J. Membrane Biol. 143, 115–122 (1995)

The Journal of
Membrane
Biology

A Heterogeneous Electrophysiological Profile of Bone Marrow-derived Mast Cells

M. Kuno[1], T. Shibata[1], J. Kawawaki[1], I. Kyogoku[2]

[1]Department of Physiology, Osaka City University, Medical School, Abeno-ku, Osaka 545, Japan
[2]Division of Internal Medicine, Juso Municipal Hospital, Yodogawa-ku, Osaka 532, Japan

Received: 22 February 1994/Revised: 20 September 1994

Abstract. Electrophysiological properties of mouse bone marrow-derived mast cells (BMMC) were studied under the whole-cell clamp configuration. About one third of the cells were quiescent, but others expressed either inward or outward currents. Inwardly rectifying (IR) currents were predominant in 14% of the cells, and outwardly rectifying (OR) currents in 24%. The rest (22%) of the cells exhibited both inward and outward currents. The IR currents were eliminated by 1 mM Ba^{2+}, and were partially inhibited by 100 μM quinidine. The reversal potential was dependent on extracellular K^+, thereby indicating that K^+ mediated the IR currents. The negative conductance region was seen at potentials positive to E_K. The OR currents did not apparently depend on the extracellular K^+ concentration, but were reduced by lowering the extracellular Cl^- concentration. The OR currents were partially blocked by 1 mM Ba^{2+}, and were further blocked by a Cl^- channel blocker, 4,4′-diisothiocyano-2,2′-stilbenedisulfonate (DIDS). In addition, the reversal potential of the OR currents was positively shifted by decreasing the ratio of external and internal Cl^- concentrations, suggesting that Cl^- was a major ion carrier. In cells exhibiting IR currents, the membrane potential varied among cells and tended to depolarize by elevating the external K^+ concentration. In cells with OR currents, the resting potential was hyperpolarized in association with an increase in conductance. These results suggest that BMMC have a heterogeneous electrophysiological profile that may underlie a variety of ion channels expressed in different phenotypes of mast cells. Activities of both the inwardly rectifying K^+ channel and the outwardly rectifying Cl^- channel seem to contribute to the regulation of the membrane potential.

Key words: Inward rectifier — K channels — Cl channels — Differentiation

Correspondence to: M. Kuno

Introduction

Mast cells originate from multipotential hematopoietic stem cells (Kitamura et al., 1981; Nakahata et al., 1982) and differentiate into different phenotypes, the mucosal and connective tissue types, in the peripheral tissues (Nakano et al., 1985). Responsiveness to antigen and secretagogues, size of secretory granules and patterns of exocytosis are different in the two types (Enerbäck, 1986). Recent electrophysiological studies noted that ion channels in rat peritoneal mast cells (the connective tissue type) and a tumor analogue of mucosal type mast cells, rat basophilic leukemia cells (RBL-2H3), were quite different: In the resting state, the inwardly rectifying K (IR_K) channels were predominant in RBL-2H3 cells (Lindau & Fernandez, 1986), but all currents were negligible in rat peritoneal mast cells (Lindau & Fernandez, 1986; Kuno et al., 1989). In the latter cells, however, secretagogues activated a variety of channels, such as Ca^{2+}-permeable channels (Kuno, Okada & Shibata, 1989; Kuno et al., 1990; Kuno & Kimura, 1992), cation-selective channels (Penner et al., 1988; Kuno et al., 1989) and an outwardly rectifying Cl^- channel (Penner, Matthews & Neher, 1988; Matthews, Neher & Penner, 1989), via actions of second messengers.

Bone marrow-derived mast cells (BMMC) obtained by an in vitro culture of mouse bone marrow stem cells in the presence of mast cell growth factors (Ihle et al., 1983; Razin et al., 1984; Fujita et al., 1988) have the potency to differentiate into the different phenotypes in appropriate microenvironments (Nakano et al., 1985; Fujita et al., 1988). Thus, electrophysiological studies of BMMC are intriguing in understanding the significance of ion channel expression in either proliferation or differentiation of mast cells. Here we report that BMMC have a heterogeneous electrophysiological profile: either an inwardly rectifying K^+ channel or an outwardly rectifying Cl^- channel is expressed in subpopulations of BMMC. A preliminary account has been made (Kuno, Kyogoku & Kawawaki, 1993).

Materials and Methods

Cells

Bone marrow cells were obtained from the femoral and tibial bones of male, 10-week-old mice (Balb/ca). 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% CO_2 atmosphere with pokeweed mitogen (1:300 dilution, GIBCO) in α-MEM (Flow Laboratories) supplemented with 10% fetal calf serum (FCS) (Flow Laboratories), 10^{-4} M 2-mercaptoethanol (Sigma), streptomycin (0.1 mg/ml, Sigma), penicillin (100 U/ml, Sigma) and amphotericin B (0.25 μg/ml, Sigma). After five days of incubation, the supernatant (PWM-SCM) was filtered with a Millipore filter (0.22 μm) and stored at −85°C. Bone marrow cells were plated at 10^6/ml in 35 mm petri dishes containing a few cover glasses (Matsunami #1) and incubated at 37°C in a 95% air-5% CO_2. The culture medium contained α-MEM, 10^{-4} M 2-mercaptoethanol, 20% horse serum (Flow Laboratories) and 10% PWM-SCM. In later experiments, 10% FCS was added as a substitute for horse serum. Half of the medium was changed every week. Cells cultured for more than four weeks were used in the experiments.

Recordings

Current signals were recorded from the whole-cell voltage clamp configuration and the membrane potential was measured under the current clamp mode (Hamill et al., 1981) at room temperature (20–24°C). The reference electrode was an Ag-AgCl wire connected to the bath solution through a Ringer-agar bridge. Pipette resistance ranged between 5–20 MΩ. The zero-current potential before formation of the gigaseal was taken as 0 mV. Pipettes contained (mM): 150 K-glutamate, 7 $MgCl_2$, 1 EGTA, 10 HEPES-KOH (pH, 7.3). In most experiments, 1 mM Na_2ATP was added to the internal medium. Similar results were obtained both with and without ATP. Several solutions were used as the external medium. Standard Ringer solution contained (mM): 145 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 glucose, 0.1% bovine serum albumin (pH = 7.3). NaCl was replaced by KCl in some experiments to make K-rich (30 and 150 mM) solutions. A low Cl^- solution was prepared by replacing NaCl with Na-isethionate. When cells were perfused with external solutions containing different ionic compositions, the liquid junction potentials were measured after the seal was broken, and the membrane potential was corrected to compensate for the difference. The difference between the junction potentials measured before the gigaseal was formed and after it was broken in the same solution was within a few millivolts, as far as the pipette resistance remained in the similar range. A further correction was not made in each experiment.

The current signals, led into a patch clamp amplifier (EPC7, LIST) without series resistance compensation, were filtered at 1 kHz by a low-pass filter with Bessel characteristics with 48 dB/octave slope attenuation (P-84, NF), digitized at 1–4 kHz with an analog-digital converter (MacLab/4, Analogue Digital Instruments) and stored on a personal computer. Sampling rate at 1 kHz reduced capacitance transients but did not apparently distort the results described herein. Cell capacitance was estimated with capacitive cancellation circuitry on the amplifier. Capacitance transients evoked by a −5 mV voltage pulse were sampled at 4 kHz during the cancellation. To obtain conductances of IR and OR currents, a linear leak current estimated from the current-voltage (*I-V*) curve negative to E_K in the presence of Ba^{2+} was subtracted. All data were expressed as mean ± SD.

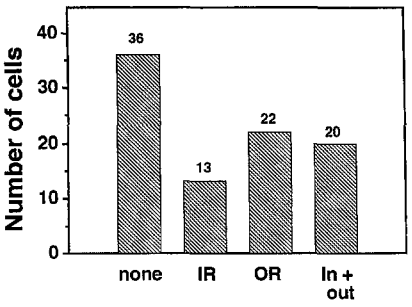

Fig. 1. Bone marrow-derived mast cells (BMMC) with different current patterns. The current patterns are classified into four types: no current activity (*none*), inwardly rectifying (*IR*) currents, outwardly rectifying (*OR*) currents, and inward plus outward currents (*In + out*) (from left to right).

Substances

Condensed stock solution of Na_2ATP (Sigma, 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 quinidine (Sigma), $BaCl_2$ and 4,4′-diisothiocyano-2,2′-stilbenedisulfonate (DIDS, Sigma) were dissolved in distilled water.

Staining of Cells

Bone marrow cells cultivated on a coverslip (15 mm in diameter) were fixed in Carnoy's solution and stained with alcian blue as described elsewhere (Worthington, 1962). Densely stained cells identified as BMMC appeared within 2–4 weeks in the presence of PWM-SCM.

Results

Whole-Cell Currents of Bone Marrow-derived Mast Cells (BMMC)

Within 2–4 weeks of culture of bone marrow cells with PWM-SCM, homogeneous nonadherent cells with a diameter of 7–11 μm proliferated and were maintained for more than 10 weeks. Since the cells were stained with alcian blue, they were identified as bone marrow-derived mast cells (BMMC) (Nakano et al., 1985). We recorded whole-cell membrane currents from BMMC cultured for more than four weeks. Although about one-third of the cells were quiescent, other cells showed inwardly rectifying (IR; in 14% of the cells), outwardly rectifying (OR; 24%), or both inward and outward currents (22%) (Fig. 1). Figure 2 shows examples of whole-cell currents recorded from three cells bathed in a K-rich (150 mM K^+) medium: IR (*A*), inward plus outward (*B*) and OR currents (*C*). A part of currents with both inward and outward directions may be the sum of the OR and IR currents, while currents without apparent rectification were seen in some cells. Those currents were seen in subpopulations over the culture period up to 15 weeks.

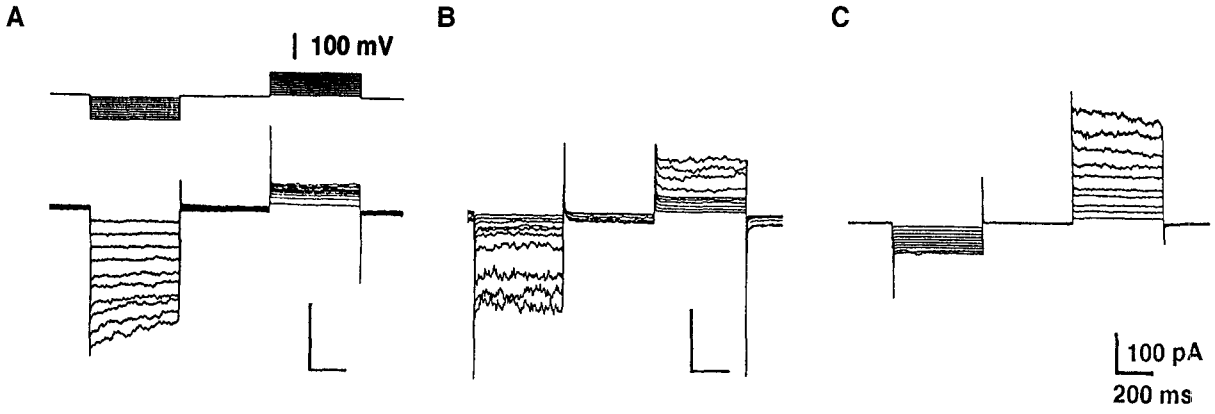

Fig. 2. Whole-cell currents recorded in the K-rich (150 mM KCl) medium. Superimposed currents evoked by step voltage pulses in three cells. (*A*) Inwardly rectifying (IR) currents. (*B*) Both inward and outward rectifying currents. (*C*) Outwardly rectifying currents. Command voltage pulses were applied from the holding potential (+13 mV in *A*, 0 mV in *B* and *C*) in ±10 mV increments. Leak currents were not subtracted.

Inwardly Rectifying (IR) Currents

Figure 3 shows current-voltage (*I-V*) relations of IR currents obtained by applying voltage ramps in the K^+-rich (150 mM) medium. IR currents were eliminated by the addition of 1 mM $BaCl_2$ into the external medium (Fig. 3*A*) and were reduced to 30–50% of the controls by 0.1 mM Ba^{2+} (*data not shown*). Quinidine (100 μM) partially inhibited the IR currents (Fig. 3*B*). Representative *I-V* relations of the Ba^{2+}-sensitive currents, obtained by subtracting the currents after addition of Ba^{2+} from that before, are shown in Fig. 4*A*. When the concentration of external K^+ was increased from 2.8 to 150 mM, the reversal potential of the current-voltage relation was positively shifted with an enhancement of the inward currents (Fig. 4*A*). The right figure is the *I-V* curve at higher gain in the presence of 2.8 mM K^+, indicating a slightly negative slope region positive to the reversal potential, similar to that reported in the RBL-2H3 cell line (Lindau & Fernandez, 1986). The reversal potentials of the IR currents were −89.6 ± 9.8 mV (mean ± SD, $n = 4$) with 2.8 mM K^+, −73.6 ± 16.4 mV ($n = 7$) with 5 mM K^+, −34.6 ± 8.3 mV ($n = 4$) with 30 mM K^+ and 6.9 ± 6.2 mV ($n = 10$) with 150 mM K^+ (Fig. 4*B*). A linear fit of the mean reversal potentials to extracellular K^+ on a semilogarithmic scale (a line in Fig. 4*B*) had a slope of 55 mV per tenfold change in the extracellular K^+, indicating that the IR currents were mediated by K^+ (IR_K).

The conductance of IR_K was greater with higher concentrations of extracellular K^+ in the same cell (Fig. 4*A*) similar to IR_K in RBL-2H3 cells (Lindau & Fernandez, 1986), but varied among different cells. Mean and SD of the conductance was 0.26 ± 0.17 nS ($n = 12$) with 5 mM K^+, 0.62 ± 1.05 nS ($n = 6$) with 30 mM K^+ and 1.66 ± 2.07 nS ($n = 12$) with 150 mM K^+. Larger cells tended to possess IR of larger conductance, but factors other than the cell membrane area also seemed to cause this variation as the conductance in cells with similar cell capacitance greatly varied (Fig. 4*C*).

Outwardly Rectifying (OR) Currents

Outwardly rectifying (OR) currents were more frequently seen than IR currents. Lowering the external K^+ concentration from 150 to 5 mM did not always change the OR (Fig. 5*A*), although reduction to some extent was observed in some cells. In the Ba^{2+}-free K^+-rich (150 mM) medium, the conductance was 1.43 ± 1.27 nS ($n = 12$), and 1 mM Ba^{2+} partially blocked the OR currents (Fig. 5*A*) by 14 ± 23% ($n = 4$). OR currents, however, still remained (0.62 ± 0.28 nS, $n = 7$) even in the presence of 50 mM $BaCl_2$ (Fig. 5*B*, control). Addition of 20 μM of DIDS, a Cl^- channel blocker, further reduced the OR currents (Fig. 5*B*). With pipettes containing Cs (150 mM CsCl or 150 mM Cs-methanesulfonate), the conductance was 0.35 ± 0.22 nS ($n = 3$) in the presence of 50 mM $BaCl_2$ and 1.71 ± 0.37 nS ($n = 3$) in the Ba^{2+}-free medium.

The reversal potential of the OR currents recorded in a K^+-rich (150 mM KCl) medium was −41.7 ± 13.2 mV ($n = 11$), which estimated the permeability ratio of Cl^- to K^+ as 7.8 from the Goldmann-Hodgkin-Katz equation. The OR currents were markedly reduced when the external Cl^- was substituted by an impermeant anion, isethionate in five cells examined (Fig. 6*A*). The conductance in the low Cl^- medium was 0.16 nS in one cell and negligible in four cells. Figure 6*B* shows the reversal potential of the OR currents plotted against the semilogarithmic scale of the ratio of the external and internal Cl^- concentrations ($[Cl^-]_e/[Cl^-]_i$). Squares and circles represent data recorded in the presence and absence of Ba^{2+}, and data recorded with Cs in pipettes are shown as filled symbols. The difference of reversal potential

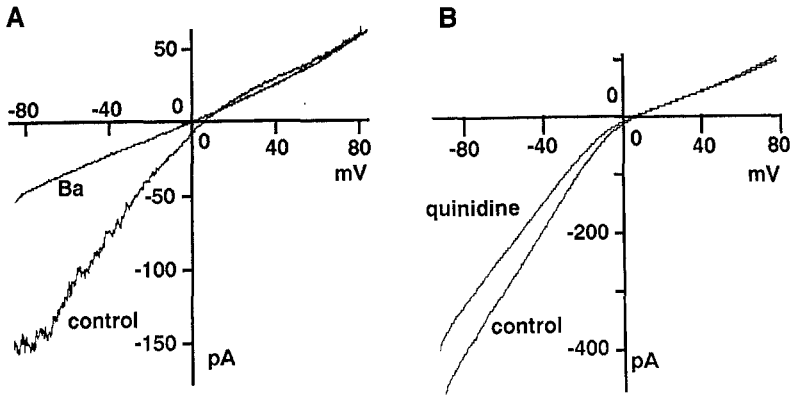

Fig. 3. Effects of K^+ channel blockers on the IR currents. Current-voltage relations obtained by applying voltage ramps at the holding potential of 0 mV. IR currents (controls) were eliminated by 1 mM $BaCl_2$ (*A*) and partially blocked by 100 μM quinidine (*B*). The external medium contained 150 mM KCl. Leak currents were not subtracted.

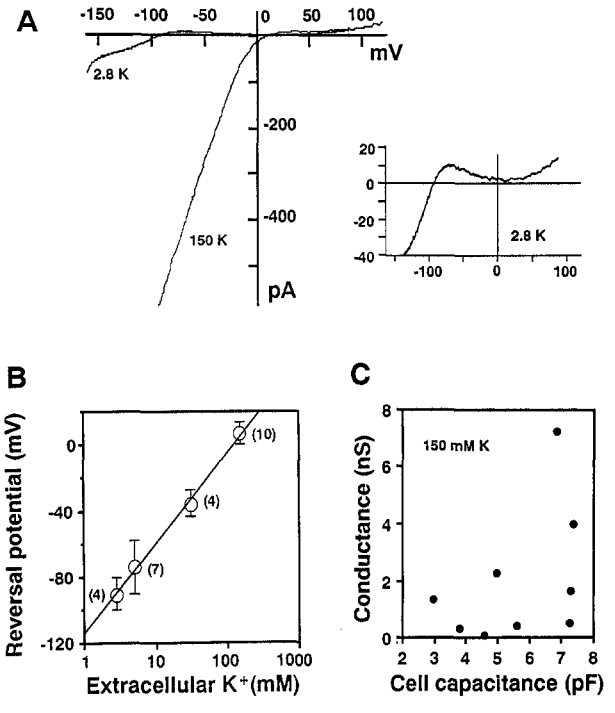

Fig. 4. IR currents and extracellular K^+ concentrations. (*A*) Current-voltage relations of Ba-sensitive IR currents in a cell when the external K^+ concentration was increased from 2.8 to 150 mM. Currents after addition of 1 mM $BaCl_2$ were subtracted. The inset figure is the relation with 2.8 mM K^+ at an expanded scale along the vertical axis. (*B*) Reversal potentials (mean ± SD) for the IR currents plotted against the external K^+ concentrations in logarithmic scale. The regression line indicates a least squares fit for data with 2.8 mM ($n = 4$), 5 mM ($n = 7$), 30 mM ($n = 4$) and 150 mM ($n = 10$) K^+ obtained from 23 cells. The internal K^+ concentration was 150 mM. (*C*) Relation between the cell capacitance and the IR conductance recorded in 150 mM K^+. Data were obtained from nine cells.

for the OR currents by a tenfold change in the ratio was 45 mV from the least squares fit for all data (indicated by a continuous line). The reversal potential was also measured from tail currents with prepulse of +100 mV. The reversal potentials obtained by the two methods did not apparently change. These results suggest that most of the OR currents seen in the presence of Ba^{2+}, and at least a part of those in the absence of Ba^{2+}, were mediated by Cl^-.

The Membrane Potential and Current Activities

In cells without any appreciable currents, the resting membrane potential recorded under the current clamp mode in the standard Ringer solution containing 5 mM K^+ was close to 0 mV (3.3 ± 7.7; $n = 5$). Cells exhibiting IR currents tended to depolarize with an increase in the concentration of external K^+ in association with an increase in the conductance measured by injecting constant currents (±10 pA) (Figure 7*A* and data linked by lines in Fig. 7*B*). The resting potential in the standard Ringer solution containing 5 mM K^+ ranged from –62 to 6.4 mV (–23 ± 25 mV, $n = 11$) (Fig. 7*B*). Addition of 1 mM $BaCl_2$ produced depolarization with a decrease in the input conductance (*data not shown*). Spontaneous fluctuations of the membrane potential, reported in macrophages with an IR_K channel (Gallin & Livengood, 1981), were not apparent.

In cells exhibiting OR currents, the resting potentials ranged from –66 to –10 mV (–28 ± 19 mV, $n = 5$) in standard Ringer solution. A spontaneous hyperpolarization was occasionally seen, in association with a development of OR currents even in the K^+-rich (150 mM K) medium (Fig. 8*A*). Voltage responses to injected currents were rectified and the slope resistance in the *I-V* curve was decreased by outward-going current pulses (Fig. 8*B*).

These findings suggest that both IR_K and OR_{Cl} channel activities more or less contribute to set the membrane potential. Low resting membrane potentials, compared with E_K or E_{Cl}, might be explained by the relatively small conductance compared to leak conductance. Leak currents may shift cells to more depolarized levels and real resting potentials without leakage may be more negative.

Discussion

Ion channels of mast cells have been investigated using rat peritoneal mast cells and a tumor analogue of mu-

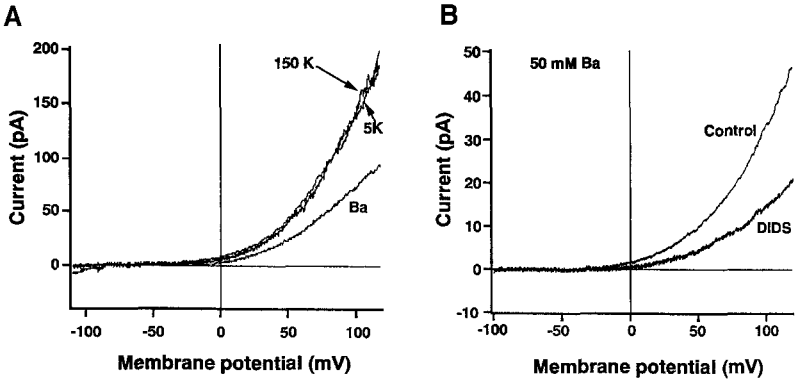

Fig. 5. The outwardly rectifying (OR) currents evoked by voltage ramps applied at the holding potential of 0 mV. (*A*) OR currents in K-rich (150 mM K) and standard Ringer (5 mM K) solutions. One millimolar $BaCl_2$ was added into the standard Ringer. (*B*) Effects of DIDS (20 μM) on the OR currents remained in the presence of 50 mM Ba^{2+}. The bath contained 50 mM $BaCl_2$ and 78 mM glucamine. Leak currents were subtracted.

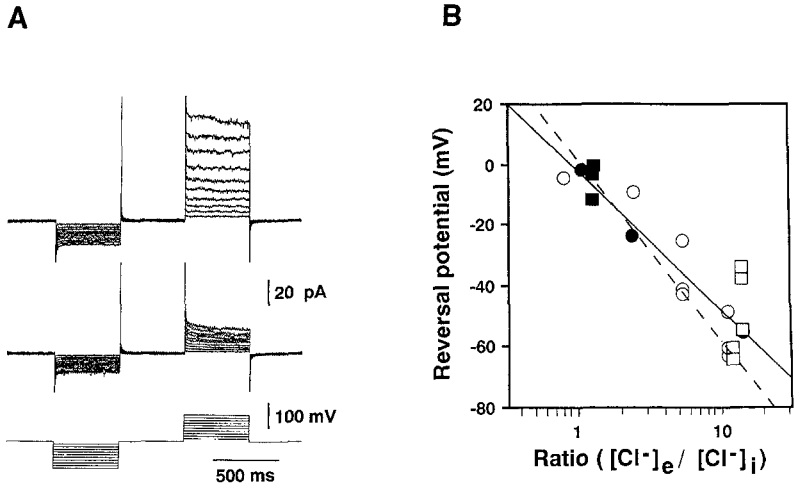

Fig. 6. The OR currents and Cl^- concentrations. (*A*) A family of currents evoked by voltage steps applied at the holding potential of 0 mV in standard Ringer (upper) and the low Cl^- (lower) solutions. In the low Cl^- solution, 145 mM NaCl was replaced by 145 mM Na-isethionate. (*B*) Semi-logarithmic plot of the reversal potential of OR currents against the ratio between extracellular and intracellular Cl^- concentrations. Squares and circles represent data recorded in the presence and absence of Ba^{2+}, respectively, and filled symbols, data recorded with Cs in the pipette. Data were obtained from 17 cells. The continuous line indicates a least squares fit for data, and the dashed line, the theoretical line for the Cl^- selective channel.

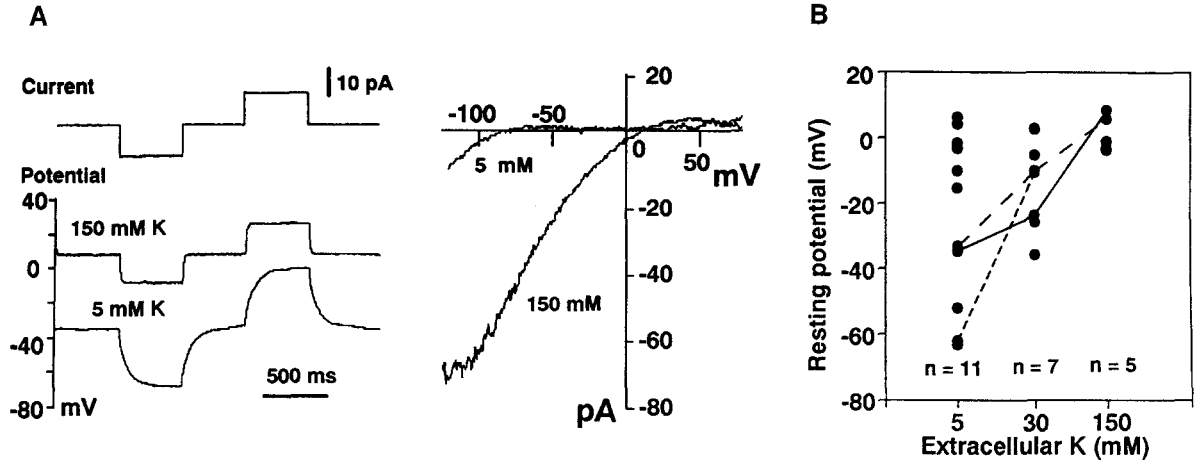

Fig. 7. Membrane potentials of cells exhibiting IR currents. (*A*) Current clamp (left) and voltage clamp (right) records in a cell. The membrane potential was positively shifted in association with an increase in input conductance by increasing the extracellular K^+ concentration from 5 to 150 mM. (*B*) Distribution of resting membrane potentials recorded under current clamp mode in the medium containing 5, 30 or 150 mM K^+. Data obtained from the same cell were linked by the same kind of line.

cosal type mast cells, RBL-2H3 cells. Since peritoneal mast cells and RBL-2H3 cells differ not only in the phenotype but also in the functional state, it is not surprising that their electrophysiological properties are different as reported previously (Lindau & Fernandez, 1986; Penner et al., 1988; Kuno et al., 1989, 1990; Matthews et al., 1989; Kuno & Kimura, 1992). BMMC have been expected to exhibit an IR_K channel similar to RBL-2H3 cells (Lindau & Fernandez, 1986) as BMMC are classified as a mucosal type from their biochemical characteristics (Haig et al., 1982; Razin et al., 1982; Sredni et al., 1983). IR_K currents found in BMMC had a negative

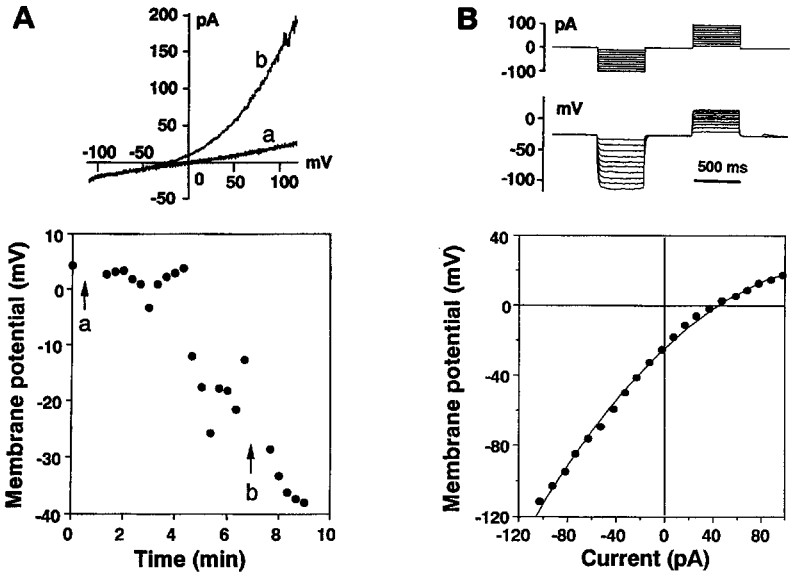

Fig. 8. Membrane potentials in cells exhibiting OR currents. (*A*) A spontaneous hyperpolarizing shift in the resting potential (lower) was accompanied by appearance of OR currents (upper); *a* and *b* are *I-V* relations obtained by applying voltage ramps in the voltage clamp mode. This cell was suspended in K^+-rich (150 mM) solution. (*B*) Voltage responses (upper) and a *I-V* relation (lower) of a cell suspended in the standard Ringer solution under the current clamp mode. Current steps in ±10 pA increments were applied at the resting potential (–25.3 mV). Membrane potentials were measured at the end of each 500 msec current step. The curve is a least squares fit for data.

conductance region at membrane potentials positive to E_K and were blocked by Ba^{2+}, similar to IR_K in RBL-2H3 cells. We report here, however, that apparent IR_K channels are seen only in a subpopulation of BMMC and that an electrophysiologial profile of BMMC in the resting state is heterogeneous.

Outwardly rectifying currents were more often seen in BMMC than in IR currents. Cl^- seemed to be a major current carrier, since the current amplitude depended on the extracellular Cl^- concentration and the reversal potential was positively shifted by decreasing the ratio between extracellular and intracellular Cl^- concentrations ($[Cl^-]_e/[Cl^-]_i$). In addition, the OR currents were inhibited by a Cl^- channel blocker, DIDS. Outwardly rectifying Cl^- currents have yet to be identified in the resting state of both mucosal and connective tissue types of mast cells, although a small conductance (~1 pS) OR_{Cl} channel was found in rat peritoneal mast cells when activated by secretagogues (Penner et al., 1988; Matthews et al., 1989). In RBL cells, crosslinking of type I FC_ϵ receptors activated a Cl^- channel that had a conductance of 32 pS and no strict outward rectification (Romanin et al., 1991). Thus, various types of Cl^- channels may exist in activated mast cells.

The OR currents were more or less reduced by 1 mM Ba^{2+}. In RBL-2H3 cells, an outwardly rectifying K^+ channel, partially blocked by Ba^{2+}, was activated by the introduction of a nonhydrolyzable GTP analogue into the cells (McCloskey & Cahalan, 1990). In the absence of K^+ channel blockers, the reversal potential of the OR currents of BMMC with the K^+ rich (150 mM) external medium was –41.7 ± 13.2 mV (n = 11), negative to E_K (0 mV). When the external K^+ concentration was decreased from 150 to 5 mM, the OR currents remained constant or decreased without appreciable changes in the reversal potential. These results suggest that contribution of K^+ currents to OR currents in the resting state of BMMC is minor if any.

The resting potential of rat peritoneal mast cells, which do not exhibit current activities, was –20 to around –30 mV or more positive (Lindau & Fernandez, 1986; Kuno et al., 1989). On the other hand, in RBL-2H3, the membrane potential was around –90 mV in the resting state and was determined by the IR_K channel (Lindau & Fernandez, 1986). IR_K channels were expressed in other peripheral blood cells originated from hematopoietic stem cells, like granulocytes (Kawa, 1989) and macrophages (Gallin & McKinney, 1988), to set the membrane potential. In BMMC without any apparent current activities, the resting potential was near 0 mV in standard Ringer solution. BMMC with IR_K tended to depolarize by elevating the external K^+ concentration and by adding Ba^{2+}, a blocker of IR_K, although the membrane potential greatly varied over a wide range in the standard Ringer solution. High resistance transitional region of the membrane potential was reported in several cell types which possess IR_K conductance with a negative conductance region (Miyazaki, Ohmori & Sasaki, 1975; Gallin, 1981). The membrane potential of most of BMMC with IR_K seemed to be in the high resistance region, at positive to E_K, and may shift to either depolarized or hyperpolarized state by a small change in the current activity as reported in other cells (Miyazaki et al., 1975; Gallin, 1981; Gallin & Livengood, 1981). However, spontaneous fluctuations of the membrane potential between the two states were not apparent in the present study. The IR_K channel may be stable in either open or closed state and may need some unknown factors to change its activity. In BMMC exhibiting OR currents, the resting potential ranged from –66 to –10 mV and an increase in OR conductance was accompanied by hyperpolarization. Thus, both IR and

OR conductances are likely to contribute to setting of the membrane potential, although the extent of regulated membrane potential may be limited by the magnitude of the conductances. It is also noteworthy that the real membrane potential may be more negative, if the leakage is comparatively negligible.

In the present study, BMMC were currently classified into four subpopulations according to the electrophysiological profile: cells with no current activity, IR currents, OR currents and inward plus outward currents. Currents in both directions remain to be characterized. Heterogeneous channel expression was also reported in bone marrow-derived macrophages (Banati et al., 1991). Macrophages have different electrophysiological properties in different phenotypes (Kettenmann et al., 1990; Banati et al., 1991). The heterogeneity in BMMC may underlie expression of various ion channels in different phenotypes of mast cells or reflect differences in the functional state. In rat peritoneal mast cells and RBL-2H3 cells, a variety of ion channels are activated during stimulus-secretion couplings (Penner et al., 1988; Kuno et al., 1989; Romanin et al., 1991). A lack of appropriate stimulating signals might explain the existence of quiescent BMMC. As has been suggested in peritoneal mast cells and RBL-2H3 cells (Penner et al., 1988; Kuno et al., 1989; Romanin et al., 1991), openings of both K^+ and Cl^- channels could support Ca^{2+} influx by increasing the electromotive force for Ca^{2+} currents due to hyperpolarization, although further studies are needed to clarify physiological significance of the channels in BMMC.

We thank Dr. S. Matsuura for critically reading the manuscript and Drs. Y. Kitamura and T. Jippo for helpful advice on culture of BMMC. This study was supported by grants from The Ministry of Education, Science, Culture, Japan and from Uehara Memorial Foundation, and by an Advanced Research Grant from the Osaka City University Medical Research Foundation.

References

Banati, R.B., Hoppe, D., Gottmann, K., Kreutzberg, G.W., Kettenmann, H. 1991. A subpopulation of bone marrow-derived macrophage-like cells shares a unique ion channel pattern with microglia. *J. Neurosci. Res.* **30:**593–600

Enerbäck, L. 1986. Mast cell heterogeneity: the evolution of the concept of a specific mucosal mast cell. *In:* Mast Cell Differentiation and Heterogeneity. A.D. Befus, J. Bienenstock and J.A. Denburg, editors. pp. 1–26. Raven, New York

Fujita, J., Nakayama, H., Onoue, H., Kanakura, Y., Nakano, T., Asai, H., Takeda, S., Honjo, T., Kitamura, Y. 1988. Fibroblast-dependent growth of mouse mast cells in vitro: Duplication of mast cell depletion in mutant mice of W/W^V Genotype. *J. Cell. Physiol.* **134:**78–84

Gallin, E.K. 1981. Voltage clamp studies in macrophages from mouse spleen cultures. *Science* **214:**458–460

Gallin, E.K., Livengood, D.R. 1981. Inward rectification in mouse macrophages: evidence for a negative resistance region. *Am. J. Physiol.* **241:**C9–C17

Gallin, E.K., McKinney, L.C. 1988. Patch-clamp studies in human macrophages: Single-channel and whole-cell characterization of two K^+ conductances. *J. Membrane Biol.* **103:**55–66

Haig, D.M., McKee, T.A., Jarrett, E.E.E., Woodbury, R., Miller, H.R.P. 1982. Generation of mucosal mast cells is stimulated *in vitro* by factors derived from T cells of helminth-infected rats. *Nature* **300:**188–190

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution current recordings from cells and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100

Ihle, J.N., Keller, J., Oroszlan, S., Henderson, L.E., Copeland, T.D., Fitch, F., Prystowsky, M.B., Goldwasser, E., Schrader, J.W., Palaszynski, E., Dy, M., Lebel, B. 1983. Biological properties of homogeneous interleukin 3: I. Demonstration of WEHI-3 growth factor activity, mast cell growth factor activity, P cell-stimulating factor activity, colony stimulating factor activity, and histamine-producing cell-stimulating factor activity. *J. Immunol.* **131:**282–287

Kawa, K. 1989. Electrophysiological properties of three types of granulocytes in circulating blood of the newt. *J. Physiol.* **415:**211–231

Kettenmann, H., Hoppe, D., Gottmann, K., Banati, R., Kreutzberg, G.W. 1990. Cultured microglial cells have a distinct pattern of membrane channels different from peritoneal macrophages. *J. Neurosci. Res.* **26:**278–287

Kitamura, Y., Yokoyama, M., Matsuda, H., Ohno, T., Mori, K.J. 1981. Spleen colony-forming cell as common precursor for tissue mast cells and granulocytes. *Nature* **291:**159–160

Kuno, M., Kawaguchi, J., Mukai, M., Nakamura, F. 1990. PT pretreatment inhibits 48/80-induced activation of Ca^{2+}-permeable channels in rat peritoneal mast cells. *Am. J. Physiol.* **259:**C715–C722

Kuno, M., Kimura, M. 1992. Noise of secretagogue-induced inward currents dependent on extracellular calcium in rat mast cells. *J. Membrane Biol.* **128:**53–61

Kuno, M., Kyogoku, I., Kawawaki, J. 1993. Membrane currents in mouse bone marrow-derived mast cells. *Proc. Int. Union Physiol. Sci.* **32:**204.30 (*Abstr.*)

Kuno, M., Okada, T., Shibata, T. 1989. A patch-clamp study: secretagogue-induced currents in rat peritoneal mast cells. *Am. J. Physiol.* **256:**C560–C568

Lindau, M., Fernandez, J.M. 1986. A patch clamp study of histamine-secreting cells. *J. Gen. Physiol.* **88:**349–368

Matthews, G., Neher, E., Penner, R. 1989. Chloride conductance activated by external agonists and internal messengers in rat peritoneal mast cells. *J. Physiol.* **418:**131–144

McCloskey, M.A., Cahalan, M.D. 1990. G protein control of potassium channel activity in a mast cell line. *J. Gen. Physiol.* **95:**205–227

Miyazaki, S., Ohmori, H., Sasaki, S. 1975. Potassium rectifications of the starfish oocyte membrane and their changes during oocyte maturation. *J. Physiol.* **246:**55–78

Nakahata, T., Spicer, S.S., Cantey, J.R., Ogawa, M. 1982. Clonal assay of mouse mast cell colonies in methylcellulose culture. *Blood* **60:**352–361

Nakano, T., Sonoda, T., Hayashi, C., Yamatodani, A., Kanayama, Y., Yamamura, T., Asai, H., Yonezawa, T., Kitamura, Y., Galli, S.J. 1985. Fate of bone marrow-derived cultured mast cells after intracutaneous, intraperitoneal and intravenous transfer into genetically mast cell-deficient W/W^V mice: Evidence that cultured mast cells can give rise to both connective tissue type and mucosal mast cells. *J. Exp. Med.* **162:**1025–1043

Penner, R., Matthews, G., Neher, E. 1988. Regulations of calcium influx by second messengers in rat mast cells. *Nature* **334:**499–504

Razin, E., Ihle, J.N., Seldin, D., Mencia-Huerta, J.-M., Katz, H.R., Leblanc, P.A., Hein, A., Caulfield, J.P., Austen, K.F., Stevens, R.L.

1984. Interleukin 3: A differentiation and growth factor for the mouse mast cell that contains chondroitin sulfate E proteoglycan. *J. Immunol.* **132:**1479–1486

Razin, E., Stevens, R.L., Akiyama, F., Schmid, K., Austen, K.F. 1982. Culture from mouse bone marrow of a subclass of mast cells processing a distinct chondroitin sulfate proteoglycan with glycosaminoglycans rich in N-acetylgalactosamine-4,6-disulfate. *J. Biol. Chem.* **257:**7229–7236

Romanin, C., Reinsprecht, M., Pecht, I., Schindler, H. 1991. Immunologically activated chloride channels involved in degranulation of rat mucosal mast cells. *EMBO J.* **10:**3603–3608

Sredni, B., Friedman, M.M., Bland, C.E., Metcalfe, D.D. 1983. Ultrastructural, biochemical, and functional characteristics of histamine-containing cells cloned from mouse bone marrow: tentative identification as mucosal mast cells. *J. Immunol.* **131:**915–922

Worthington, W.C., Jr. 1962. Dual staining of mast cell cytoplasmic constitution by alcian blue and safranin. *J. Histochem. Cytochem.* **10:**503