J. Membrane Biol. 185, 75–81 (2002)
DOI: 10.1007/s00232-001-0112-3

The Journal of
Membrane
Biology

Chloride Conductance Determining Membrane Potential of Rabbit Articular Chondrocytes

K. Tsuga[1,2], **N. Tohse**[1], **M. Yoshino**[1], **T. Sugimoto**[2], **T. Yamashita**[2], **S. Ishii**[2], **H. Yabu**[1]
Department of Physiology[1] and Orthopaedic Surgery[2], Sapporo Medical University School of Medicine, S-1 W-17 Chuo-Ku, Sapporo, 060-8556, Japan

Received: 1 February 2001/Revised: 6 August 2001

Abstract. Membrane conductance of cultured rabbit articular chondrocytes was characterized by means of the patch-clamp technique. The resting membrane potential of the articular chondrocytes was about −42 mV. The membrane potential shifted in accordance with the prediction by the Nernst equation for Cl^- when intracellular and extracellular concentrations of Cl^- were changed. On the other hand, change in extracellular concentration of K^+ produced no shift in the membrane potential of chondrocytes. The Cl^- channel blocker 4-acetamido-4′-isothiocyanatostilbene-2′2-disulfonic acid (SITS) depolarized the membrane potential. These findings suggest that the membrane potential of the chondrocytes is determined mainly by Cl^- conductance. Using the cell-attached patch-clamp method, a large unitary conductance of 217 pS was observed in the articular chondrocytes. The unitary current was reversibly blocked by SITS. Therefore, the unitary current was carried by Cl^-. The Cl^- channel showed voltage-dependent activation and the channels exhibited long-lasting openings. Therefore, the membrane potential of rabbit cultured articular chondrocytes was mainly determined by the activities of the large-conductance and voltage-dependent Cl^- channels.

Key words: Chloride channel — Membrane potential — Articular chondrocytes — SITS — Patch clamp — Rabbit

Introduction

Articular cartilage has extraordinary biomechanical properties of elasticity and a self-lubricating mechanism to protect the joint from pressure and shearing forces. Articular cartilage consists of articular chondrocytes and articular matrix. The chondrocytes are responsible for producing and maintaining the articular matrix. Therefore, the function of the chondrocytes has been studied in order to understand their role in the pathogenesis of major bone and joint diseases. Several studies using the patch-clamp methods (Grandolfo et al., 1990, 1992; Hall, Horwitz & Wilkins, 1996; Sugimoto et al., 1996; Mozrzymas, Martina & Ruzzier, 1997) have been performed to disclose the electrophysiological aspects of chondrocytes. Recently, pharmacological modulators on ion channels have been reported to have an influence on chondrocyte proliferation (Wohlrab & Hein, 2000).

Chondrocytes have been reported to have relatively low resting membrane potential (Sugimoto et al., 1996), similar to other nonexcitable cells, including red blood cells (Freedman, 1998), and endothelial cells (Nilius, Viana & Droogmans, 1997). Several reports have already shown the existence of K^+ channels in articular chondrocytes. K^+ channels are generally essential to maintain the membrane potential of most types of cells (Grandolfo et al., 1990, 1992; Mozrzymas et al., 1997). However, it is not likely that K^+ channels solely construct the membrane potential of the chondrocytes because their membrane potential (about −40 mV) (Sugimoto et al., 1996) is far less than the equilibrium potential of K^+ (about −90 mV). On the other hand, Cl^- channels and Na^+ channels were also observed in the chondrocytes (Sugimoto et al., 1996). In the present study, we investigated ionic conductance of the membrane potential of the chondrocytes cultured from rabbit articular cartilages. The present results indicate that Cl^- conductance and not K^+ conductance determines the membrane potential.

Correspondence to: N. Tohse; email:touse@sapmed.ac.jp

Materials and Methods

Cell Preparation

Animal preparation and isolation of chondrocytes were carried out as described previously (Nagao et al., 1991; Sugimoto et al., 1996). In brief, Japanese white rabbits (4-week old, weight: 0.8–1 kg) were sacrificed by an application of pentobarbital (i.v.). Articular cartilages from knee or shoulder joints of the rabbits were removed by shaving under sterile conditions. The cartilage slices were minced at about 1 mm^3 after rinsing 3 times in phosphate buffered saline (PBS). After enzymatic disaggregation of the tissue with 0.2% collagenase, cells were dispersed by incubating tissue in a medium containing Ham F12 (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% fetal calf serum (FCS) at 37°C for 4 hours in a humidified atmosphere with 5% CO_2. The cell suspension was filtered through a sterile #80 platinum mesh and centrifuged for five minutes at 1,500 revolutions per minute. The cell pellet was resuspended in the medium. The viability of the cells was determined by the trypan blue-exclusion method and was greater than 99%. The freshly isolated chondrocytes were plated at a density of 2–3×10^5 cells/ml in 35-mm Falcon tissue culture dishes. After 3–4 days, cultures were taken from the dishes and used for experiments. The cells were rinsed twice in the medium solution and then bathed in an extracellular solution. In experiments of the present study, a total of 58 separate isolations of cells were used (17 for whole-cell recordings; 41 for single-channel recordings).

Whole-Cell Recording

Membrane potential of the chondrocytes was measured with the whole-cell patch configuration. Patch pipettes were made by a two-step pulling of capillary tubes. Their resistance and diameters were 2–4 MΩ and 1–5 μm. Whole-cell currents were measured with an Axopatch-1D patch clamp amplifier (Axon Instruments Foster City, CA). The recorded membrane potentials were filtered by a 6-pole Bessel filter (2 kHz), then digitized, and stored on an IBM-compatible computer. Data were acquired and analyzed using pClamp software (Axon Instruments), and results were displayed on a digital plotter (Hewlett-Packard Laserjet IIIP printer).

The cells were bathed in standard extracellular solution with a composition of (in mM): 140 NaCl, 5 KC1, 2 $CaCl_2$, 1 $MgC1_2$, Tris (pH 7.4) (at a temperature of 22–25°C). In experiments of changing the extracellular concentration of K^+, NaCl was substituted with equimolar KCl. On the other hand, when extracellular concentration of Cl^- was changed, NaCl was substituted with equimolar Na-glutamate. The patch pipettes contained (in mM) 115 K-aspartate, 26 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 2 ATP, 10 EGTA, and Tris (pH 7.4) (total $[Cl^-]$ = 30 mM). When the intrapipette concentration of Cl^- was 20 mM, concentrations of KCl and K-aspartate were 16 and 125 mM, respectively. The concentration of free Ca^{2+} in both pipette solutions was calculated at 6.7×10^{-9} M.

Single-Channel Recording in Cell-Attached Patches

Cell-attached patch recordings were performed according to the standard techniques. Data were stored on an IBM-compatible computer. Current signals were analyzed on pClamp software (Axon Instruments). The current signals were filtered by a 6-pole Bessel filter at 2 kHz, and then digitized at 5 kHz. Open probability (P_o) was calculated from the averaged current divided by the single-channel amplitude or by using the fraction of total time in each dwell class. The cells were then bathed in a high-potassium solution composed of (in mM): 140 KCl, 2 $CaCl_2$, 1 $MgCl_2$, Tris (pH 7.4) (22–25°C). The patch pipettes were filled with solution containing (mM): 140 KCl, 1 $CaCl_2$, 1 $MgCl_2$, Tris (pH 7.4). In this condition, we assumed the membrane potential to be −50 mV because the intracellular concentration of Cl^- seems to be 20 mM, which is known as a normal value of most types of cells, including cardiomyocytes (Hume & Harvey, 1991), skeletal muscle (Aickin, 1990), and neurons (Alvarez-Leefmans et al., 1988) (also *see* Table 1). The values of potential in the single-channel recording were corrected by this resting membrane potential.

Table 1. Membrane potentials of the chondrocytes at intracellular concentration of Cl^- of 20 and 30 mM

Intracellular Cl^-	Mean (mV)	SD	*n*
30 mM	−42.2	13	104
20 mM	−48.1	11.3	38

The difference between membrane potential with 30 and 20 mM Cl^- is statistically significant by unpaired Student's *t*-test ($p < 0.01$).

Data and Statistics

The experiments were carried out at room temperature (22–25°C). All values are expressed as mean ± SD. Statistical analyses of changes were performed by use of Student's paired and unpaired *t*-test. Statistical significance was established when the probability value was less than 0.05.

Chemical Agents

The following chemical agents were used: Collagenase Type I (Sigma, St.Louis, MO), 4-Acetamide-4′-isothiocyanatostilbene-2,2′-disulfonic acid (SITS) (Sigma).

Results

Membrane Potential of Articular Chondrocytes

Membrane potential of the chondrocytes was recorded under current-clamp condition with the conventional patch configuration (Table 1). The mean value of the membrane potential was close to the equilibrium potential for Cl^- predicted by the Nernst equation (−41.4 mV). When the intrapipette concentration of Cl^- was decreased from 30 mM to 20 mM, the membrane potential was also close to the equilibrium potential for Cl^- (−51.8 mV). Therefore, the Cl^- conductance seems to contribute to the membrane potential of chondrocytes.

Effect of Cl^- Channel Blocker

To investigate the contribution of the Cl^- conductance to the membrane potential, 4-acetamido-4′-isothiocynatostilben-2′2-disulfonic acid (SITS), a blocker of Cl^- channels, was applied to the chon-

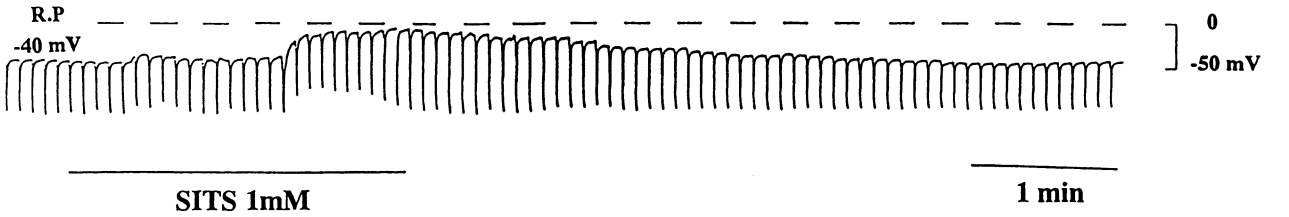

Fig. 1. Effect of SITS, a blocker of Cl^- channels, on the membrane potential of a chondrocyte. *R.P.* indicates control resting membrane potential of a chondrocyte. Constant current pulses (100 pA in amplitude; 50 msec in duration) were frequently applied to measure membrane conductance. After an application of SITS (1 mM), the membrane depolarized and membrane conductance decreased (*see* also Table 2). The cells were perfused with the standard bath solution (5 mM K^+). Pipettes contained 30 mM Cl^-.

Table 2. Changes in passive membrane characteristics by SITS (1 mM)

	Control	SITS	*n*	*p*
Membrane potential (mV)	−46.3 ± 13.2	−15.6 ± 9.3	33	<0.001
Membrane conductance (nS)	2.3 ± 1.1	2.0 ± 0.9	5	<0.02

Mean ± SD. Effects of SITS are statistically by paried Student's *t*-test. Constant current pulse to measure membrane conductance is 100 pA in amplitude, and 50 msec in duration.

drocytes (Fig. 1). The membrane potential was recorded by use of the current-clamp mode. Constant current pulses (100 pA in amplitude; 50 msec in duration) were applied through the pipettes to monitor changes in membrane conductance. On application of 1 mM SITS, the membrane potential markedly depolarized (Fig. 1 and Table 2). This depolarization was finally followed by a decrease in membrane conductance (Table 2).

Contribution of Cl^- and K^+ on Membrane Potential

The contribution of Cl^- and K^+ to membrane conductance for constructing membrane potential was examined by studying the concentration-potential relationship of the chondrocytes. The intrapipette solution containing 20 mM Cl^- was used. At first, when the concentration of K^+ in external solution was changed by substitution of equimolar Na^+, the membrane potential was not changed (Fig. 2*A*). During the experiment, the concentration of Cl^- was constant. K^+ conductance may not contribute to the membrane potential of the chondrocytes. On the other hand, the concentration of Cl^- in external solution was changed from 150 to 80, 40, or 20 mM by substitution with glutamate ion. In this experiment, the concentrations of Na^+ and K^+ were not changed. The membrane potential was depolarized as the concentration of Cl^- was decreased (Fig. 2*B*). A semilogarithmic plot showed a linear relationship between extracellular concentration of Cl^- and membrane potential. The relation was close to the theoretical line of the equilibrium potential of Cl^- deduced from the Nernst equation ($[Cl^-]_i$ = 20 mM). These data indicate that Cl^- conductance is relatively large in the membrane of chondrocytes.

Single-Channel Activities of Cl^- Channels

The present data about the membrane potential suggest that Cl^- channels are present in the articular chondrocytes. In order to characterize Cl^- channels, the cell-attached patch-clamp recording was applied to the chondrocytes. When the membrane potential was held at +30 mV constantly, outward unitary currents were frequently observed (Fig. 3). These channel activities were almost abolished by an application of 1 mM SITS, a blocker of Cl^- channels (n = 3). The channel activities were restored after washing out the SITS. Therefore, the activities were produced by the openings of the Cl^- channels. In the present experimental conditions, only one type of unitary conductance was observed. Figure 4 shows the voltage-current relationship of the Cl^- channels. The unitary conductance deduced from the voltage-current relationship was 218 pS. The mean value from 11 different cells was 217.2 ± 21.6 pS. Figure 5 shows the voltage-dependency of open probability (P_o) of the Cl^- channels. The P_o was markedly increased in accord with membrane depolarization. Figure 6 shows representative data of open-closed kinetics of Cl^- channels at +30 mV. The open-time distribution of the Cl^- channels matched two exponential components closely (Fig. 6*A*). The mean value of fast and slow time constants were 2.7 ± 1.8 and 24.3 ± 17.5 msec, respectively (n = 19). The relatively slow time constant indicated that the Cl^- channels undergo long-lasting openings. The closed-time distribution of the Cl^- channels was also matched closely by two exponential components (Fig. 6*B*). The mean value of fast and slow time constants were 0.8 ± 0.3 and 39.3 ± 32.7 msec, respectively (n = 15). These time constants of closed-time distribution indicate that the Cl^- channels undergo a bursting of openings interrupted by a relatively long closing, as shown in Fig. 4.

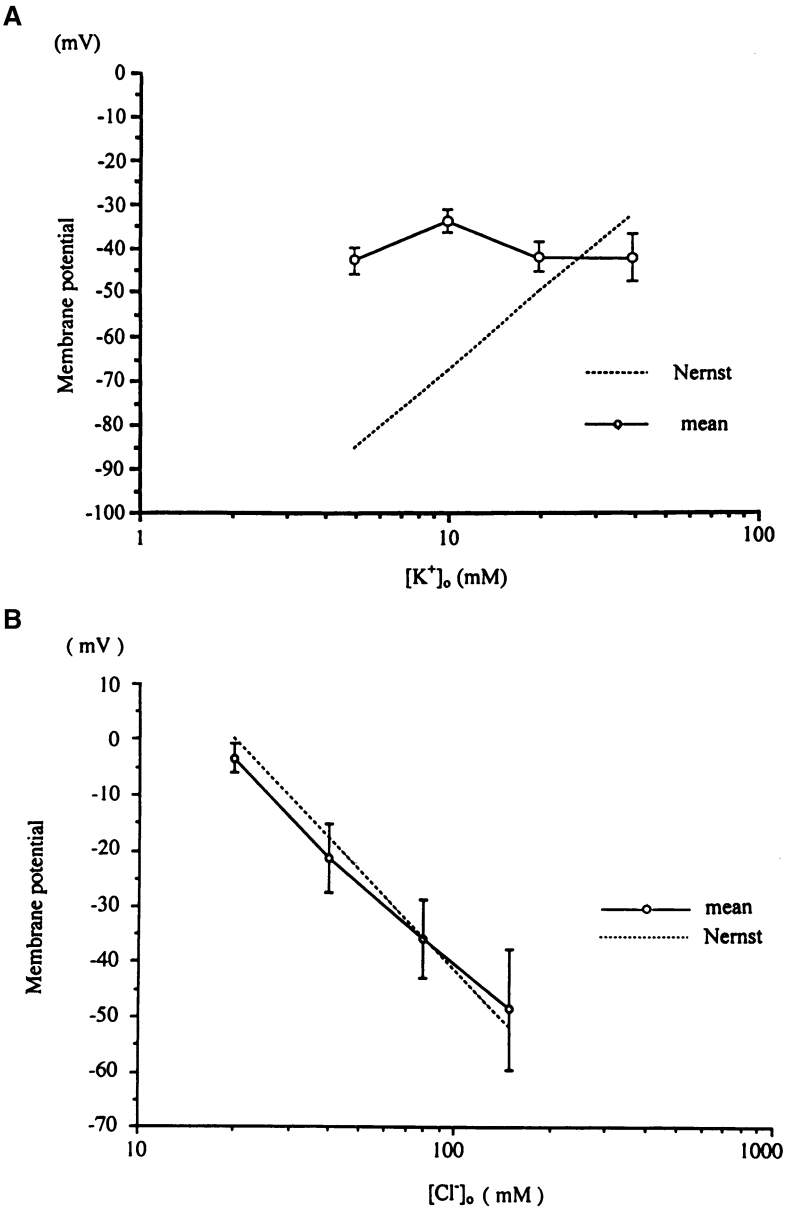

Fig. 2. (*A*) Relationship between extracellular concentration of K^+ and membrane potential of chondrocytes. Data are shown as mean ± SD. Broken line indicates K^+ equilibrium potential predicted by the Nernst equation. Pipette contained 20 mM Cl^-. (*B*) Relationship between extracellular concentration of Cl^- and membrane potential of chondrocytes. Data are shown as mean ± SD. Broken line indicates Cl^- equilibrium potential predicted by the Nernst equation. Pipette contained 20 mM Cl^-.

Discussion

Membrane Potential

In the present study, the membrane potential of the cultured chondrocytes was about −40 to −50 mV. This value was close to that reported by the previous study (Sugimoto et al., 1997). A low membrane potential was observed in several types of nonexcitable cells, including osteoblasts (Dixon, Aubin & Dainty, 1984; Ypey et al., 1988; Fritsch, Edelman & Balsan, 1987). On the other hand, osteoclasts had a more negative (deep) membrane potential (Smith, Kelly & Dixon, 1991). The low membrane potential seems not to be produced by K^+ conductance because the equilibrium potential of K^+ was about −90 mV in the physiological condition. In fact, we observed that an alteration in the extracellular concentration of K^+ produced no change in the membrane potential of chondrocytes. The present results indicate that the production of shallow membrane potential may be due to Cl^- conductance. In the present study, the membrane potential shifted in accordance with the Nernst equation for Cl^- when intrapipette and extracellular [Cl^-] were changed. In addition, the membrane potential was reversibly depolarized by an application of the Cl^- channel blocker SITS. SITS also decreased the membrane conductance of the chondrocytes. All these results indicate that the membrane potential of articular chondrocytes is mainly produced by Cl^- conductance. We have already reported a Cl^- current in rabbit articular

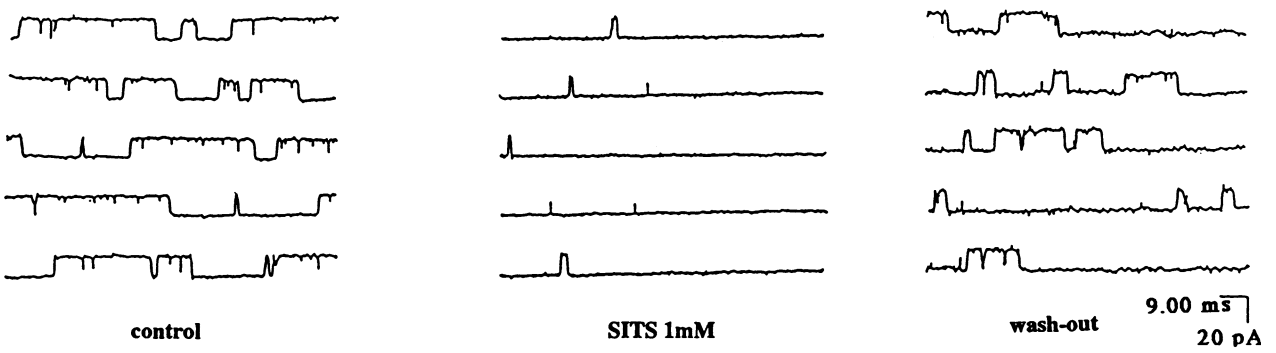

Fig. 3. Single-channel recording in rabbit articular chondrocytes. Activities of single channels were recorded by the cell-attached patch clamp with high-Cl^- intrapipette solution. Potential of the patch membrane was held at +30 mV. Outward unitary currents were frequently observed in control condition (*control*). When 1 mM SITS was applied to the bath solution, the channel activities were markedly inhibited. After a washout of SITS, the channel activities recovered. The data indicate that the channel activities were produced by openings of Cl^- channels.

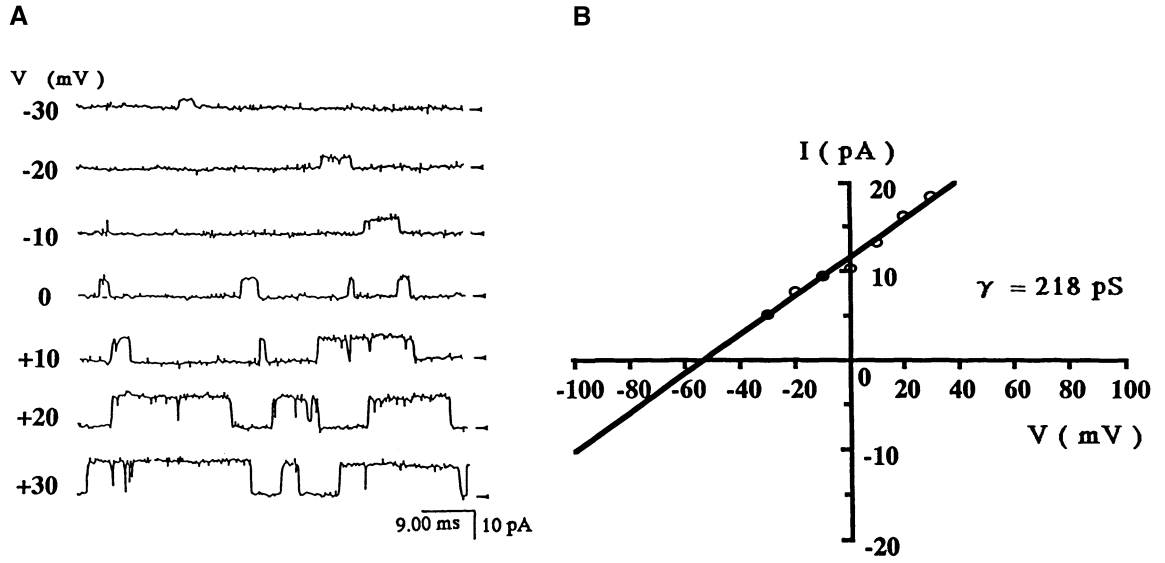

Fig. 4. The current-voltage relationship of the Cl^- channel of rabbit articular chondrocytes. Panel *A* shows representative recordings of the Cl^- channel. The recordings were obtained by cell-attached patch clamp. Values to the left of the current traces indicate the transmembrane potential of the patch membrane. A cell membrane potential of −50 mV was assumed to calculate the patch membrane potential (*see* Materials and Methods). Arrows to the right of the current traces indicate the zero-current level. Panel *B* shows the unitary current-voltage relationship. The theoretical line was deduced by the least-square regression analysis. The line indicates a unitary conductance of 218 pS.

chondrocytes (Sugimoto et al., 1997). The current decreased when 0.25 mM SITS was applied to the cells. Therefore, this current may determine the membrane potential.

It is still likely that the value of membrane potential in the present study is changed because of culturing. Recently, we have measured the membrane potential of the freshly-isolated chondrocytes (Okubo, Yamada, Tsuga & Tohse, 2001, unpublished observation). The membrane potential of the freshly-isolated cells was −44 ± 10.8 mV (n = 9). Therefore, keeping the cells in culture did not seem to change their membrane potential. However, attention should be still paid to the fact that chondrocytes in situ are exposed to higher osmolarity of matrix. In the present study, we used isotonic solutions. Influence of extracellular osmolarity should be examined in further study.

K^+ Channels

Some reports have provided evidence for the existence of K^+ channels in articular chondrocytes. One type of K^+ channel is of high conductance and dependent on Ca^{2+} (Grandolfo et al., 1990). Another type of K^+ channel with high conductance is dependent on membrane potential (Mozrzymas et al., 1997). In addition, a type of K^+ channel having low

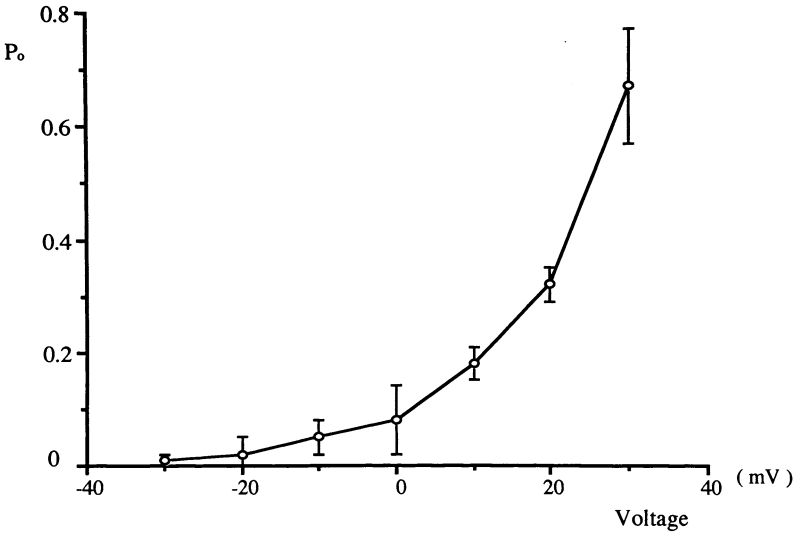

Fig. 5. The voltage dependency of open probability of the Cl^- channels. The open probability was increased by depolarization of the patch membrane. Data are shown as mean ± SD.

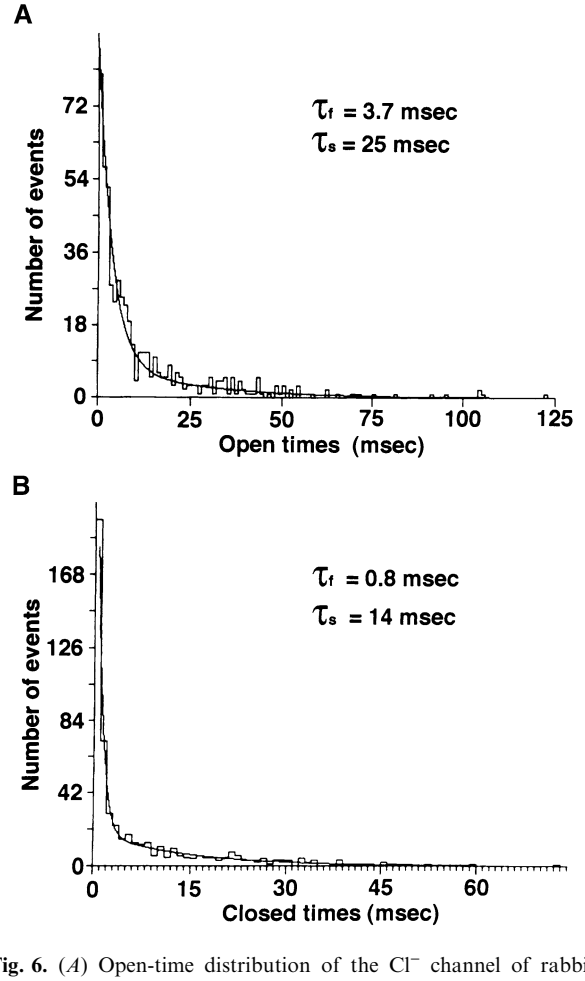

Fig. 6. (*A*) Open-time distribution of the Cl^- channel of rabbit articular chondrocytes at +30 mV. The histogram was fitted by a two-exponential function. The time constants of fast and slow component were 3.7 and 24 msec, respectively. (*B*) Closed-time distribution of the Cl^- channel of rabbit articular chondrocytes at +30 mV. The histogram matched a two-exponential function. The time constants of the fast and slow component were 0.8 and 14 msec, respectively.

conductance has been reported (Mozrzymas, 1994). In our previous report, a substantial amount of K^+ current was observed in the chondrocytes (Sugimoto et al., 1997). However, those reports did not examine a contribution of K^+ channel activity to membrane potential. The present results indicate that K^+ conductance does not contribute to determining the (resting) membrane potential of the chondrocytes. In addition, Wright et al. (1996) showed that several K^+ channel blockers did not change membrane potential of cultured chondrocytes. These K^+ channels may act to modulate membrane potential when pressure stress (Wright et al., 1996) or chemical mediators are applied to the chondrocytes.

Cl^- Channels

Cl^- channels contribute to various cellular functions in nonexcitable cells. It is well known that Cl^- channels play an important role in transport function, particularly in epithelial and endothelial cells. On the other hand, Cl^- channels are probably present in every animal cell (Nilius et al., 1997). Their major functional impact includes regulation of cell volume, regulation of the intracellular pH in cooperation with a variety of transporters, and setting of the membrane potential and the transport of amino acids (Nilius, 1998). In the present study, activities of Cl^- channels with a large unitary conductance (217 pS) were observed in rabbit articular chondrocytes. The channel activities were enhanced at depolarizing potential (voltage dependency), and blocked by an application of the Cl^- channel blocker SITS. These characteristics of the Cl^- channels accorded with those of the whole-cell Cl^- current that we previously reported (Sugimoto et al., 1997). Cl^- channels with large conductance have been found in cultured rat myocytes (Blatz & Magleby, 1983), A6 renal cells

(Nelson, Tang & Palmer, 1984), rat Schwann cells (Gray, Bevan & Ritchie, 1984), and rat pulmonary alveolar cells (Schneider et al., 1985). These Cl^- channels have also shown strong voltage dependency. Oyama & Walker (1986) and Stea & Nurse (1989) reported that large-conductance Cl^- channels contribute to membrane potential in cultured glomus cells. Therefore, it seems likely that the large-conductance, voltage-dependent Cl^- channels determine the membrane potential of the articular chondrocytes.

Physiological Significance

The present study shows that the large-conductance Cl^- channels keep the membrane potential of the chondrocytes at low potential (about −40 mV). This low membrane potential may create a situation where other types of channels can easily regulate cellular function of articular chondrocytes. Activities of the Ca^{2+}-dependent K^+ channel have been observed in articular chondrocytes (Grandolfo et al., 1990). If some influences increase the intracellular Ca^{2+} of the chondrocytes, the K^+ channels hyperpolarize the membrane potential toward the equilibrium potential of K^+ (−90 mV). The impact of this hyperpolarization from the low membrane potential may facilitate some cellular functions. On the other hand, some types of channels (i.e., nonspecific cation channels, Na^+ channels, Ca^{2+} channels) may depolarize the membrane potential from −40 mV, then producing an impact on the chondrocytes.

References

Aickin, C.C. 1990. Chloride transport across the sarcolemma of vertabrate smooth and skeletal muscle. *In:* Chloride Channels and Carriers in Nerve, Muscle and Glial Cells. F.J. Alvarez-Leefmans, J.M. Russell, editors. pp. 209–249. Plenum Press, New York

Alvarez-Leefmans, F.J., Gamiño, S.M., Giraldez, F., Noguerón, I. 1988. Intracellular chloride regulation in amphibian dorsal root ganglion neurones studied with ion-selective microelectrodes. *J. Physiol.* **406:**225–246

Blatz, A.C., Magleby, K.L. 1983. Single voltage-dependent chloride-sensitive channels of large conductance in cultured rat muscle. *Biophys. J.* **43:**237–241

Dixon, S.J., Aubin, J.E., Dainty, J. 1984. Electrophysiology of a cloned osteoblast-like cell line: Evidence for the existence of a Ca-activated K conductance. *J. Membrane Biol.* **80:**49–58

Freedman, J.C. 1998. Membrane transport in red blood cells. *In:* Cell Physiology Source Book, 2nd edition. N. Sperelakis, editor. pp. 325–341. Academic Press, New York

Fritsch, J., Edelman, A., Balsan, S. 1987. Comparative studies of membrane potentials of rat osteoblasts in situ and in primary culture. *Bone Mineral.* **2:**463–478

Grandolfo M., D'Andrena P., Martina M., Ruzzier, F., Vittur, F. 1992. Calcium-activated potassium channels in chondrocytes. *Biochem. Biophys. Res. Comm.* **182:**1429–1434

Grandolfo, M., Martina, M., Ruzzier, F., Vittur, F. 1990. A potassium channel in cultured chondrocytes. *Calcified Tissue Int.* **47:**302–307

Gray, P.T., Bevan, S., Ritchie, J.M. 1984. High-conductance anion-selective channels in rat cultured Schwann cells. *Proc. R. Soc. B London* **221:**395–409

Hall, A.C., Horwitz, E.R., Wilkins, R.J. 1996. Physiological Society Symposium: Ion transport in health and disease; the cellular physiology of articular cartilage. *Exp. Physiol.* **81:**535–545

Hume, J.R., Harvey, R.D. 1991. Chloride conductance pathways in heart. *Am. J. Physiol.* **261:**C399–C412

Mozrzymas, J.W., Martina, M., Ruzzier, F. 1997. A large-conductance voltage-dependent potassium channel in cultured pig articular chondrocytes. *Pfluegers Arch.* **433:**413–427

Mozrzymas, J.W., Visintin, M., Vittur, F., Ruzzier, F. 1994. Potassium channels of pig articular chondrocytes are blocked by propofol. *Biochem. Biophys. Res. Comm.* **202:**31–37

Nagao, M., Ishii, S., Murata, Y., Akino, T. 1991. Effect of extracellular fatty acids on lipid metabolism in cultured rabbit articular chondrocytes. *J. Orthopaed. Res.* **9:**341–347

Nelson, D.J., Tang, J.M., Palmer, L.G. 1984. Single-channel recordings of apical membrane chloride conductance in A6 epithelial cells. *J. Membrane Biol.* **80:**81–89

Nilius, B. 1998. Ion channels in nonexcitable cells. *In:* Cell Physiology Source Book, 2nd edition. N. Sperelakis, editor, pp. 436–455. Academic Press, New York

Nilius, B., Viana, F., Droogmans, G. 1997. Ion channels in vascular endothelium. *Ann. Rev. Physiol.* **59:**145–170

Oyama, Y., Walker, J.L. 1986. The intracellular chloride activity of glomus cells in the isolated rabbit carotid body. *Brain Res.* **368:**167–169

Schneider, G.T., Cook, D.I., Gags, P.W., Young, J.A. 1985. Voltage sensitive, high-conductance chloride channels in the luminal membrane of cultured pulmonary alveolar (type II) cells. *Pfluegers Arch.* **404:**354–357

Smith, S.M., Kelly, M.E., Dixon, S.J. 1991. K and Cl current in freshly isolated rat osteoclasts. *Pfluegers Arch.* **419:**358–370

Stea, A., Nurse, C.A. 1989. Chloride channels in cultured glomus of the rat carotid body. *Am. J. Physiol.* **257:**C174–181

Sugimoto, T., Yoshino, M., Nagao, M., Ishii, S., Yabu, H. 1996. Voltage-gated ionic channels in cultured rabbit articular chondrocytes. *Comp. Biochem. Physiol. C* **115:**223–232

Wright, M., Jobanputra, P., Bavington, C., Salter, D.M., Nuki, G. 1996. Effects of intermittent pressure-induced strain on the electrophysiology of cultured human chondrocytes: evidence for the presence of stretch-activated membrane ion channels. *Clin. Sci.* **90:**61–71

Wohlrab, D., Hein, W. 2000. Effect of ion channel modulators on the membrane potential of human chondrocytes. *Orthopäde* **29:**80–84

Ypey, D.L., Ravesloot, J.H., Buisman, H.P., Nijweide, P.J. 1988. Voltage-activated ionic channels and conductances in embryonic chick osteoblast cultures. *J. Membrane Biol.* **101:**141–150