 1989; Bernheim et al., 1993; DeCoursey and Cherny, 1993). In isolated patches of alveolar epithelial cells, H\(^+\) permeation mechanisms through voltage-activated H\(^+\) channels suggest a difference from those permeated through water-filled ion channels (DeCoursey and Cherny, 1995). The current noise was small and was not augmented by depolarizations in BMMC as well. Little noise, high sensitivity to temperature, and a slow activation rate raises a possibility that H\(^+\)-conducting molecules in BMMC may be intermediate between channel and carrier proteins, although this is only conjecture.

**Effects of ATP on the H\(^+\) Current**

A vacuolar type H\(^+\)-ATPase is known as an electrogenic H\(^+\) transport system. The H\(^+\)-ATPase, generally localized in membranes of intracellular organelles, is found in the plasma membrane of osteoclasts (Väänänen et al., 1990) and macrophages (Swallow et al., 1988). The F\(_o\) component of the H\(^+\)-ATPase in osteoclasts forms a channel protein to secrete H\(^+\) (Junge, 1989). As osteoclasts generate from bone marrow cells, it is conceivable that BMMC would possess a similar plasmalemmal H\(^+\)-ATPase. However, the H\(^+\) current in BMMC cannot be well explained by a vacuolar type H\(^+\)-ATPase for the following reasons: First, the current is recorded in some cells even when ATP is omitted from the intracellular milieu. Second, bafilomycin A\(_1\), a selective and potent blocker for the vacuolar type H\(^+\)-ATPase (Bowman et al., 1988), does not block the current. Third, immunohistochemical studies documented that the antibody for the vacuolar H\(^+\)-ATPase labels osteoclasts but not other cell types of bone marrow (Väänänen et al., 1990). Fourth, the ATPase can pump H\(^+\) against a 10,000-fold uphill concentration gradient (Heldrich et al., 1989), but only outward currents are activated at potentials positive to \(E_{\text{pp}}\) in BMMC. On this basis and together with the electrophysiological properties (steep voltage-dependence, gating kinetics, the \(V_{\text{rev}}\)), the H\(^+\)-ATPase is unlikely to mediate the H\(^+\) current.

In many cell types, H\(^+\) currents are recorded in the absence of ATP (Byerly and Suen, 1989; DeCoursey, 1991; DeCoursey and Cherny, 1993; Kapus et al., 1993), and a dependency on ATP has not been described. Although the H\(^+\) current was activated in some BMMC even without ATP, the current amplitude was smaller...
Measurements of pH in using BCECF provide evidence that in some phagocytes but is roughly comparable to that reported in various cell types (DeCoursey and Cherny, 1994). It is conceivable that metabolic acidosis accompanies cell growth of BMMC as reported in other cell types (Gerson and Kiefer, 1982; Grinstein and Dixon, 1989). Otherwise, maintenance of pH may be challenged by releasing large quantities of chemical mediators during anaphylactic actions or in bacterial infection (Echtenacher et al., 1996; Malaviya et al., 1996). The H⁺ efflux mechanism dissipates metabolic acids generated during these cellular activities (Lukacs et al., 1993). In addition, many mast cell activities are pH dependent: for example, exocytosis is influenced by pH (Alfonso et al., 1994), and enzymes, such as chinas, are strongly pH dependent (McEuen et al., 1995). Thus the H⁺ conductance may play a significant role in pH regulation of mast cells in various functional states, as in other cells (Hoffman and Simonsen, 1989).

Electrophysiological studies are often conducted at room temperature, which may cause one to overlook the H⁺ current. Our results imply that H⁺ conductive pathways are expected to exist more widely.

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