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Molecular and pharmacological properties of inwardly rectifying K^+ channels of human lung cancer cells

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Abstract

Properties of inwardly rectifying K^+ channels in small-cell lung cancer (SCLC) cells have not been clarified in detail. Here, we found inwardly rectifying K^+ channels in a human SCLC cell line (RERF-LC-MA), which expresses no multidrug resistance-associated protein 1 (MRP1) and multidrug resistance P-glycoprotein (MDR1). Extracellular Ba^{2+} and Cs^+ inhibited inwardly rectifying K^+ currents of RERF-LC-MA cells in a concentration-dependent manner, but tetraethylammonium ion and glibenclamide were ineffective. Okadaic acid, an inhibitor of phosphatases 1 and 2A, and phorbol-12,13-dibutyrate, an activator of protein kinase C, significantly decreased the inwardly rectifying K^+ current. Lowering the intracellular pH but not the extracellular pH decreased the K^+ current. Reverse transcriptase-polymerase chain reaction (RT-PCR) and Western blotting analysis showed that RERF-LC-MA cells express Kir2.1 mRNA and protein. The inwardly rectifying K^+ current is suggested to be generated by Kir2.1 protein in the human small-cell lung cancer cell, and that the K^+ channel is negatively regulated by protein kinase C and the intracellular acidic pH.

Keywords: K^+ channel; Small-cell lung cancer, human; Protein kinase C; Multidrug resistance protein

1. Introduction

Small-cell lung cancer (SCLC) accounts for 20–25% of all bronchogenic carcinomas and has distinctive pathological, biological and clinical features (Cook et al., 1993). Human SCLC cells are suggested to be of neuroectodermal origin and exhibit electrophysiological characteristics typical of neuroendocrine cells (Pancrazio et al., 1993). Whole-cell patch-clamp studies have shown the presence of voltage-dependent Ca^{2+}, Na^+ and K^+ channels in human SCLC cell lines such as NCI-H128, NCI-H69 and NCI-H146 (Pancrazio et al., 1989). Action potentials in SCLC cells result from the Na^+ channels (Blandino et al., 1995). Resting membrane potentials of SCLC cells are regulated by the K^+ channels (Pancrazio et al., 1993), and K^+ channel blockers inhibit SCLC cell proliferation through a membrane depolarization (Pancrazio et al., 1993).

Inwardly rectifying K^+ channels, which maintain the resting membrane potential, were essentially absent in NCI-H69 cells (Jirsch et al., 1993). However, the NCI-H69-derived H69AR cells that overexpress multidrug resistance-associated protein 1 (MRP1) showed a large inwardly rectifying K^+ current (Jirsch et al., 1993), suggesting that expression of the K^+ channels are deeply associated with multidrug resistance of the cells. So far, properties of the inwardly rectifying K^+ channels in H69AR cells and other SCLC cells have not been clarified in detail, and the channel molecules have not been identified.

Herein, we used another human SCLC cell line (RERF-LC-MA; Mitsuhashi et al., 1992). In the present study, we found that neither MRP1 nor multidrug resistance P-glycoprotein (MDR1) was expressed, but large inwardly rectifying K^+ currents were present in RERF-LC-MA cells, in contrast to the case of NCI-H69 cells. We confirmed that RERF-LC-MA cells express Kir2.1 mRNA and protein. The

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regulatory mechanisms of human Kir2.1 channel in the cells were also studied.

2. Materials and methods

2.1. Cells

Human SCLC cells (RERF-LC-MA) established by Radiation Effects Research Foundation (Hiroshima, Japan) were obtained from Japanese Cancer Research Resources Bank (Tokyo, Japan). Passage number of the cells used in the present study was 10–27. The cells were maintained in Eagle's Minimum Essential Medium (Cosmo Bio, Tokyo, Japan) supplemented with 0.18% $NaHCO_3$, 4 mM HEPES, 10 units/ml of penicillin G, 10 μg/ml of streptomycin and 10% fetal calf serum at 37 °C under 95% O_2–5% CO_2. For Western blotting, C-A120 cells (Sumizawa et al., 1994) supplied by Dr. Shinichi Akiyama (Kagoshima University, Kagoshima, Japan) were used as positive control for expression of MRP1. K562/40VCR cells (Hirose and Kuroda, 1998) supplied by Dr. Masao Hirose (Tokushima University, Tokushima, Japan) were used for positive control for expression of MDR1.

2.2. Patch-clamp recordings

For whole-cell patch-clamp recordings, RERF-LC-MA cells were seeded on a glass coverslip and incubated for 2–7 h in Eagle's Essential Medium. Whole-cell currents were recorded from the seeded cells, using an EPC-7 (List Electronic, Darmstadt, Germany) or EPC-9 (HEKA elektronik Dr.-Ing P. Schulze, Lambrecht, Germany) patch-clamp system as described previously (Sakai and Takeguchi, 1993). Whole-cell membrane capacitance was 25–45 pF. The compositions of extracellular bathing and intracellular pipette solutions are shown in Table 1. Unless otherwise noted, the pH values of these bathing and pipette solutions were 7.3. All the pipette solutions did not contain Mg^{2+} or ATP. To obtain the current–voltage relationship, command pulses were superposed on the holding potential (0 to ± 100 mV, voltage jump by ± 20 mV, duration of 400 ms). The pulse was delivered from a microcomputer equipped with a D/A converter, and resultant currents were recorded on a tape-recorder. Experiments were performed at 35–37 °C.

2.3. Sodium dodecylsulfate (SDS)-polyacrylamide gel electrophoresis and Western blotting

Membrane fractions of RERF-LC-MA cells were prepared as described previously (Asano et al., 1996). Membrane preparations (30 μg of protein) were incubated in a sample buffer containing 2% SDS, 2% β-mercaptoethanol, 10% glycerol and 10 mM Tris–HCl (pH 6.8) at room temperature for 2 min and applied to the SDS-polyacrylamide gel. Western blotting was carried out as described previously (Asano et al., 1996). QCRL-1, a monoclonal antibody against human MRP1 (Hipfner et al., 1994), was supplied by Dr. Susan P.C. Cole (Queen's University, Kingston, Canada). Ab-2, a monoclonal antibody against human MDR1, was obtained from NeoMarkers (Union City, CA, USA). Anti-Kir2.1, a polyclonal antibody against human Kir2.1, was from Alomone labs (Jerusalem, Israel). These primary antibodies were used with 1:100 dilution. Horseradish peroxidase-conjugated anti-mouse or anti-rabbit immunoglobulin G was used as a secondary antibody (1:1000 dilution).

2.4. RNA isolation, reverse transcriptase-polymerase chain reaction (RT-PCR) and subcloning

Total RNA was isolated from RERF-LC-MA cells by acidic phenol method (Chomczynski and Sacchi, 1987). RT-PCR was performed by GeneAmp RNA PCR Kit (Perkin Elmer; Foster City, CA, USA) including oligo$(dT)_{16}$-primer, MuLV reverse transcriptase and AmpliTaq DNA polymerase. Total RNA (1.3 μg) was oligo-(dT)-primed (2.5 μM) in a final volume of 20 μl in the presence 50 units of the MuLV reverse transcriptase (42 °C for 15 min and heated at 99 °C for 5 min). Twenty microliters of the RT

Table 1
Compositions of solutions for patch-clamp experiments (in mM)

Name of solutions	pH	KCl	NaCl	HCl	K-aspartate	Na-aspartate	Tris	HEPES	PIPES
125 K^+–140 Cl^- [a]	7.3	125		15			15	5	
125 K^+–140 Cl^- (pH 6.3)[a]	6.3	125		15			15		10
125 K^+–140 Cl^- (pH 6.8)[a]	6.8	125		15			15		10
7 K^+–140 Cl^- [b]	7.3	7		133			133	5	
30 K^+–140 Cl^- [b]	7.3	30		110			110	5	
30 K^+–140 Cl^- (pH 6.3)[b]	6.3	30		110			110		5
100 K^+–140 Cl^- [b]	7.3	100		40			40	5	
7 K^+–5 Cl^- [b]	7.3	5			2	133		5	
7 K^+–25 Cl^- [b]	7.3	7	18			115		5	
7 K^+–140 Cl^- [b]	7.3	7	133					5	

[a] Intracellular pipette solution.
[b] Extracellular bathing soluition.

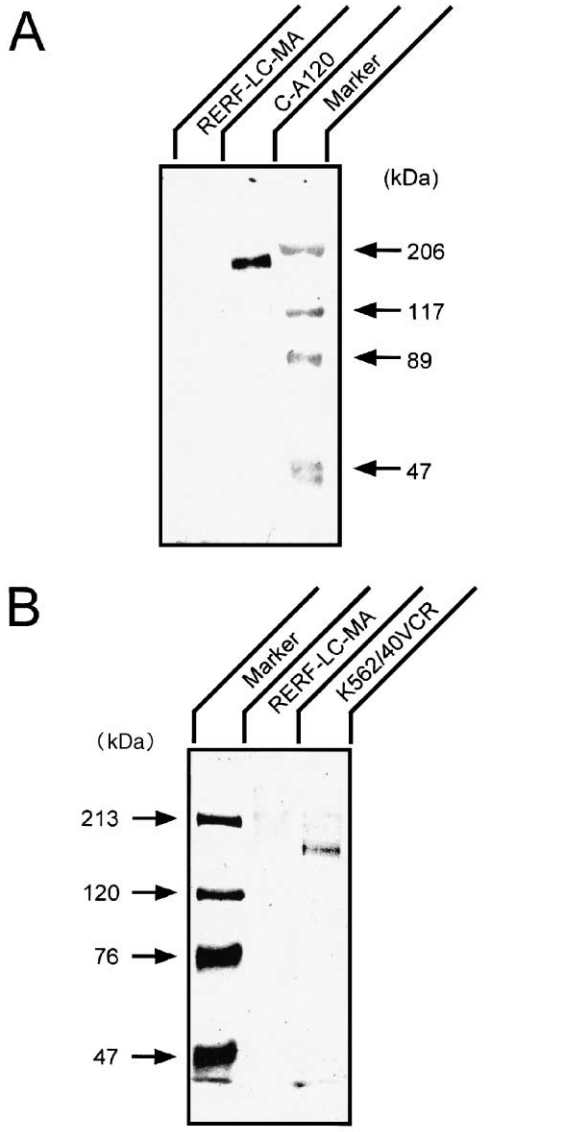

Fig. 1. Absence of MRP1 and MDR1 proteins in RERF-LC-MA cells. (A) Western blotting was performed with 30 μg of membrane protein from RERF-LC-MA and C-A120 cells using a monoclonal anti-human MRP1 antibody. A single band of 190 kDa was detected only in C-A120 cells. (B) Western blotting was performed with 30 μg of membrane protein from RERF-LC-MA and K562/40VCR cells using a monoclonal anti-human MDR1 antibody. A single band of 170 kDa was detected only in K562/40VCR cells.

sample was incubated with 2.5 units of the Taq DNA polymerase and the sense primer (150 nM; 5′-GC(A/G)GACATCTTCACCAC(G/C)TG-3′) and the antisense primer (150 nM; 5′-CCATAGCC(T/G)ATGGT(T/C)GTCTG-3′) in a 100 μl reaction mixture. These primers correspond to sequences of human Kir2.1 (Raab-Graham et al., 1994) and Kir2.3 channels (Makhina et al., 1994); the sense primer matches nucleotide 208–227 of Kir2.1 and 130–149 of Kir2.3, and the antisense primer matches nucleotide 418–437 of Kir2.1 and 394–413 of Kir2.3. After the PCR reaction (95 °C for 15 s, 60 °C for 30 s; 35 cycles), 2 μl of the products was loaded on 8% polyacrylamide gel and electrophoresed. The purified 230-bp products were subcloned into pCR-Script™ Amp SK(+) cloning vector (Stratagene). For DNA sequencing, an Autoread DNA sequencing kit and the ALF-II DNA sequencer (Amersham Pharmacia Biotech) were used.

2.5. Chemicals

Okadaic acid was obtained from Research Biochemical International (Natick, MA, USA). Phorbol-12,13-dibutyrate (PDB) was from Wako (Osaka, Japan). 1,2-Bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) was from Dojindo Laboratories (Kumamoto, Japan). *N*-[2-(*p*-

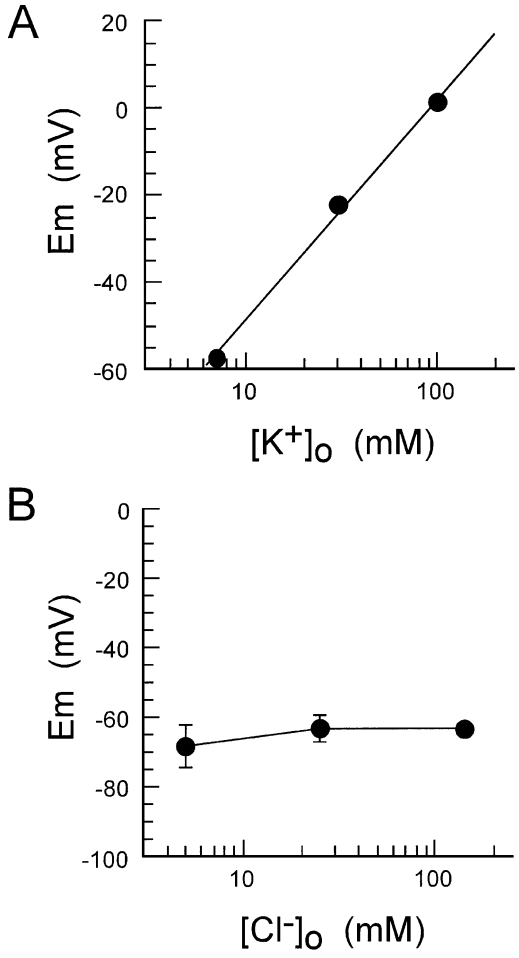

Fig. 2. The membrane potential at zero current of RERF-LC-MA cells as a function of the K^+ and Cl^- concentrations in the bathing solution. (A) The membrane potential at zero current (Vm) was measured with the 125 K^+–140 Cl^- pipette solution and three different bathing solutions (7 K^+–140 Cl^-, 30 K^+–140 Cl^- and 100 K^+–140 Cl^-) (n=5–8). (B) Vm was measured with the 125 K^+–140 Cl^- pipette solution and three different bathing solutions (7 K^+–5 Cl^-, 7 K^+–25 Cl^- and 7 K^+–140 Cl^-) (n=4).

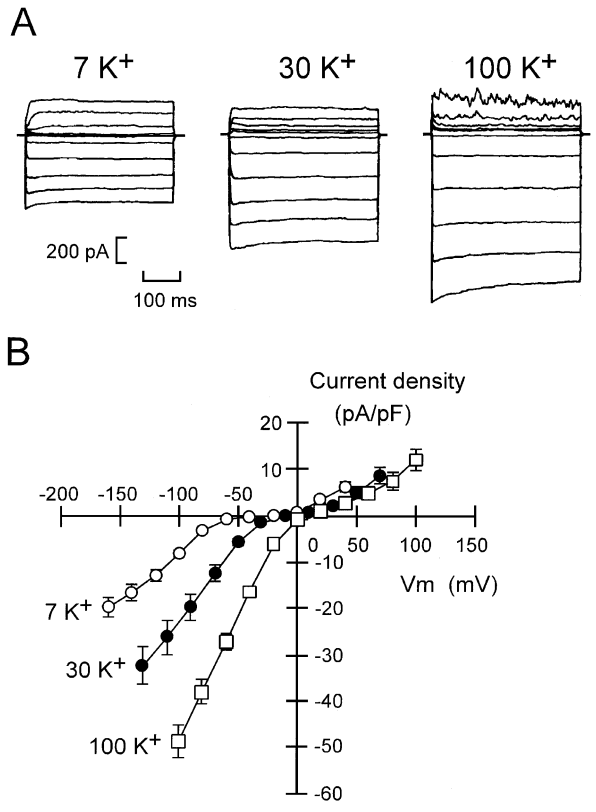

Fig. 3. Whole-cell K^+ currents in RERF-LC-MA cells. Whole-cell currents were measured with the 125 K^+ – 140 Cl^- pipette solution and three different bathing solutions (7 K^+ – 140 Cl^-, 30 K^+ – 140 Cl^- and 100 K^+ – 140 Cl^-). (A) Typical current traces evoked by voltage pulses in 20-mV steps from the holding potential of −60, −30 and 0 mV for the bathing solutions of 7 K^+ – 140 Cl^-, 30 K^+ – 140 Cl^- and 100 K^+ – 140 Cl^-, respectively. (B) Average current density–voltage relationships (n = 5–8).

Bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide dihydrochloride (H-89) was from BIOMOL Research Laboratories (Plymouth Meeting, PA, USA).

2.6. Data analysis

The data are presented as means ± S.E.M. Differences between groups were analyzed by one-way analysis of variance (ANOVA), and correction for multiple comparisons was made by using Tukey's multiple comparison test. Comparison between the two groups was made with Student's t-test. Statistically significant differences were assumed at $P < 0.05$.

3. Results

3.1. Absence of MRP1 and MDR1 in RERF-LC-MA cells

Before studying the properties of K^+ channels in the human SCLC cell (RERF-LC-MA), expression of multidrug resistance proteins such as MRP1 and MDR1 in the ATP-binding cassette superfamily was checked, because overexpression of MRP1 was suggested to be associated with expression of inwardly rectifying K^+ channels in another SCLC cell (H69AR cells). Fig. 1A shows that a monoclonal antibody against human MRP1 protein is free of reaction with any membrane proteins prepared from RERF-LC-MA cells, while the antibody bound to a 190-kDa MRP1 protein in C-A120 cells. Fig. 1B shows that a monoclonal antibody against human MDR1 protein has no reaction with any membrane proteins of RERF-LC-MA cells, while it bound to a 170-kDa MDR1 protein in K562/40VCR cells. These results indicate that RERF-LC-MA

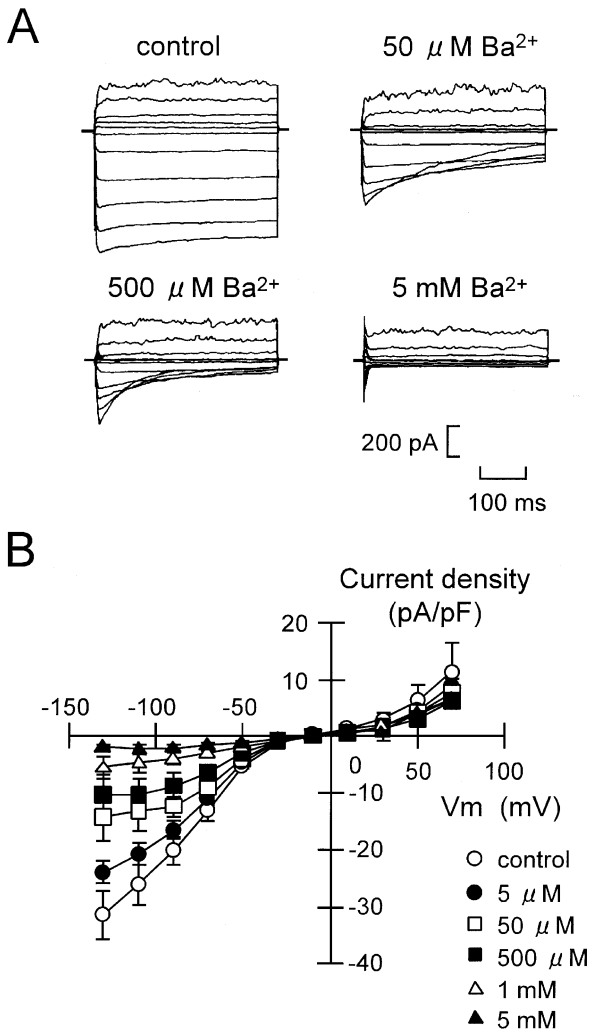

Fig. 4. Inhibitory effect of Ba^{2+} on the inwardly rectifying K^+ current. (A) Typical current traces measured with the 125 K^+ – 140 Cl^- pipette and 30 K^+ – 140 Cl^- bathing solutions. The bathing solution contained different concentrations of Ba^{2+}. Whole-cell currents were generated by voltage pulses in 20 mV steps from the holding potential of −30 mV. (B) Each symbol shows the average current density measured at 350–400 ms of each voltage pulse in the presence of different concentrations of Ba^{2+} (n = 4).

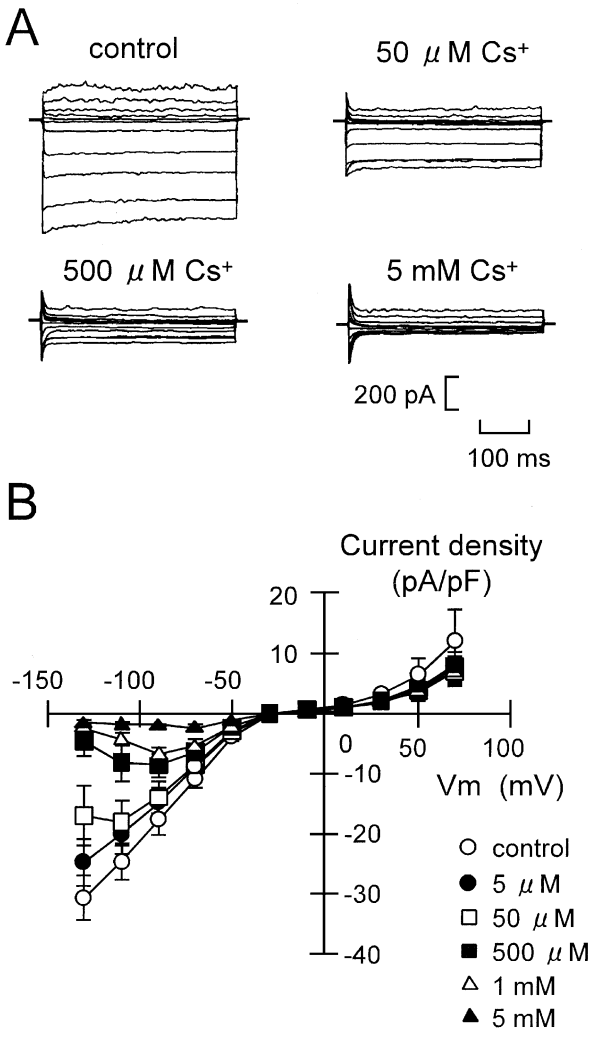

Fig. 5. Inhibitory effect of Cs^+ on the inwardly rectifying K^+ current. (A) Typical current traces measured with the 125 K^+ – 140 Cl^- pipette and 30 K^+ – 140 Cl^- bathing solutions. The bathing solution contained different concentrations of Cs^+. Whole-cell currents were generated by voltage pulses in 20 mV steps from the holding potential of − 30 mV. (B) Each symbol shows the average current density measured at 350–400 ms of each voltage pulse in the presence of different concentrations of Cs^+ (n = 4).

cells express no significant amount of MRP1 and MDR1 proteins.

3.2. Effects of altered extracellular concentrations of either K^+ or Cl^- on the membrane potential of human SCLC cells

In the whole-cell configuration at the current clamp mode, we measured the membrane potential (E_m) of the RERF-LC-MA cells. Increasing the extracellular K^+ concentration ($[K^+]_o$) at a constant $[Cl^-]_o$ depolarized the cell systematically (Fig. 2A). The slope of Fig. 2A is about 51 mV per decade, close to the value calculated from the Nernst equation under the assumption that only K^+ conductance contributes to E_m (61 mV at 36 °C). In contrast, decreasing $[Cl^-]_o$ at a constant $[K^+]_o$ did not significantly affect the E_m (Fig. 2B).

3.3. Whole-cell K^+ currents in RERF-LC-MA cells

Fig. 3A shows typical whole-cell current traces obtained from RERF-LC-MA cells. The intracellular pipette solution contained 125 mM K^+ (Table 1). When K^+ concentration in the extracellular bathing solution was elevated (7 → 30 → 100 mM), inwardly rectifying currents in response to hyperpolarizing pulses increased systematically (Fig. 3A,B) and the reversal potential of the current shifted in parallel with the predicted change in E_K (Fig. 3B). The inward rectifying K^+ current was stable at least for 10 min: that is, the current density recorded 0 and 10 min after achievement of whole-cell configuration with the 30 K^+ – 140 Cl^- bathing solution at − 130 mV were − 33.1 ± 2.7 and − 31.8 ± 2.4 pA/pF (n = 7), respectively (not significant; $P > 0.05$).

3.4. Effects of K^+ channel blockers on the inwardly rectifying K^+ current

Classical inwardly rectifying K^+ channels (Kir2.x or IRK) are well known to be blocked by external Ba^{2+} and

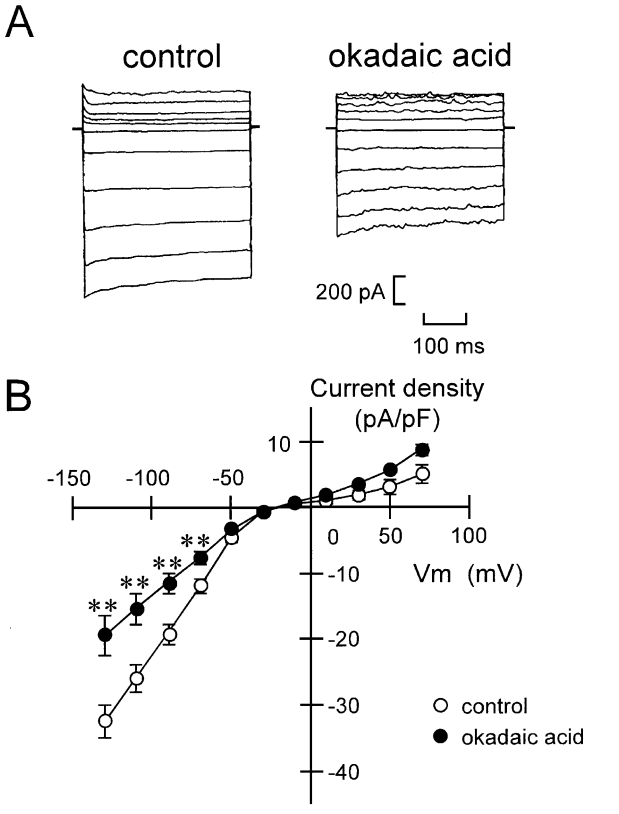

Fig. 6. Inhibitory effect of okadaic acid on the inwardly rectifying K^+ current. (A) Typical current traces measured in the absence (left) and presence (right) of 0.5 μM okadaic acid in the bathing solution. The 125 K^+ – 140 Cl^- pipette and 30 K^+ – 140 Cl^- bathing solutions were used. The cells were pre-incubated with (right) or without (left) okadaic acid for 30 min at 33 °C. (B) Each symbol shows the average current density measured at 350–400 ms of each voltage pulse in the absence (○) and presence (●) of 0.5 μM okadaic acid (n = 7). ** $P < 0.01$.

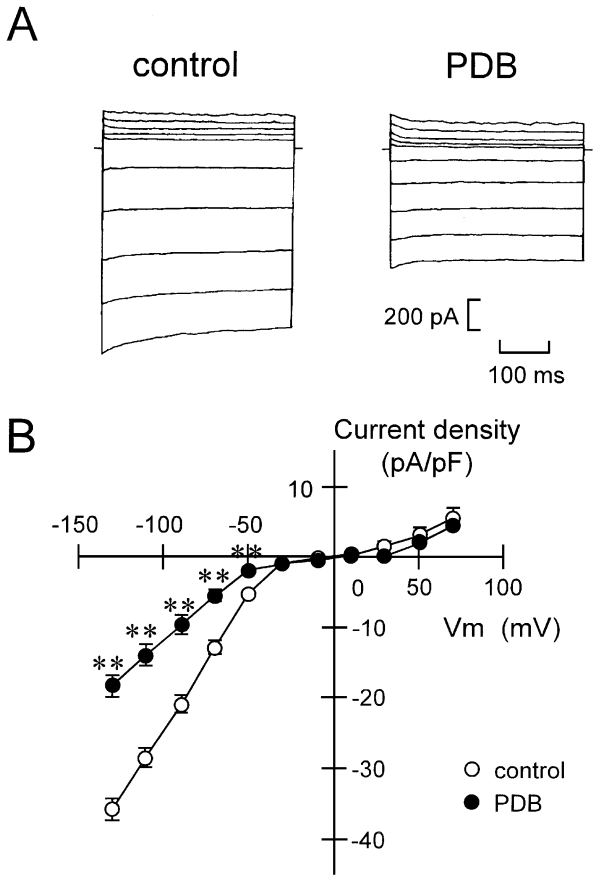

Fig. 7. Inhibitory effect of phorbol-12,13-dibutyrate (PDB) on the inwardly rectifying K^+ current. (A) Typical current traces measured in the absence (left) and presence (right) of 1 μM PDB in the bathing solution. The 125 K^+ – 140 Cl^- pipette and 30 K^+ – 140 Cl^- bathing solutions were used. The cells were pre-incubated with (right) or without (left) PDB for 30 min at 33 °C. (B) Each symbol shows the averaged current density measured at 350–400 ms of each voltage pulse in the absence (○) and presence (●) of 1 μM PDB ($n=6$). ** $P<0.01$.

Cs^+ (Isomoto et al., 1997). The present inwardly rectifying K^+ currents of the RERF-LC-MA cells were inhibited by extracellular Ba^{2+} (Fig. 4) and Cs^+ (Fig. 5) in a concentration-dependent manner. The Ba^{2+}-induced inhibition of the inward currents was time- and voltage-dependent (Fig. 4A). The Cs^+-induced inhibition of the currents was also voltage-dependent, but no clear time-dependent inactivation was observed (Fig. 5A). Although Ba^{2+}- and Cs^+-insensitive outwardly rectifying current was observed (Figs. 4B and 5B), we did not explore further this current in the present study.

Extracellular addition of tetraethylammonium ion (5 mM), which is an inhibitor of a number of K^+ channels such as voltage-gated K^+ channels (Mathie et al., 1998) and Ca^{2+}-activated K^+ channels (Nelson and Brayden, 1993), had no effect on the inwardly rectifying K^+ current ($n=5$, data not shown). Extracellular addition of glibenclamide (100 μM), an inhibitor of ATP-sensitive K^+ channels (Inagaki et al., 1996), also induced no inhibition of the inward K^+ current ($n=3$, not shown).

The channel activity of GTP-binding protein-coupled inwardly rectifying K^+ channels (Kir3.x or GIRK) has been reported to be sensitive to BAPTA (Rogalski et al., 1999). Intracellular addition of BAPTA (2 mM) had no effect on the inwardly rectifying K^+ currents in the present human SCLC cells ($n=4$, data not shown).

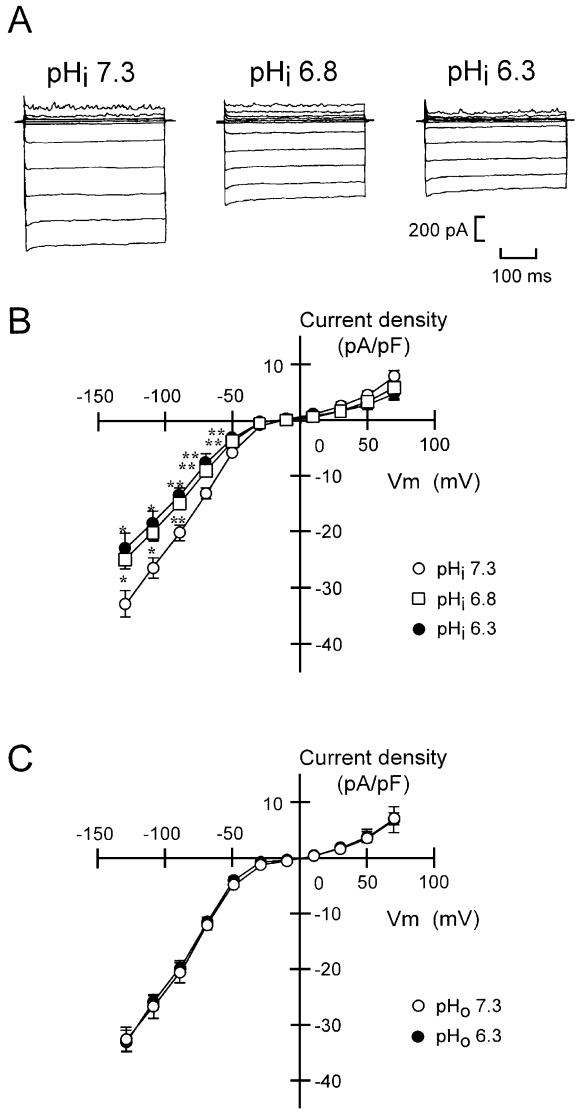

Fig. 8. Sensitivities to intracellular and extracellular pH of the inwardly rectifying K^+ current. (A) Typical current traces recorded with the 30 K^+ – 140 Cl^- bathing solution (pH 7.3) and the 125 K^+ – 140 Cl^- pipette solutions of pH 6.3, 6.8 and 7.3. (B) Whole-cell currents were recorded with the 30 K^+ – 140 Cl^- bathing solution (pH 7.3) and the 125 K^+ – 140 Cl^- pipette solutions of pH 6.3 (●), 6.8 (□) and 7.3 (○). The current density was measured at 350–400 ms of each voltage pulse ($n=6$). * $P<0.05$ and ** $P<0.01$, significantly different from the value of pH_i 7.3 (○). (C) Whole-cell currents were recorded with the 125 K^+ – 140 Cl^- pipette solution (pH 7.3) and the 30 K^+ – 140 Cl^- bathing solutions of pH 6.3 (●) and 7.3 (○). The current density was measured at 350–400 ms of each voltage pulse ($n=4$).

3.5. Protein kinase C mediated inhibition of the inwardly rectifying K^+ current

Okadaic acid (0.5 μM), an inhibitor of phosphatases 1 and 2A, significantly decreased the inwardly rectifying K^+ current (Fig. 6). Lower concentration of it (10 nM) also significantly decreased the inward K^+ current: that is, the current density recorded in the absence and presence of 10 nM okadaic acid with the 30 K^+–140 Cl^- bathing solution at −130 mV were -33.7 ± 2.9 and -25.3 ± 1.2 pA/pF, respectively ($n=4$, $P<0.05$). Phorbol-12,13-dibutyrate (PDB; 1 μM), a potent protein kinase C activator, also decreased the inward current (Fig. 7). H-89 (10 μM), a protein kinase A inhibitor, did not affect the current ($n=6$, data not shown). These results suggest that protein kinase C-mediated phosphorylation of K^+ channels and/or the K^+ channel-associated proteins in RERF-LC-MA cells is an essential event for inhibition of the channel activity.

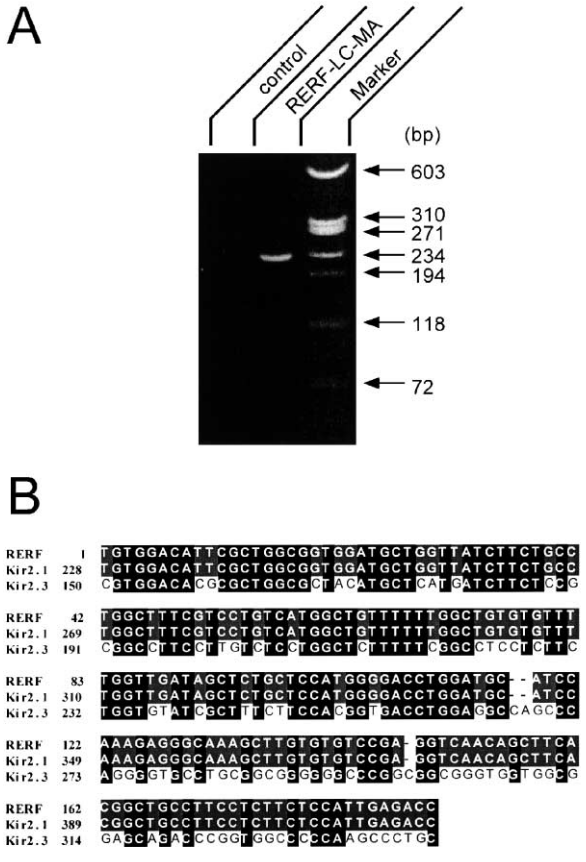

Fig. 9. Expression of Kir2.1 mRNA in RERF-LC-MA cells. (A) Gel analysis of the RT-PCR product from RERF-LC-MA cells. A single band of 230 bp was detected by ethidium bromide staining (RERF-LC-MA). No band was detected in a negative control experiment without reverse transcriptase (control). (B) Nucleotide sequence of a subcloned RT-PCR product from RERF-LC-MA cells (RERF). Sequences of the primers are omitted. The sequence is aligned with the corresponding part of human Kir2.1 and Kir2.3. Identical residues are printed white on black or gray.

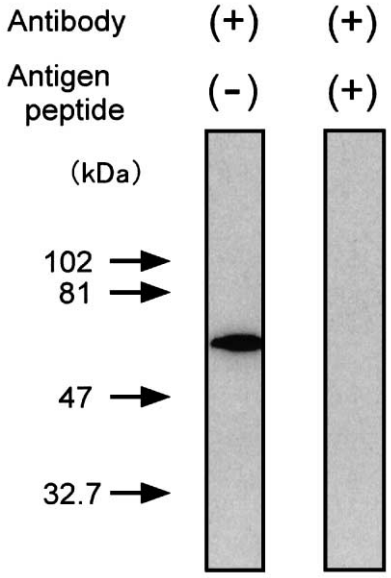

Fig. 10. Expression of Kir2.1 protein in RERF-LC-MA cells. Western blotting was performed with 30 μg of membrane protein from RERF-LC-MA cells using a polyclonal anti-human Kir2.1 antibody. A single band of 64 kDa was detected (left lane). For negative control, 1 μg of the antibody was pre-incubated with 1 μg of the antigen peptide that corresponded to amino acid residues 392–410 of human Kir2.1 (right lane).

3.6. Inhibition of the inwardly rectifying K^+ current by the intracellular acidic pH

The inwardly rectifying K^+ current significantly decreased when the pH of the intracellular pipette solution (pH_i) was lowered from 7.3 to 6.8 and 6.3 (Fig. 8A,B). In contrast, lowering the extracellular pH (pH_o) from 7.3 to 6.3 induced no change of amplitudes of the inward current (Fig. 8C).

3.7. Expression of Kir2.1 in RERF-LC-MA cells

In the RT-PCR experiment, a set of primers that can amplify partial cDNA fragments of both human Kir2.1 and Kir2.3 were used. The predicted sizes of PCR products for Kir2.1 and Kir2.3 were 230 and 284 bp, respectively. Fig. 9A shows that RT-PCR from total RNA of RERF-LC-MA cells gives only a 230-bp product. Sequencing revealed that the 230-bp product had 100% homology (except the primers' sequences) with the human Kir2.1 (HH-IRK1; GenBank U12507) (Fig. 9B). Expression of Kir2.1 protein in RERF-LC-MA cells was confirmed by Western blotting with an antibody against Kir2.1 (Fig. 10). In this experiment, a single band of 64 kDa was detected. This band was derived from Kir2.1 protein because it disappeared in the presence of the antigen peptide (Fig. 10).

4. Discussion

In the present study, we have recorded the inwardly rectifying K^+ current in the human SCLC cell, RERF-LC-MA. Inwardly rectifying K^+ channels so far reported have been classified into four subfamilies: (1) classical inwardly rectifying K^+ channels (Kir2 subfamily), (2) GTP-binding protein-activated K^+ channels (Kir3 subfamily), (3) ATP-dependent K^+ channels (Kir1 and Kir4 subfamilies) and (4) ATP-sensitive K^+ channels (Kir6 subfamily) (Isomoto et al., 1997). Our electrophysiological and pharmacological data suggest that the inwardly rectifying K^+ channels in RERF-LC-MA cells do not belong to either (1) Kir3 subfamily, because the channels were not sensitive to BAPTA, (2) Kir1 subfamily, because the rectification of the channels was much stronger than that of Kir1 (Doupnik et al., 1995) or (3) Kir6 subfamily, because the channels were not inhibited by glibenclamide.

The most probable candidate for a molecule of the present K^+ channel is, therefore, Kir2 subfamily. In fact, the present RT-PCR experiments showed that Kir2.1 is expressed in the human SCLC cell, RERF-LC-MA (Fig. 9). Furthermore, the present Western blot analysis confirmed the expression of Kir2.1 protein in RERF-LC-MA cells (Fig. 10).

So far, the properties of mouse Kir2.1 but not human Kir2.1 have been well characterized. We found in the present study that sensitivities to extracellular Ba^{2+} (Fig. 4) and Cs^+ (Fig. 5) of human inwardly rectifying K^+ channels in RERF-LC-MA cells are comparable with those of the cloned mouse Kir2.1 expressed in *Xenopus* oocytes (Kubo et al., 1993). In addition, lowering the intracellular pH (pH_i) inhibited the present inwardly rectifying K^+ channels as reported previously for mouse Kir2.1 (Sabirov et al., 1997; Shieh et al., 1996): that is, the present inward K^+ currents of the RERF-LC-MA and the previous K^+ currents of mouse Kir2.1-expressed oocytes were decreased by 30% (pH_i 7.4 → 6.3; Fig. 8B) and 30% (pH_i 7.2 → 6.4; Shieh et al., 1996), respectively.

Concerning the protein kinase C-dependent regulation of mouse Kir2.1 activity, contradicting results using heterologous expression systems have been reported: Fakler et al. (1994) and Jones (1996) reported that activation of protein kinase C leads to inhibition of the Kir2.1 activity, whereas Henry et al. (1996) reported that activation of protein kinase C did not affect the Kir2.1 activity. In the present study using the human SCLC cell line, we found that protein kinase C-dependent phosphorylation leads to inhibition of the inwardly rectifying K^+ current (Figs. 6 and 7).

MRP1 and MDR1 are well known as multidrug resistance proteins included in the ATP-binding cassette superfamily. Campling et al. (1997) reported that 1 of 23 SCLC cell lines expressed MDR1 mRNA, whereas MRP1 expression was detected in 19 of 23 cell lines. They suggested that expression of MRP1 and MDR1 may not be the only factor accounting for the drug resistance of SCLC cell lines, and that the drug resistance is unlikely to be explained by a single resistance mechanism. In RERF-LC-MA cells, no expression of MRP1 and MDR1 proteins was observed (Fig. 1); however, the cells were reported to be fairly resistant to nimustine hydrochloride, a cell cycle phase nonspecific agent, indicating that the cells have some drug resistance mechanisms (Mitsuhashi et al., 1992). In contrast, NCI-H69 cells, lacking expression of MRP1 and MDR1, were highly sensitive to nimustine hydrochloride (Mitsuhashi et al., 1992).

Interestingly, whole-cell patch-clamp experiments of NCI-H69 cells, which are parental cells of the H69AR, showed no inwardly rectifying K^+ currents, and the predominant channel in NCI-H69 cells was a voltage-activated delayed rectifier K^+ channel (Pancrazio et al., 1993). However, MRP1-overexpressed H69AR cells, which were established by culturing NCI-H69 cells with doxorubicin, showed Ba^{2+}-sensitive inwardly rectifying K^+ currents (Jirsch et al., 1993). The extent of the inward K^+ conductance in H69AR cells (231 pS/pF in 5 mM external K^+; Jirsch et al., 1993) is comparable with that in RERF-LC-MA cells (200 pS/pF in 7 mM external K^+; Fig. 3B). We newly selected RERF-LC-MA cells that were resistant to doxorubicin (120 ng/ml), cepharanthine (1 μg/ml) and mezerin (100 nM), and found they expressed no MRP1 protein (H. Sakai, T. Shimizu, A. Ikari and N. Takeguchi, unpublished observation). Taken together, expression of inwardly rectifying K^+ channels may be involved in the drug resistance mechanisms of human SCLC cells. It is possible that RERF-LC-MA cells express other multidrug resistance proteins that are associated with Kir2.1.

Kir channels play essential roles in maintaining the resting membrane potential of cells (Isomoto et al., 1997). In fact, the resting membrane potential of RERF-LC-MA cells was governed by the K^+ concentration (Fig. 2). Therefore, Kir2.1 may be one of the housekeeping channels in the SCLC cell.

In summary, we found that the inwardly rectifying K^+ current of the human SCLC cell line is suggested to be generated by Kir2.1 protein, and that opening of the K^+ channel is regulated by protein kinase C and intracellular pH, and the expression of the channel is not regulated by MRP1 or MDR1. Apparently, further study using an antisense strategy is necessary to examine whether Kir2.1 is only the channel that generates the inwardly rectifying K^+ current in the SCLC cell line.

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