Voltage-gated K⁺ currents in mouse articular chondrocytes regulate membrane potential

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Abbreviations: AC, articular chondrocyte; r.m.p., resting membrane potential; ATP, adenosine 5'-triphosphate

Membrane currents and resting potential of isolated primary mouse articular chondrocytes maintained in monolayer cell culture for 1–9 days were recorded using patch clamp methods. Quantitative RT-PCR showed that the most abundantly expressed voltage-gated K⁺ channels was for K⁺1.6, and immunological methods confirmed the expression of K⁺1.6 α-subunit proteins. These chondrocytes expressed a large time- and potential-dependent, Ca²⁺-independent ‘delayed rectifier’ K⁺ current. Steady-state activation was well-fit by a Boltzmann function with a threshold near -50 mV, and a half-activation potential of -34.5 mV. The current was 50% blocked by 1.48 mM tetraethylammonium, 0.66 mM 4-aminopyridine and 20.6 nM α-dendrotoxin. The current inactivated very slowly at membrane potentials in the range of the resting potential of the chondrocytes. Resting membrane potential of the chondrocytes at room temperature (19–21°C) and in 5 mM external K⁺ was -46.4 ± 1.3 mV (mean ± s.e.m; n = 23), near the ‘foot’ of the activation curve of this K⁺ current. Resting potential was depolarized by an average of 4.2 ± 0.8 mV by 25 mM TEA, which blocked about 95% of the K⁺ current. At a membrane potential of -50 mV, the apparent time constant of inactivation (tauᵦ) was 37.9 s, and the ‘steady-state’ current level was 19% of that at a holding potential of -90 mV, at -40 mV, tauᵦ was 20.3 s, and ‘steady-state’ current was 5% of that at -90 mV. These results demonstrate that in these primary cultured, mouse articular chondrocytes steady-state activation of a voltage-gated K⁺ current contributes to resting membrane potential. However, this current is also likely to have a significant physiological role in repolarizing the chondrocyte following depolarizing stimuli that might occur in conditions of membrane stretch. For example, activation of TRPV1 (‘transient receptor potential’ non-specific cation channels in these cells during cyclic loading and unloading of the joint cartilage, or in response to hypertonic challenge is expected to result in depolarization and Ca²⁺ entry. Potassium currents are required to maintain the resting membrane potential.

Introduction

Chondrocytes constitute the only cell type in diarthrodial joint cartilage. Due to normal loading and unloading of the joint chondrocytes are subject to repetitive mechanical and osmotic stresses.¹ Since chondrocytes synthesize extracellular matrix proteins (e.g., aggregans and collagens), these cells are central to the maintenance of the integrity of the cartilage.² At present the molecular bases of the transduction between external stimuli and production of the constituents of the cartilage are not completely understood. Metabolism of the cartilage constituents by the chondrocytes is sensitive to both extracellular³ and intracellular⁴–⁸ ionic concentrations. Thus, significant functional roles of plasma membrane ion channels are expected.⁹,¹⁰ As membrane potential governs ionic fluxes through ion channels, clarification of the factors responsible for generating the membrane potential of articular chondrocytes is essential for an understanding of the mechanical and osmotic transduction processes, and excitation-secretion coupling in these cells.

Articular chondrocytes from a variety of mammalian species are known to express several types of ion channels. A number of these could contribute to membrane potential. For example, electrophysiological studies in isolated chondrocytes have directly identified voltage-gated¹¹–¹⁴ Ca²⁺-activated¹⁵ ATP-sensitive¹⁶ and stretch-activated¹⁷ K⁺ channels. Non-specific cation channels,¹⁸ voltage-activated¹⁹ and swelling-activated²⁰,²¹ Cl⁻ and voltage-gated H⁺²²,²³ channels have also been recorded in isolated chondrocytes. Other experimental work has provided indirect evidence for the possible presence of stretch-activated non-selective cation channels²⁴–²⁶ and transient receptor potential vanilloid, or TRPV²⁷–²⁹ channels. All of these types of channels could contribute to altering membrane potential under appropriate conditions.
Significant expression of mRNA of eight K V channel subtypes (KV1.1, KV1.2, KV1.6, KV2.1, KV3.3, KV4.1, KV4.2, KV4.3) was detected (data not shown). The levels of expression of these K V channel transcripts were further examined using quantitative real-time RT-PCR analysis (Fig. 1A). The data in Figure 1A show that mRNA for 4 K V channel α-subunits, namely, KV1.6, KV2.1, KV3.3 and KV4.1 was relatively abundant in mouse AC, with KV1.6 exhibiting much the largest expression level relative to the 'housekeeping' gene GAPDH.

Current-voltage relations in mouse articular chondrocytes. Figure 2A shows an example of a family of membrane currents recorded from a mouse AC after 8 days in culture. Currents over the potential range from -110 mV to about -60 mV were circumstances. For example, previous in vitro electrophysiological studies have shown that voltage-gated K+ channels contribute to membrane potential of ‘resting’ rat4 and dog12 articular chondrocytes. Activation of ‘small conductance’ Ca2+-activated K+ channels was suggested to underlie the apamin-sensitive membrane hyperpolarization of human articular chondrocytes that followed repetitive mechanical stimulation.23,28

The mouse is often the experimental animal of choice because of the relative ease with which genetic manipulations can be performed on this species.29 In spite of this, there is very little information concerning ion channels, pumps and transporters in mouse articular chondrocytes. These data are necessary for an understanding of normal and pathophysiology of chondrocytes. The purpose of this study was to examine membrane currents in primary cultured mouse articular chondrocytes (AC) using patch clamp methods. Our results demonstrate that a prominent current in these cells is a voltage-gated K+ current with ‘delayed rectifier’ characteristics, and is not dependent on internal Ca2+ concentration for its activation. The voltage-dependence of activation and pharmacological properties of this current strongly suggests that it plays a role in establishing and modulating the membrane potential of mouse AC.

Results

Expression of voltage-gated K+ channels in mouse articular chondrocytes. The expression levels of mRNA for 15 subtypes of voltage-gated K+ (Kv) channel α-subunits were initially examined in mouse AC using non-quantitative RT-PCR analysis. Significant expression of mRNA of eight Kv channel subtypes (Kv1.1, 1.2, 1.6, Kv2.1, Kv3.3, Kv4.1, 4.2, 4.3) was detected (data not shown). The levels of expression of these Kv channel transcripts were further examined using quantitative real-time RT-PCR analysis (Fig. 1A). The data in Figure 1A show that mRNA for 4 Kv channel α-subunits, namely, Kv1.6, 2.1, 3.3 and 4.1 was relatively abundant in mouse AC, with Kv1.6 exhibiting much the largest expression level relative to the ‘housekeeping’ gene GAPDH.

Western blots for Kv1.6 α-subunits in mouse AC showed a prominent band near 50 kDa, corresponding to the expected molecular weight of Kv1.6 α-subunit (arrow). (C) Immunohistochemical identification of K1.6 α-subunit (red) and α smooth muscle actin (green) proteins in an isolated mouse AC. Superposition of Kv1.6 and α-smooth muscle actin staining is shown at the extreme right. White scale bar indicates 10 µm.
inactivation of this current, especially at strongly depolarized membrane potentials.

Figure 2B shows the mean current density-voltage (IV) relation for 12 mouse AC after 6–8 days in culture. All of these cells had a similar pattern of membrane currents to that shown in Figure 2A. The zero-current potential of the IV was approximately -50 mV, which was close to the membrane potentials where the IV relation became distinctly non-linear due to activation of the time and voltage-dependent outward current. These results identified the most prominent transmembrane current in mouse AC, and suggested that this large time- and voltage-dependent

![Figure 2](image)

**Figure 2.** (A) Current-voltage relation of a mouse articular chondrocyte (AC). Family of membrane currents from an AC, after 8 days in culture, is shown. Cell capacitance was 14.4 pF. Cell was held at -90 mV, and stepped for 500 ms to membrane potentials between -110 mV and +70 mV. (B) Mean (±s.e.m.) current-voltage (IV) relation from 12 cells after 6–8 days in culture. Currents were normalized to cell capacitance; mean capacitance was 16.8 pF (±1.6). (C) Reversal potential of voltage-dependent outward current. (A) Example of voltage-clamp protocol (inset) and membrane currents used to determine reversal potential of outward current. (D) Plot of tail current amplitude vs. membrane potential from currents in (C). • indicates ‘peak tail current’ (see arrow in C); ○ indicates ‘steady-state tail current’. Reversal potential for this example, namely the potential at which peak and steady-state lines crossed, was determined to be -82.5 mV by linear interpolation.

time-independent, with a linear current-voltage relation. The mean apparent input resistance (not ‘corrected’ for pipette seal resistance) for 22 cells was 3.44 ± 0.26 GΩ (recorded with soda-lime patch pipettes). In contrast, voltage steps positive to about -50 mV produced time-dependent outward currents whose rate of activation and magnitude increased markedly in response to progressively larger membrane depolarizations. The general characteristics of this outward current resembled that of a ‘delayed rectifier’. In the example shown in Figure 2A, the currents inactivated very little during the 500 ms voltage-clamp steps. However, some cells showed a small (~10%) amount of inactivation of this current, especially at strongly depolarized membrane potentials.

Figure 2B shows the mean current density-voltage (IV) relation for 12 mouse AC after 6–8 days in culture. All of these cells had a similar pattern of membrane currents to that shown in Figure 2A. The zero-current potential of the IV was approximately -50 mV, which was close to the membrane potentials where the IV relation became distinctly non-linear due to activation of the time and voltage-dependent outward current. These results identified the most prominent transmembrane current in mouse AC, and suggested that this large time- and voltage-dependent
outward current could play a significant role in setting the resting membrane potential of mouse AC.

The outward current is a $K^+$ current. Figure 2C shows an example of currents produced in a mouse AC by a voltage-clamp protocol that was used to determine the reversal potential of the time- and voltage-dependent outward current. The reversal potential of the outward current in this example was -82.5 mV (Fig. 2D). The mean reversal potential of the outward current from 13 different cells, in culture for 1–9 days, was -84.1 ± 0.6 mV. The Nernst potential for $K^+$ in the internal and external solutions was -83 mV, which confirmed that the outward rectifying current in mouse AC was a $K^+$ current. In seven cells, the shift in reversal potential of the current when external $K^+$ was increased from 5 to 15 mM was +26.1 ± 0.7 mV, not significantly different than the shift of +27.5 mV expected for a purely $K^+$ selective ion channel ($p = 0.09$).

Inhibition of outward current by $K^+$ channel blockers TEA, 4-AP and α-DTX. The $K^+$ current in mouse AC was blocked by three selected $K^+$ channel blockers. Two of these are rather non-specific blockers, namely TEA and 4-AP. However, the much more specific blocker, the snake toxin α-DTX was also effective and potent. Figure 3A shows block of the $K^+$ current by 5 mM and 25 mM TEA. These IV relations were produced with voltage-clamp ‘ramps’. Activation of the time- and voltage-dependent $K^+$ current was observed positive to about -50 mV, as can be seen by the significant non-linearity in the IV relation which occurred near this membrane potential (cf. Fig. 2). Five mM TEA significantly reduced the magnitude of the outward current at all membrane potentials, and 25 mM TEA almost completely abolished the non-linearity in the IV. Note that the zero-current potential of the IV shifted to slightly more depolarized membrane potentials as the concentration of TEA was increased. This finding suggests that block of this $K^+$ current would be expected to result in a depolarization of the resting membrane potential. The dose-response relation for TEA-induced block of the $K^+$ current, shown in inset to Figure 3A, was fitted to the equation

$$I = A/(1 + [TEA]/K_d) + (1 - A)$$

where $A$ is the fraction of current that is sensitive to TEA and $K_d$ is the 50% blocking dose of TEA. The best-fit line had $A = 0.77 ± 0.02$ and $K_d = 1.48 ± 0.14$ mM. Figure 3B compares currents elicited by a series of voltage-clamp steps in control conditions and in the presence of 25 mM TEA. The current remaining in the presence of 25 mM TEA exhibited little detectable time-dependence. This is consistent with the TEA dose-response relation (Fig. 3A), which predicts that 25 mM TEA would block about 95% of the drug-sensitive component (‘$A$’, in equation (i)) of the total membrane current of the chondrocytes.

There was no effect of TEA on the membrane currents at potentials negative to about -60 mV. For 18 cells which were exposed to 25 mM TEA, the linear slope conductance measured between -60 and -100 mV in control conditions was 0.44 ± 0.05 nS, and 0.40 ± 0.04 nS during exposure to 25 mM TEA ($p = 0.18$; paired t-test).

Experimental protocols identical to those in Figure 3A showed that the $K^+$ channel blocker 4-aminopyridine (4-AP) inhibited the $K^+$ current with a $K_d$ of 0.66 ± 0.07 mM. The 4-AP-sensitive fraction of current was 0.75 ± 0.02 (data not shown; see Suppl. Materials).

Figure 3C and D provide important additional information concerning the pharmacological profile of this mouse AC $K^+$ current. These results show that time-dependent $K^+$ current can be blocked by low concentrations of the snake toxin α-DTX. Figure 3C shows families of membrane currents in control conditions, and in the presence of 10 and 100 nM α-DTX. Note that 100 nM α-DTX almost completely blocked the time-dependent portion of the current, leaving a ‘pedestal’ qualitatively very similar to that which remained in the presence of 25 mM TEA (cf. Fig. 3B). Figure 3D is the dose-response relation for α-DTX block of the $K^+$ current, at +50 mV, fitted to equation (i). The best fit values of $A$ (α-DTX-sensitive fraction of current) and $K_d$ (50% blocking dose of α-DTX) were 0.84 ± 0.03 and 20.6 ± 1.7 nM, respectively.

Voltage-dependence of mouse AC $K^+$ current activation. The membrane-potential dependence of activation of the TEA-sensitive $K^+$ current is shown in Figure 4. An example of a family of TEA-sensitive $K^+$ current produced by a series of 500 ms voltage-clamp steps is shown in Figure 4A. This family of currents was similar to that in Figure 2A, but these currents were corrected at each membrane potential for the current remaining in the presence of 25 mM TEA (cf. Fig. 3B). Figure 4B summarizes data pooled from 17 different AC, in which TEA-corrected slope conductance density (conductance/cell capacitance) is plotted against membrane potential. Slope conductance density was obtained by dividing membrane current density at each potential $V_m$ by the quantity $(V_m - E_R)$, where $E_R$ is the reversal potential of the current, which was set at -84 mV. This value was consistent with the reversal potential of the $K^+$ current. Figure 4B shows a Boltzmann function fitted to the mean slope conductance-vs-voltage relation. The function was:

$$G = G_{max}/(1 + \exp[-(V_m - V_{h})/S_h])$$

where $G_{max}$ is the maximal conductance, $V_{h}$ is the potential for half-activation of the current, and $S_h$ is a slope factor at $V_{h}$. The best-fit Boltzmann function plotted in Figure 5B had $G_{max} = 0.59 ± 0.005$ nS/pF, $V_{h} = -34.5 ± 0.4$ mV and $S_h = 6.9 ± 0.4$ mV.

Slow inactivation of mouse AC $K^+$ current. The $K^+$ current inactivated relatively little during 500 ms voltage-clamp steps used in the IV protocols in Figures 2 and 4. However, significant inactivation occurred during much longer membrane depolarizations. Figure 5 shows examples of data from two different voltage-clamp protocols. The data in Figure 5A shows examples of currents produced from one cell during 30 s depolarizing steps over the membrane potential range from -20 to +40 mV, i.e., potentials at which large $K^+$ currents were generated. The magnitude of the $K^+$ current declined by >90% during these long depolarizing steps. Similar results were obtained from 4 other cells. Figure 5B shows data from a different cell using a voltage-clamp protocol which was designed.
Figure 6 shows the time course of development of slow inactivation of K⁺ current at two membrane potentials, namely -40 and -50 mV. This range of potentials was chosen because it spanned the range of resting membrane potentials recorded from the majority of isolated mouse AC under our experimental conditions (see below). The voltage-clamp protocol in these experiments consisted of a train of depolarizing ‘test’ steps (+10 mV, 500 ms) applied at 10 s intervals. The time course of development of inactivation of K⁺ current was measured from the magnitude of ‘test’ currents. Membrane potential was held at either -40 mV or -50 mV during the train of steps. Figure 6A is an example of a family of ‘test’ step currents recorded at holding potential of -40 mV. In this example, the ‘test’ current amplitude to demonstrate slow inactivation over a membrane potential range (-110 to -50 mV) where relatively little or no K⁺ current was activated. Identical ‘test’ steps were applied at the beginning and end of 30 s ‘conditioning’ steps. Inactivation of K⁺ current was measured by the ratio of peak current produced by the second ‘test’ step (P2, in Fig. 5B) to peak of the initial ‘test’ step (P1). These data show that there was no significant difference in the magnitude of first and step ‘test’ steps for conditioning potentials of -110 mV and -90 mV (i.e., no inactivation), whereas significant inactivation was produced for conditioning potentials between -70 and -50 mV. Similar results were obtained from 6 other cells.
The time course of decline of the normalized ‘test’ current magnitude was well-described by a single exponential function of the form

$$I(t) = A \times \exp \left( -\frac{t}{\tau_{in}} \right) + B$$

decreased by about 50% during the first 30 s of the protocol, and continued to decline to about 14% of its initial amplitude by the end of the 190 s protocol. (The slowly decaying ‘tail currents’ at the end of the depolarizing steps arose because of the depolarized holding potential). Figure 6B compares the time course of inactivation at holding potentials of -40 mV and -50 mV. Data from 6 cells were averaged (different groups of cells at each potential). The time course of decline of the normalized ‘test’ current magnitude was well-described by a single exponential function of the form

$$I(t) = A \times \exp \left( -\frac{t}{\tau_{in}} \right) + B$$

**Figure 4.** Potential-dependence of activation of the TEA-sensitive K⁺ current in mouse AC. (A) Example of a family of currents produced by a series of voltage-clamp steps between -130 mV and +40 mV, from a holding potential of -90 mV. The voltage-clamp protocol was run twice, in the presence and absence of 25 mM TEA in the bathing solution. The net TEA-sensitive current, normalized to cell capacitance (12.4 pF) is shown. (B) Slope conductance-voltage relation for TEA-sensitive K⁺ current, averaged (mean ± s.e.m.) from 17 different AC, with mean capacitance of 11.6 ± 0.75 pF. Slope conductance was obtained from membrane currents by dividing current density at each membrane potential, Vₘ, by (Vₘ - E), where E is reversal potential of the current. The solid line is the best-fit Boltzmann function, equation (ii) (see text for details).

**Figure 5.** Slow inactivation of mouse AC K⁺ current. (A) Family of currents produced by 30 s depolarizing steps from -20 mV to +30 mV (20 mV increments), from a holding potential of -90 mV. The interval between successive steps was 60 s. Currents in the presence of 25 mM TEA were subtracted. Inset shows first 1 s of currents at onset of depolarizing steps. Cell capacitance was 11.2 pF. (B) Family of currents produced by a 3-step voltage-clamp protocol. Two identical ‘test’ steps (+10 mV, 0.2 s), P1 and P2, were applied at the start and end, respectively, of a 30 s ‘conditioning’ step. The ‘conditioning’ steps ranged from -110 mV to -50 mV (20 mV increments). Each 3-step sequence was applied at 60 s intervals, from a holding potential of -90 mV. The families of currents produced by P1 and P2 are shown in inset. For ‘conditioning’ potentials between -110 mV and -70 mV the magnitude of P2 was almost identical to P1, i.e., there was little inactivation of K⁺ current, but there was significant inactivation at potentials between -70 mV and -50 mV. Cell capacitance was 10.1 pF.
negative to -50 mV. In contrast, outward K+ current was reduced significantly at membrane potentials more positive than about -45 mV, resulting in a shift of the zero-current potential from -46.7 mV to -42.0 mV in the presence of TEA. This finding indicated that this voltage-gated K+ current can make a contribution to the r.m.p. of mouse AC. This possibility was tested using current-clamp recordings of the change in r.m.p. of mouse AC produced by block of the K+ current with 25 mM TEA. Figure 7B is a scattergram of the r.m.p. of 23 different mouse AC and accompanying change in membrane potential in the presence of 25 mM TEA. Although the scattergram appears to show a trend for more depolarized r.m.p. to be accompanied by larger TEA-induced changes in r.m.p., there was no statistically significant correlation between these quantities (p > 0.09). The mean r.m.p. of this group of cells was -46.4 ± 1.3 mV, and the mean change in r.m.p. in the presence of 25 mM TEA was 4.2 ± 0.8 mV. Both of these numbers are in good agreement with the zero-current potentials measured in voltage-clamp conditions, as shown in Figure 7A; the mean change in r.m.p. of 4.2 mV was not significantly different than the change in zero-current potential of the mean IV (p = 0.58). These data show that the voltage-gated K+ current in mouse AC makes a small contribution to the r.m.p., under the conditions of these experiments. Perhaps more importantly these results also reveal that this K+ current can serve to stabilize the resting membrane potential in response to perturbations (e.g., stretch) which would be expected to depolarize the chondrocyte.

In summary, these voltage-clamp data identify a prominent time- and voltage-dependent K+ current in mouse AC from the femoral-hip joint, and demonstrate that it can contribute to the resting membrane potential of these cells.
in mammalian articular chondrocytes have been reported. Polyclonal antibodies against α-subunits of voltage-gated K⁺ channels Kᵥ1.1-Kᵥ1.5 have provided evidence for the presence of only Kᵥ1.4 K⁺ channel proteins in equine and elephant articular chondrocytes. KV channel expression in mouse AC appears to differ from that of equine and elephant, as Kᵥ1.4 RNA was essentially undetectable in mouse. In other studies, polyclonal antibodies against Kir6.1 were used in human and equine articular cartilage as an indicator of ATP-sensitive K⁺ channels, and functional ATP-sensitive K⁺ channels were identified in parallel electrophysiological studies.

Discussion

Summary of results. This study provides a comprehensive analysis of several key electrophysiological and pharmacological properties of primary AC from two-week old mice, maintained in nonconfluent, monolayer cell culture for 1 to 9 days. The main findings were:

(i) Mouse AC expresses mRNA for several voltage-gated K⁺ channels (Kᵥ), with the delayed rectifier K⁺ channel isoform Kᵥ1.6 being the most abundant.

(ii) Expression of Kᵥ1.6 α-subunit proteins in mouse AC was shown using western blots and single cell immunohistological methods.

(iii) A prominent membrane current in mouse AC is a time- and voltage-dependent delayed rectifier K⁺ current that is blocked by relatively low doses of TEA (50% block at 1.5 mM), 4-AP (0.66 mM) and the snake toxin α-DTX (20.6 nM).

(iv) This K⁺ current inactivates very slowly, with an exponential time course with time constants in the range of 20–40 s at membrane potentials in range of the r.m.p. of the cells in vitro.

(v) Block of the current (with TEA) results in depolarization of the r.m.p. of the AC, demonstrating that this K⁺ current has a role in setting and modulating the membrane potential of mouse AC.

Expression of K⁺ channel genes in chondrocytes. Mouse articular chondrocytes express mRNA for a variety of voltage-gated K⁺ channel isoforms of the Kᵥ1 to Kᵥ4 subtypes (Fig. 1A). The most abundantly expressed isoform was Kᵥ1.6, which has delayed rectifier properties in heterologous expression systems. Immunological methods confirmed that Kᵥ1.6 α-subunit proteins were expressed in mouse AC (Fig. 1B and C). Very few other molecular characterizations of K⁺ channel expression in mammalian articular chondrocytes have been reported. Polyclonal antibodies against α-subunits of voltage-gated K⁺ channels Kᵥ1.1-Kᵥ1.5 have provided evidence for the presence of only Kᵥ1.4 K⁺ channel proteins in equine and elephant articular chondrocytes. KV channel expression in mouse AC appears to differ from that of equine and elephant, as Kᵥ1.4 RNA was essentially undetectable in mouse. In other studies, polyclonal antibodies against Kir6.1 were used in human and equine articular cartilage as an indicator of ATP-sensitive K⁺ channels, and functional ATP-sensitive K⁺ channels were identified in parallel electrophysiological studies.

K⁺ currents and channels in chondrocytes. A number of electrophysiological studies using whole-cell patch clamp techniques have identified K⁺ currents with delayed rectifier-like properties in articular chondrocytes of dog, rat, horse and elephant, pig, and rabbit. Currents in these chondrocytes all have qualitatively similar membrane potential-dependent properties to those in mouse AC, but they differ somewhat in their detailed biophysical properties. For example, the membrane potential for half-activation of the current varied from -25.2 mV for dog, -22.0 mV for elephant, -18.0 mV for rat, to -12.5 for horse. All of these half-activation potentials were somewhat more positive than that observed for mouse, which was about -35 mV (Fig. 4). The density of the fully-activated K⁺ current also varied considerably amongst these five species. For mouse, the current density at +40 mV was 71.5 ± 8.5 pA/pF, which was much larger than that of horse (18 pA/pF), dog (20 pA/pF), or rat (30 pA/pF), but was considerably smaller than that of elephant (150 pA/pF). Chicken and porcine growth plate chondrocytes also express voltage-gated, delayed rectifier-like K⁺ currents. The half-activation potential of the K⁺ current in chicken epiphyseal growth plate chondrocytes in cell culture was -7 mV, which
is a much more depolarized membrane potential than for mammalian articular chondrocytes. Delayed-rectifier K’ currents recorded from porcine costal growth plate chondrocytes in situ in cartilage was half-activated at -11.3 mV.

**Pharmacological properties of chondrocyte K’ currents.** The pharmacological properties of voltage-gated delayed rectifier-like K’ currents in vertebrate chondrocytes are diverse. The non-specific K’ channel blocker TEA blocked K’ current in all mammalian articular chondrocytes, with K’ s in the range of a few mM. The K’ for block of mouse AC K’ current by TEA (1.48 mM: Fig. 3) was in the same concentration range as that for dog (0.66 mM), and rat (1.45 mM), but 50% blocking concentrations were somewhat greater for horse (2.6 mM), and pig (5 mM) chondrocytes. In contrast, K’ current in chicken growth plate chondrocytes was unaffected by 10 mM TEA. 4-AP blocked horse, rat and chicken K’ currents with K’ s of approximately 1 mM or less, very similar to the inhibition of mouse AC K’ current.

The molecular expression of Kv isoforms and the pharmacological profile of the voltage-gated K’ current in mouse AC strongly suggests that K’,6,16 channels underlie the majority of the current. Dendrotoxin is a specific and potent blocker of K’ channel blockers TEA and 4-AP, charybdoxin and dendrotoxin, respectively. Although RNA expression for block of mouse AC K’ current by TEA (1.48 mM) for α-DTX blocked K’ current with a K’ of <100 nM. In contrast, equine chondrocyte K’ currents were unaffected by 100 nM of the related peptide dendrotoxin-I. Rat and chicken K’ currents were also blocked by low (K’ < 10 nM) concentrations of the scorpion toxin charybdoxin.

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**Resting membrane potential of chondrocytes.** Previous studies have shown that K’ channels are importantly involved in the ionic basis of chondrocyte membrane potential. This has been most clearly shown in a study of acutely isolated rat chondrocytes. Resting membrane potential was progressively depolarized with increasing external [K’], but the observed 24 mV change per 10-fold change in extracellular [K’] over the range of 5 to 100 mM was much less than the 58 mV change expected for a purely K’-permeable membrane. These results may suggest that while K’ ions make a significant contribution to resting membrane potential, there is likely an additional ionic conductance involved. Consistent with the data on rat articular chondrocytes, potential-sensitive optical dye measurements of membrane potential of cultured human articular chondrocytes support the hypothesis that a K’ conductance is involved in setting the r.m.p.

Pharmacological block of the voltage-gated K’ current in mouse AC resulted in a variable depolarization of the r.m.p. (Fig. 7). Sufficient TEA to block the voltage-gated K’ current in dog chondrocytes depolarized the cells by an average of 14.8 mV, from a mean r.m.p. of -38.1 mV. In rat chondrocytes, the K’ channel blockers TEA, 4-AP, charybdoxin and dendrotoxin, at concentrations sufficient to completely block the voltage-gated K’ current, depolarized the cells between 19.5 to 27.2 mV. All of these data imply that persistent activation of voltage-gated K’ currents can contribute to r.m.p. of articular chondrocytes in these in vitro conditions. The relatively small contribution by K’ current to r.m.p. in mouse AC may arise from its strong steady-state inactivation (Fig. 5) in the range of r.m.p. (-40 to -50 mV). This inactivation reduced the peak current to about 5–19% of its fully activated magnitude. It is possible that the much larger membrane depolarization of dog and rat AC that accompanied block of K’ currents in these cells was a result of smaller steady-state inactivation of the currents in dog and rat chondrocytes. However, neither of these studies reported slow inactivation properties of the voltage-gated K’ currents in the chondrocytes.

Calcium-activated K’ channels may play a role in modulating chondrocyte r.m.p. Human chondrocytes hyperpolarized in response to repetitive mechanical stimulation. This was attributed to ‘small’ conductance (apamin-sensitive) Ca2+-activated K’ channels, activated via mechanically-induced release of ATP and subsequent elevation of intracellular Ca2+ concentration. Other types of Ca2+-activated K’ channels may also be activated in chondrocytes in response to elevation of intracellular Ca2+ concentration. For example, in OUMS-27 cell line, derived from human
chondrosarcoma, stimulation of histamine receptors increased intracellular Ca2+ concentration, resulting in membrane hyperpolarization that was produced by activation of three different types of Ca2+-activated K+ channels.47

Limitations to this study. There are a number of limitations on the interpretation of the results of this study. A significant source of uncertainty is the use of chondrocytes in dissociated cell culture, rather than acutely dissociated chondrocytes used in several previous studies. Dissociated chondrocytes are known to dedifferentiate in monolayer cell culture. For example, matrix proteins such as collagen and proteoglycans,48-51 surface marker molecules30,52 and a variety of chondrocyte-associated genes44 undergo time and passage-dependent changes in expression during monolayer cell culture of articular chondrocytes. However, significant changes in many of these parameters generally occur only following several passages of the cells (reviewed in ref. 55). However, properties of K+ channels recorded from chondrocytes in monolayer cell culture have been reported to change after relatively short periods in culture. Potassium channels recorded from cultured pig growth plate chondrocytes increased in conductance as cells progressed from isolated to confluent,56 and stretch-activated K+ channels in pig articular chondrocytes were recorded more frequently after 4–6 days in culture.37 In this study, cultured mouse AC were used only during first expansion of the cells, up to a maximum of 9 days in culture. The majority (~80%) of recordings was made on cells between 1 and 5 days in culture, and only non-confluent isolated cells with a round morphology were chosen for recording. These procedures likely minimized many of the effects of cell dedifferentiation that may occur in vitro.

It is difficult to extrapolate the results of this study to the in situ environment of articular chondrocytes, because the extracellular osmotic and ionic environment of articular chondrocytes in intact cartilage is very different than that of in vitro conditions. Due to the presence of immobile negative charges on the cartilage matrix proteoglycans the concentration of extracellular cations is considerably elevated compared with anions, a condition which is very difficult to replicate in monolayer cell culture. The high concentrations of Na+ (250–350 mM), K+ (8–10 mM) and Ca2+ (15–20 mM), but much lower concentration of Cl− (60–90 mM) in the synovial fluid result in osmotic pressure of 380–450 mosmol (kg H2O)-1 (reviewed in ref. 57), which is considerably greater than that of the culture medium or the external recording saline. Moreover, the pH of the cartilage fluid is 6.9–7.1, somewhat more acidic than normal serum pH of about 7.4. It is not clear how the properties of the K+ current recorded in culture conditions would change under the much different extracellular ionic and osmotic conditions found in intact cartilage.

Accurate recording of resting membrane potential of small cells such as chondrocytes is subject to a number of possible artifacts. Both high-resistance microelectrode and patch-clamp recordings may distort resting membrane potential measurements, although the sources of error with the two techniques are different. The major limitation of microelectrode recordings is the introduction of a transmembrane electrical ‘shunt’ conductance created by the hydration mantle surrounding the wall of the impaled microelectrode. The magnitude of this shunt resistance may be as little as 200 MΩ,58 which depending upon the input resistance of the cell, leads to a substantial depolarization of the resting potential. More accurate estimates of the ‘true’ resting potential can be obtained by examining the transient change in potential that occurs during microelectrode impalement,32 but ‘steady-state’ measurements of resting potential made with high-resistance microelectrodes may be subject to substantial errors due to the shunt resistance resulting from microelectrode impalement. Based on the high input resistance of in vitro mouse AC, microelectrode impalement would be expected to have a very significant depolarizing effect on r.m.p. Patch clamp recordings of resting potential are much less compromised by a shunt resistance, because the electrode-membrane seal resistance generally is much greater than the cell membrane resistance (but see ref. 59). However, the patch clamp electrode readily perfuses the inside of the cell with the solution in the patch clamp electrode.60 If the ionic composition of the electrode solution differs greatly from that of the cell cytosol, resting potential may be altered, depending on the ionic conductances in the cell membrane that determine resting potential. Similarly, because high-resistance microelectrodes contain a high concentration of KCl (e.g., 2–3 M/l), leakage of K+ and Cl− into the cell cytosol may alter resting potential.59 Interestingly the resting potentials of mouse, dog12 and rat14 articular chondrocytes were very similar, in spite of the fact that two different recording techniques were used, patch clamp for dog and mouse, and microelectrode for rat. However, it is possible that this agreement is merely coincidental, and that different recording artifacts gave rise to similar apparent resting potentials.

Methods

Cell isolation and culture. Animal care and use protocols were approved and enforced by the institutional Animal Care and Use Committee of GlaxoSmithKline, Collegeville, PA, USA. Two-week old mice were euthanized using CO2 in a sealed box. Articular chondrocytes (AC) were isolated from femoral head cartilage, and stored frozen in liquid nitrogen until use. The detailed protocols for isolation, cryopreservation and cell culturing of mouse AC are presented in Supplementary Materials. Briefly, cells were cultured at 37°C in DMEM/F12, supplemented with 10% fetal calf serum, 2 mM L-glutamine and 1% penicillin/streptomycin. Cells were plated in 35 mm dishes on pieces of glass coverslip, and were used only during first expansion (i.e., plating from frozen sample), and were not passaged. These chondrocytes were used up to 9 days after plating, but ~50% of the cells used in this study were in culture for only 1 to 3 days, and ~80% were from 1 to 5 days.

Adult rat cardiac fibroblasts were isolated and cell-cultured as previously described.30

Real-time RT-PCR. Total RNA extraction, reverse-transcription (RT), and real-time RT-PCR were performed as previously reported.31 Total RNA was extracted from a frozen sample of primary mouse chondrocytes by the acid guanidium thiocyanate-phenol-chloroform method, and RT was performed according
Channels

MO, USA) in PBS and 0.2% Triton X-100 for 20 min at room temperature. Following three rinses in 1% goat serum in PBS, the cells were incubated for ~8 hours at 4°C with anti-Kv1.6 rabbit polyclonal antibody, diluted 1:500 (Chemicon AB5148; Millipore Corp., Billerica, MA, USA) and anti-α smooth muscle actin mouse monoclonal antibody, diluted 1:500 (Sigma-Aldrich, St. Louis, MO, USA). Following three rinses with PBS, the cells were incubated for a minimum of 1 hour at room temperature in 1% goat serum PBS containing Alexa Fluor 568 goat anti-rabbit IgG (Molecular Probes InVitrogen; Carlsbad, CA, USA) and Alexa Fluor 488 goat anti-mouse IgG (Molecular Probes InVitrogen; Carlsbad, CA, USA), both diluted 1:200. Images were obtained using an Olympus Fluoview 1000 confocal microscope (Olympus; Tokyo, Japan), with a 60x water immersion objective. Emission spectra were recorded between 487–541 nm for Alexa Fluor 488 and 562–662 nm for Alexa Fluor 568. Images were acquired and analyzed using Olympus software FV10-ASW, ver. 2.0.

Electrophysiology. Pieces of coverslip with attached mouse AC were transferred from culture dishes to a recording chamber on an inverted microscope, and superfused with HEPES-buffered solution at a rate of about 1 ml/min. Temperature was 19–21°C. Standard whole-cell patch clamp measurements were made on single mouse AC. Many of the cells aggregated, spread and flattened during culture, but care was taken to choose only single, isolated cells with a round appearance for recordings. Patch pipettes were fabricated from either thin-walled borosilicate or soda-lime glass capillaries. The d.c. resistance of the pipettes when filled with intracellular solutions was in the range 2–5 MΩ. The resistance of the ‘on-cell’ seal before breaking into the cells for whole-cell recordings depended on the type of glass used for the patch pipettes, with the soda-lime glass forming considerably higher seal resistances than the borosilicate. For the borosilicate glass the mean seal resistance was 3.1 ± 0.2 GΩ; n = 73, whereas for the soda-lime glass it was 16.5 ± 0.7 GΩ; n = 144 (p < 10⁻⁴). Current clamp measurements of resting membrane potential were made with soda-lime glass pipettes to minimize the effect of ‘shunt’ resistance on membrane potential.²² Pipette series resistance in whole-cell current recordings was about 2x pipette resistance, and 80–90% of the series resistance was electronically compensated.

Electrophysiological recordings of current and membrane potential were made with a Multi-Clamp 700A patch-clamp amplifier (Molecular Devices; Sunnyvale, CA, USA). Current and potential signals were digitized with a 1322A Digi-Data acquisition system (Molecular Devices, Sunnyvale, CA, USA), stored on a microcomputer and analyzed offline with pClamp version 8. Plots, statistical analysis and curve-fitting of data were made with ‘Sigmaplot’ (Systat Software; San Jose, CA, USA) or ‘Prism’ (Graphpad Software; La Jolla, CA, USA). When data from several cells were pooled, membrane currents were normalized to cell capacitance (‘current density’). Capacitance was measured for each cell from the area under the current transient produced by a +5 mV step in membrane potential. Mean capacitance increased with time in culture (see Suppl. Materials). The liquid junction potential between the external and pipette
solutions was compensated by adding -10 mV to membrane potentials recorded by the patch-clamp amplifier.

**Solutions, chemicals and drugs.** Solutions were made using Analar grade chemicals (Sigma-Aldrich, Inc.; St. Louis, MO, USA). Tetraethylammonium Cl (TEA) and 4-amino pyridine (4-AP) were obtained from Sigma-Aldrich. The snake toxin α-dendrotoxin (α-DTX) was obtained from Alomone Labs (D-350; Jerusalem, Israel). ‘Standard’ external solution consisted of (mM): NaCl, 140; KCl, 5; CaCl₂, 1; MgCl₂, 1; HEPES, 10; glucose, 5.5; mannitol, 15. pH was adjusted to 7.4 with NaOH. Osmolality was measured with a freezing-point depression osmometer (Model 3250, Advanced Inst., Norwood, MA, USA) and was in the range 300–305 mOsm. ‘Internal’ (pipette) solution consisted of (mM): K-aspartate, 100; KCl, 20; MgCl₂, 1; Na₂ATP, 4; CaCl₂, 0.85; EGTA, 5; HEPES, 10. pH was adjusted to 7.2 with KOH. The approximate pCa of this solution was 7.9. Osmolality was 284–287 mOsm.

**Statistics.** Averaged data and parameters from curve fitting are shown as mean ± standard error of the mean (s.e.m.). Paired or unpaired Student’s t test was used to test for statistical differences between mean values. A p value of <0.05 was taken to indicate a significant difference.

**Conclusions.**

The available electrophysiological evidence shows that voltage-gated, delayed rectifier-like K⁺ currents are common to most mammalian chondrocytes, although these currents differ somewhat in their molecular, biophysical and pharmacological properties from species to species. In mouse AC the K⁺ currents appear to arise primarily from expression of α-DTX-sensitive K⁺,1,6 channels. Our electrophysiological data strongly suggest that activation of the voltage-gated K⁺ current characterized in this study can contribute to establishing the r.m.p. of mouse AC. This voltage-gated K⁺ current may serve to moderate the effects of activation of non-selective cation channels on chondrocyte membrane potential. Stretch-activated non-selective cation channels,24,25,26 and/or TRPV4 cation channels,24,26,27 may be activated by mechanical stresses and osmotic challenges that chondrocytes experience during the compression and relaxation of cartilage which accompanies the motion of diarthrodial joints. Activation of these cation channels could depolarize the chondrocyte; in turn this depolarization would activate the delayed rectifier K⁺ current and thus repolarize the cell to a more negative membrane potential. Hyperpolarization of the chondrocyte membrane via activation of this K⁺ current would be self-limiting because of the voltage-dependent nature of the current. Deactivation of the K⁺ channels at membrane potentials near the foot of the current’s activation curve potentially restricts the range of effect of the K⁺ current to between about -60 mV and the reversal potential of the cation channel current.

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**Note**

Supplementary materials can be found at: [http://www.landesbioscience.com/journals/channels/ClarkCHAN4-3-Sup.pdf](http://www.landesbioscience.com/journals/channels/ClarkCHAN4-3-Sup.pdf)
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