Expression of Voltage Dependent Potassium Currents in Freshly Dissociated Rat Articular Chondrocytes

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Key Words

Chondrocyte • Cartilage • Whole cell • Patch clamp
• Potassium channel

Abstract

The electrophysiological properties of voltage dependent potassium channels from freshly dissociated rat articular chondrocytes were studied. The resting membrane potential (-42.7 ± 2.0 mV) was significantly depolarized by increasing concentrations of external potassium. No change was observed when external chloride concentration was varied. Addition of TEA, 4AP, α -Dendrotoxin and charybdotoxin depolarized resting membrane potential. Whole cell patch clamp studies revealed the presence of outwardly rectifying currents whose kinetic and pharmacological properties suggest the expression of voltage dependent potassium channels. Two kinds of currents were observed under the same experimental conditions. The first one, most frequently observed (80%), starts activating near -50 mV, with $V_{1/2} = -18$ mV, $G_{\max} = 0.30$ pS/pF. The second kind was observed in only 10% of cases; It activates near -40 mV, with $V_{1/2} = +28.35$ mV, $G_{\max} = 0.28$ pS/pF pA/pF and does not inactivates. Inactivating currents were

significantly inhibited by TEA ($IC_{50} = 1.45$ mM), 4AP ($IC_{50} = 0.64$ mM), CTX ($IC_{50} \approx 10$ nM), α -Dendrotoxin ($IC_{50} < 100$ nM) and Margatoxin ($IC_{50} = 28.5$ nM). These results show that rat chondrocytes express voltage dependent potassium currents and suggest a role of voltage-dependent potassium channels in regulating membrane potential of rat chondrocytes.

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Introduction

Articular chondrocytes are the only type of cells present in the cartilage of diarthrodial joints. They play a crucial role in the production and maintenance of this tissue, mainly by synthesizing extracellular matrix such as aggrecan, and collagens, as a response to mechanical loading on the joints, as well as osmotic and ionic changes in the extracellular media. [1-3]. The molecular components and mechanisms that render chondrocytes responsive to such stimuli are not fully understood, but include process that occur in the plasma membrane, where

ion channels may be playing a crucial role [4]. Despite this fact, the knowledge of diversity of ion channels of articular chondrocytes is scarce, as compared to other types of cells [5-7]. Electrophysiological studies have revealed the presence of voltage-gated Na⁺ channels [8]; Voltage-gated K⁺ channels [9-12]; Calcium-dependent K⁺ channels [13]; stretch activated K⁺ channels [14, 15], and voltage-gated Cl⁻ channels [8,16]. Pharmacological studies suggest the presence of a wider diversity that includes, besides the aforementioned, voltage-gated calcium channels [17-19]; TRPV channels [18] and aquaporins [20]. A few immunocytochemical studies have been made on chondrocytes, showing the expression of epithelial Na⁺ channels [21] and Kv1.4, a shaker-related voltage-dependent K⁺ channel [12]. Ion channels are also involved in the proliferation and differentiation capacities of chondrocytes, as has been shown by several pharmacological studies in human chondrocytes, using distinct channel blockers [22, 23, 17].

The current knowledge of chondrocyte ion channel diversity has been obtained from a variety of animal species, including chicken [9], cow [18], rabbit [16], dog [11], pig [10,15], horse, elephant [12] and human [22, 23], but so far, no similar analysis has been carried out in rat, despite the value of this small mammal as a biological model. Therefore, the aim of this job was to study the presence of voltage-dependent potassium channels in rat articular chondrocytes by electrophysiological procedures, such as membrane potential measurements and whole cell patch clamp and compare the results with findings in other species.

Materials and Methods

Isolation and dissection of rat articular chondrocytes

Articular chondrocytes were obtained from rat hip joint cartilage as follows: Juvenile Wistar male rats, weighting 160-180 grams, were anesthetized by intramuscular injection of pentobarbital. Under sterile conditions, cartilages were removed and minced before enzymatic digestion with 0.2% type II collagenase in PBS for 2-3 hrs at 37°C. After digestion, cells were pelleted by centrifugation at 800 RPM and resuspended in DMEM supplemented with 10% fetal bovine serum by mechanical dissociation through a tip polished Pasteur pipette. Viability was evaluated by trypan blue incorporation. The freshly isolated chondrocytes were plated at a density of 1-3 x10⁵ cells/ml on glass coverslips, which had been immersed previously on plastic dishes, filled with culture media. These cultures were maintained under sterile conditions at 37 °C in a humidified atmosphere with 5% CO₂. A time lapse of 30 minutes in most cases was enough for settling and attachment of cells

to glass coverslips. Recordings were made right after attachment and up to 8 hours after.

Recordings of membrane potential

Each measurement of membrane potential was obtained by impaling a separate cell from those attached to coverlips that had been immersed in a saline solution for at least 5 minutes before. Membrane potential was measured using intracellular microelectrodes, pulled from glass capillary with internal filament (GT-15, Warner Instruments) to electrical resistances of 40-80 MΩ, using a Brown Flaming type puller (model p87, Sutter Company) and filled with a filtered 3M KCl solution. The pipette was attached to a holder, which in turn was attached to a piezoelectric micromanipulator (Burleigh). A TEV200A amplifier (DAGAN Corporation) was employed to measure the membrane potential after junction potential correction. The cells were bathed in a standard extracellular solution, composed of (mM): 110 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 10 HEPES (pH7.4).

Whole cell membrane current recordings

Patch pipettes were made from glass capillary without filament (34500, KIMAX), pulled using a Brown Flaming type puller (model p87, Sutter Company). The tips of the pipettes were slightly fire polished to a tip resistance of 2-6 MΩ. Ion currents were measured with a DAGAN 8900 amplifier under voltage clamp conditions. Data were acquired on a PC compatible computer through the Digidata 1200 digital board (Axon Instruments, Foster City). The stimulation protocols and the acquisition of data were controlled by the CLAMPEX module of the pCLAMP 8.0 software (Axon Instruments). The external standard solution composition was (mM): 110 NaCl, 5 KCl, 1.8 CaCl₂, 1 MgCl₂, 10 HEPES (pH 7.4). The pipette solution composition consisted of (mM): 5 NaCl, 110 KCl, 1 MgCl₂, 5 EGTA-K, 10 HEPES (pH 7.4). Seal resistance before patch rupture ranged from two to ten GΩ.

Capacitance measurements

After seal rupture, a capacitive current was induced by a hyperpolarizing step voltage from -80 to -90 mV. The membrane capacitance was calculated off-line, fitting a time constant to the decaying phase of the capacitive current. This time constant was divided by the membrane resistance (R_m), which in turn was calculated from the difference between the steady state current and the basal current, before the onset of the pulse, divided by the pulse amplitude.

Data analysis and statistics

Analysis of ion current amplitude, as well as time constant fitting was made with the CLAMPFIT module of pClamp 8.0 software. Data is reported as mean ± SD. To determine time constants (τ) of activation and inactivation, ion currents were fitted to an exponential standard function with one or two terms:

(1)

$$I(t) = \sum_{i=1}^n A_i e^{-t/\tau_i} + C$$

To determine the half activation potential ($V_{1/2}$), chord conductance values were fitted to a Boltzmann function:

$$(2) \quad G(v) = G_{\min} + \frac{G_{\max} - G_{\min}}{1 + e^{\frac{z_d F}{RT} (V - V_{1/2})}}$$

To estimate the IC_{50} , currents were fitted to a Hill equation as function of blocker concentration (x):

$$(3) \quad I(x) = I_{\min} + \frac{I_{\max} - I_{\min}}{1 + \left(\frac{[x]}{IC_{50}}\right)^h}$$

Statistical tests, such as t test and ANOVA were performed with the statistical module of MICROSOFT EXEL 2003 (Microsoft Corp).

Results

Membrane potential of freshly dissociated chondrocytes is mainly determined by K^+ but not by Cl^- ions

Under standard conditions, when chondrocytes were incubated in a standard external solution, containing (mM): 140 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES (pH7.4), the resting membrane potential was -42.7 ± 2.0 mV ($n=30$). To find out the contribution of K^+ and Cl^- to the membrane potential, the external concentration of those ions was changed and the membrane potential measured accordingly. K^+ concentration was varied replacing equimolar amounts of NaCl by KCl, while the Cl^- concentration was varied by substitution of equimolar quantities of gluconate. Increases in the external K^+ concentration produced membrane depolarization that fitted a linear relationship when membrane potential was plotted versus the log of K^+ concentration (Fig. 1A). Linear regression analysis fitted a slope of 24 mV, and ANOVA ONE-WAY revealed statistically significant difference among means ($p=8 \times 10^{-37}$). On the other hand, substitution of Cl^- by gluconate as external anion did not influence significantly on membrane potential (Fig. 1B). Although regression analysis reported a slope of -3 mV, a multiple comparison test (ANOVA ONE-WAY) revealed non statistically significant difference among means ($p=0.31$).

The participation of K^+ channels on the resting membrane potential was further assayed by measuring the membrane potential of chondrocytes incubated with different K^+ channel blockers (Table 1). Chondrocytes incubated with TEA 1 mM, had a mean membrane

Fig. 1. Ion dependence of resting membrane potential. *A.* Rat chondrocyte membrane potential becomes gradually depolarized as external K^+ is increased, from 5 to 10, 25, 50 and 100 mM. Each point represents the mean value of 40 independent cases. *B* Replacement of external Cl^- by gluconate did not significantly affected chondrocytes resting membrane potential.

potential of -27.9 ± 3.4 mV ($n=20$, $p=9.6 \times 10^{-7}$), this effect was increased with TEA 10 mM, that depolarized the membrane to -13.6 ± 1.4 mV ($n=20$, $p=4 \times 10^{-13}$). 4AP 1 mM also produced a significant depolarization (-21.4 ± 4.5 mV, $n=20$, $p=1 \times 10^{-8}$). Charybdotoxin (10 nM), a more specific blocker of some types of voltage-dependent and Ca^{2+} -dependent K^+ channels, depolarized the membrane to -17.5 ± 2.9 mV ($n=20$, $p=4 \times 10^{-11}$). α -Dendrotoxin (100 nM), a toxin known to block some types of voltage-gated K^+ channels, depolarized the membrane of chondrocytes to -18.4 ± 1.2 mV ($n=20$, $p=1 \times 10^{-11}$).

Table 1. Addition of K⁺ channel blockers to external media depolarizes the membrane of rat chondrocytes. Potassium channel blockers TEA, 4AP, Charibdotoxin and α -Dendrotoxin were added to external media at the concentrations shown and membrane potential measured on separate cells. A lapse of 5 min was allowed before measurements. Columns show mean and SD of 20 cells of each condition. P results from two tailed t student comparisons of each treatment *versus* control.

Cells	Mean Vm (mV)	St dev.	p
CONTROL	-40.87	5.29	----
TEA 1 mM	-27.97	3.14	9x10 ⁻⁷
TEA 10 mM	-13.63	1.37	4x10 ⁻¹³
4 AP 1 mM	-21.36	4.50	1x10 ⁻⁸
CTX	-17.54	2.91	4x10 ⁻¹¹
DTX	-18.40	1.24	1x10 ⁻¹¹

Fig. 2. Electrophysiological properties of inactivating outward currents from rat chondrocytes. *A.* Representative family of whole cell currents obtained after stimulating rat chondrocyte with the voltage protocol depicted below. Starting from a holding potential of -60 mV, a 1 second pulse was gradually taken, from -60 through +60 with +20 mV steps. *B.* I-V relationship. For every chondrocyte, a peak measurement was obtained for each current, then referred to membrane capacitance to obtain current density values. Values shown are the mean $\pm$ SD of 32 cases from 5 rats. *C.* Fitting of chord conductance values to a Boltzmann equation gives a g_{max} of 0.30 pS/pF and a $V_{1/2}$ of -18 mV.

Ion currents of freshly dissociated chondrocytes are heterogeneous

Ion currents from freshly dissociated chondrocytes were recorded, using the whole cell clamp configuration. A total of 40 chondrocytes, from 5 male rats were studied. Only those cases with a seal resistance greater than

2 G Ω before rupture were considered. A mean capacitance of 28.8 ± 7.0 pF was obtained. The stimulation protocol started from a holding potential of -60 mV, and thereafter consisted in a series of pulses from -60 mV through +60 mV in 20 mV steps. Under the same experimental conditions, a heterogeneous response was

Fig. 3. Electrophysiological properties of non-inactivating outward currents from rat chondrocytes. *A* Representative family of currents of a non inactivating type of outward currents, obtained from whole cell clamped rat chondrocytes, with the voltage protocol shown below. *B*. I-V relationship. Current values were measured at 500 msec after onset of the pulse and standardized to membrane capacitance. Data shown is the averaged values of 5 distinct chondrocytes. *C*. Chord conductance-voltage relationship. A Boltzmann fit produced a $V_{1/2}$ of +28.3 mV and $G_{max} = 0.28$ pS/pF.

obtained: In 10% of cases no conspicuous currents were recorded; In 80% of cases outward, inactivating currents were obtained (Figure 2A); In the remaining 10% of cases outward currents exhibited a sustained, non-inactivating behavior, as shown in figure 3A.

Figure 2B shows the relationship between the membrane potential and the mean current density of the first type of currents ($n=32$). They start activating around -50 mV and show inactivation at potentials greater than +40 mV. The rate of activation increases as the membrane depolarizes. Exponential fit of the rising phase of the current estimates an activation time constant of 5.1 ± 0.4 msec and an inactivation time constant of 272 ± 7.5 msec at +40 mV. A current density of 30.4 ± 1.9 pA/pF was averaged at the same potential. A maximal conductance of 0.308 pS/pF was achieved at +22 mV, with a half-activation potential of -18 mV (Figure 2C).

Figure 3A illustrates a representative example of a second type of currents obtained under the same

conditions. They have a sustained activation that does not inactivate as the first type does. Figure 3B depicts the relationship of mean current density *versus* membrane potential. It has an outward rectifying behavior, although it is less pronounced than the first type of currents. A maximal conductance of 0.28 pS/pF was reached at +60 mV, with a half-activation potential of +28.35 mV (Figure 3C).

To verify that these outward currents are due to the flux of potassium, tail currents measurements were made (figure 4A). These currents were obtained from a voltage protocol that started from a holding potential of -60 mV, followed by a 100 msec prepulse of +40 mV and then by a series of command potentials from -100 mV through +20 mV in steps of 20 mV. As shown in figure 4B, a linear relationship was obtained when the mean current density was plotted *versus* the membrane potential. From this curve, an inversion potential of -65.5 mV was calculated. This value roughly approaches the equilibrium

Fig. 4. Tail currents of rat chondrocytes. *A.* A representative family of tail currents obtained from a voltage protocol that starts from a holding of -60 mV, then switches to a prepulse of +40 mV by 100 msec and then changes to a pulse that goes from -100 mV through +20 mV with 20 mV steps. The arrow indicates zero current level. *B.* A linear relationship between current density and test pulse voltage. Fitting of data predicts a reversal potential of -65.5 mV. Reported data are averaged values of 8 chondrocytes expressing inactivating outward currents.

Figure 4 consists of two panels. Panel A shows a family of tail current traces obtained from a voltage protocol that starts from a holding of -60 mV, then switches to a prepulse of +40 mV by 100 msec and then changes to a pulse that goes from -100 mV through +20 mV with 20 mV steps. The arrow indicates zero current level. Panel B shows a linear relationship between current density and test pulse voltage. Fitting of data predicts a reversal potential of -65.5 mV. Reported data are averaged values of 8 chondrocytes expressing inactivating outward currents.

Fig. 5. Effect of TEA on rat chondrocyte currents. *A.* Representative current traces induced by a single voltage step, from -60 to +40 mV, before and after increased concentrations of TEA (0.5, 1, 5 and 10 mM) were added to the external medium. Traces are from the same chondrocytes. *B.* Current density *versus* membrane potential relationship of 4 chondrocytes. *C.* Relationship between the current density and TEA concentration at +40 mV. Continuous line shows the fitting to Hill equation, from which an IC_{50} (1.45 mM) is calculated.

Fig. 6. Effect of 4AP on rat chondrocyte currents. *A.* Representative current traces induced by a single voltage step, from -60 to +40 mV, before and after increased concentrations of 4AP (0.1, 0.5, 1, and 5 mM) were added to the external medium. Traces are from the same chondrocytes. *B.* Current density *versus* membrane potential relationship of 3 chondrocytes. *C.* Relationship between the current density and 4AP concentration at +40 mV. Continuous line shows the fitting to Hill equation, from which an $IC_{50}=0.64$ mM) is calculated.

Fig. 7. Effect of Charibdotoxin on rat chondrocyte currents. *A.* Representative current traces induced by a single voltage step, from -60 to +40 mV, before and after increased concentrations of CTX (1 and 10 nM) were added to the external medium. Traces are from the same chondrocytes. *B.* Current density *versus* membrane potential relationship of 3 chondrocytes.

potential for potassium.

Pharmacological properties of rat chondrocyte potassium currents

To characterize further these currents, their pharmacological sensitivity to the potassium channel blockers Tetraethylammonium (TEA), 4-aminopyridine

(4-AP), Charybdotoxin (CTX), α -Dendrotoxin (DTX) and Margatoxin (MTX) was studied. Figure 5A illustrates the effect of TEA. It shows the current obtained at +40 mV without TEA, and after TEA was added to external media. Figure 5B shows a relationship between the mean current density and the membrane potential ($n=3$). Addition of 0.5 mM TEA decreased current density at

Fig. 8. Effect of α -Dendrotoxin on rat chondrocyte currents. *A.* Representative current traces induced by a single voltage step, from -60 to +40 mV, before and after increased concentrations of DTX (10 and 100 nM) were added to the external medium. Traces are from the same chondrocytes. *B.* Current density *versus* membrane potential relationship of 3 chondrocytes.

Fig. 9. Effect of rMargatoxin on rat chondrocyte currents. *A.* Representative current traces induced by a single voltage step, from -60 to +40 mV, before and after increased concentrations of 4AP (0.1, 0.5, 1, and 5 mM) were added to the external medium. Traces are from the same chondrocytes. *B.* Relationship between the current density and MTX concentration at +40 mV. Continuous line shows the fitting to Hill equation, from which an $IC_{50}=23.4$ nM is calculated.

+40 mV, from 29.6 ± 2.6 to 22.5 ± 2.3 pA/pF. Currents were further decreased by addition of 1 mM TEA (17.8 ± 1.2 pA/pF), 5 mM TEA (13.9 ± 1.2 pA/pF) and 10 mM TEA (5.6 ± 2.3 pA/pF). From this data an $IC_{50} = 1.45$ mM was calculated (Figure 5C). Addition of 4AP also decreased current density (Figure 6), from 34.7 pA/pF at +40 mV, to 30.8 ± 2.0 pA/pF after addition of 0.1 mM 4AP. A mean current density of 20.7 ± 2.5 pA/pF was obtained for 0.5 mM 4AP, 1 mM 4AP (10.9 ± 2.2 pA/pF) and 5 mM 4AP (9.6 ± 1.4). Fitting of this data to Hill's equation estimated an $IC_{50} = 0.64$ mM (Figure 6C). Addition of CTX to external media also produced inhibition of currents (Figure 7): Control currents decreased from 35.3 ± 3.4 pA/pF to

23.1 ± 3.2 pA/pF after incubation with 1 nM CTX; Addition of 10 nM CTX produced a 57% inhibition (15.3 ± 1.7 pA/pF), thence an estimation of IC_{50} about 10 nM can be assumed. Addition of 10 nM DTX decreased current density, from 29.7 ± 1.5 to 20.9 ± 1.3 pA/pF, and 100 nM DTX decreased it further to 9.5 ± 1.2 pA/pF. From this results, an $IC_{50} < 100$ nM can be assumed (Figure 8). After addition of 2.5 nM MTX, control currents were reduced from 39.0 ± 0.9 pA/pF to 35.4 ± 1.42 , to 28.7 ± 0.84 pA/pF with 10 nM MTX and to 11.2 ± 0.4 pA/pF with 100 nM MTX. From this data, an $IC_{50} = 28.5$ nM was estimated (Figure 9).

Discussion

The electrophysiological and pharmacological properties of potassium currents of freshly dissociated rat articular chondrocytes were studied. Firstly, the membrane potential of these cells was measured using intracellular microelectrodes. The average membrane potential obtained (-42.7 ± 2.0 mV) is comparable to measurements made in chondrocytes of other species: A membrane potential of -38.1 ± 1.4 mV has been reported in canine chondrocytes [11], -41.0 ± 7.0 mV in rabbit cultured chondrocytes [8], and in rabbit growth plates, a range of -36 to -56 mV has been described [24]. The fact that the membrane of rat chondrocytes was depolarized by increases in extracellular K^+ , as well as by K^+ channel blockers, suggest a role for K^+ channels in maintaining the membrane potential. A similar result was described by Wilson *et al.* [11], who found that application of external TEA and 4-AP depolarizes canine chondrocytes. Likewise, it has been found that human chondrocytes depolarize after increasing extracellular K^+ and after addition of TEA [23]. In this work, the more specific blockers charybdotoxin and dendrotoxin were included. Both of them also effectively depolarized the membrane potential of chondrocytes. A rather different result was found in rabbit chondrocytes, where no changes were observed by variation of external potassium, and hyperpolarization resulted after increasing external chloride concentration [16]. These differences may result from the distinct conditions of the chondrocytes at the time of recording, because in the case of rabbit, measurements were done from cultured chondrocytes three to four days old, while in this work, as well as in the work with dog and human, freshly isolated chondrocytes were recorded.

In agreement with the membrane potential results, whole cell clamp experiments confirmed that rat chondrocytes have potassium currents, and certain diversity is suggested by the fact that different results were found when applying the same experimental conditions to chondrocytes. In most cases, an outward current was recorded, with characteristics of a delayed rectifier potassium current, that starts activating at around -50 mV and with slow inactivation; less frequently a sustained, non-inactivating current was observed.

Previous reports have described potassium currents from articular chondrocytes of several species. Whole cell studies identified voltage gated, Ca^{2+} independent potassium currents with slow inactivation from canine chondrocytes [11], as well as horse and elephant [12].

Delayed rectifier potassium channels have been identified from porcine chondrocytes [15]. Grandolfo *et al.* [13] reported Ca^{2+} activated potassium currents in cultured pig chondrocytes. Sugimoto *et al.* [8] reported a potassium current from cultured rabbit chondrocytes. Delayed rectifier potassium currents were reported in chicken growth plate chondrocytes [9]. Single channel studies have revealed different types of potassium channels. Mozrzymas *et al.* [10] found a 125 pS calcium activated K channel in pig cultured chondrocytes. Martina *et al.* [14] described a large conductance K potassium channel activated by stretch.

The variability observed in the expression of currents may be due, among some other things, to the differentiation state of chondrocytes, since it has been shown that these cells de-differentiate easily in culture conditions after removal of the extracellular matrix [25, 26], for that reason recordings were made within a few hours of dissociation from cartilage. These experimental conditions are similar to those employed by Wilson *et al.* [11] on canine, and Mobasher *et al.* [12] on equine and elephant articular chondrocytes and the results are somewhat similar. For instance, in this work a mean capacitance of 28.8 ± 7.0 pF was obtained, roughly comparable to 37.9 ± 9.0 pF, 23.8 ± 2.8 pF and 18.6 ± 6.4 pF from rabbit, horse and elephant respectively [8,12], but differed from 5.78 ± 0.11 pF measured on dog chondrocytes [11]. The steady state properties of rat currents are similar to those of dog, horse and elephant. Both types of currents described in this report start activating at about -50 mV. The first one has a half activation potential of -18 mV, that is close to -25.2 ± 0.3 mV reported of dog, -22.2 ± 6.4 of elephant and -12.5 ± 4.3 mV of horse. Comparing the current density at $+40$ mV, inactivating rat currents (30 pA/pF) are similar to dog measurements of 20 pA/pF, and about 18 pA/pF of horse, but lower than those of elephant (about 150 pA/pF). The rat currents activate slightly slower (5.11 ± 0.44 msec) than those of horse and elephant (3 msec), whereas the time constant of inactivation found in rat currents is faster (272 ± 7.5 msec) than 1396 and 710 msec for inactivation time constants of elephant and horse respectively.

The pharmacological profile of the potassium currents found in rat chondrocytes has some striking differences with those of other species. Rat TEA sensitivity IC_{50} (1.45 mM) is lower than those of dog ($IC_{50}=0.66$ mM), but higher than those of horse ($K_d=2.5$ mM) and pig ($K_d=5$ mM). A 5 mM TEA completely blocks rabbit K currents, and 10 mM TEA has no effect on chicken [9]. Rat currents have a higher sensitivity to

4AP ($IC_{50} = 0.64$ mM) than dog currents, where a 1 mM 4AP produced a 26% blocking of currents. In horse currents, a K_d of 1.3 mM has been reported. Rabbit currents were also sensitive to 4AP, and 1 mM 4AP has been shown to completely block currents. An $IC_{50} < 1$ mM was reported for chicken currents. Charybdotoxin (CTX) is a more specific potassium channel blocker derived from the venom of scorpion, which is known to block voltage dependent K^+ channels KV1.2 and KV1.3, as well as Ca^{2+} activated K^+ channels [27]. In rat chondrocytes, CTX blocked K^+ currents with an $IC_{50} \sim 10$ nM, comparable to chicken, although has not been probed in other species. α -dendrotoxin (DTX), a toxin derived from snake venom has been shown to block KV1.1 channels ($IC_{50} = 0.1$ nM), Kv1.6 ($IC_{50} = 23$ nM) ROMK1 inward rectifier K^+ channels ($K_d = 150$ nM) [28] (Harvey, 2001). There are no previous reports of the action of this peptide in chondrocyte currents, although I-dendrotoxin, a related peptide, specific for Kv1.1 and Kv1.2 channels [29] (Hopkins, 1998), failed to block horse currents. In our results α -DTX 100 nM blocked 68% of currents at +40 mV, thence an $IC_{50} < 100$ nM is deduced.

Nowadays a great diversity of molecular species accounting for potassium channels have been described [30] (Coetzee et al, 1999), nonetheless no report describing the molecular nature of potassium channels of articular chondrocytes has been made so far. Based on the electrophysiological and pharmacological results described herein, it can be suggested that rat chondrocytes are expressing voltage dependent potassium channels of the Kv1 family; however there is no exact match with any of the seven member subunits (Kv1.1 to Kv1.7) [30]. Considering the pharmacological profile, a best match is found with Kv1.3, which are sensitive to both CTX and DTX, also the time constant of inactivation reported for Kv1.3 (250-600 msec) fits with our finding (272 msec), although there are subtle differences, for instance the $V_{1/2}$ of Kv1.3 is more negative (-30 mV) than (-18 mV) for rat currents [30]. To support this hypothesis, the effect of margatoxin, a more specific blocker of Kv1.3 currents, was assayed and, as shown in figure 9, it decreases the currents too, suggesting further the participation of Kv1.3 subunits.

Based in immunohistochemical evidence Mobasher *et al.* [12] suggested a Kv1.4 channel subunit as a main contributor to the potassium currents observed in dog chondrocytes, although they did not find an exact match with the reported pharmacological and kinetic behavior of any Kv1 members, suggesting that this behavior may result from the combined expression of several Kv1

members. In the case of rat chondrocyte currents, a combined expression of several Kv1 subunits is also likely to account for the combined results.

As shown before, a second type of currents, exhibiting a sustained, non-inactivating behavior was obtained under the same voltage protocol, although with less frequency than the inactivating currents. This type is not likely to be due to the expression of Kv1 subunits and, because of their rarity, we did not explore its pharmacological properties, but they may result from the expression of other Kv subunits belonging to Kv2 through Kv9. We rule out the possibility of Ca^{2+} dependent potassium channels because the experiments were done with pipette solutions without Ca^{2+} . This variability may result from differences in the state of the individual cells explored such as the cell cycle, the stage of differentiation, or whether these cells were or not on the loading zone of the cartilage. It also may have to do with the position that individual chondrocytes had on the cartilage before dissociation, that is, if they were located at the superficial, middle or deep zones. None of those possibilities was explored in this first descriptive study, but they may be approached in future work.

Another possible source of variation could be dedifferentiation of chondrocytes after being dissociated and attached to glass coverslips. To minimize this chance, recordings were obtained at short times after dissociation, in most cases within 30 minutes to three hours, and in a few instances, recording was extended up to 8 hours. In those cases, however, no significant differences were found on the features of the currents and care was taken to select those chondrocytes attached to glass that had not changed shape, considering only cells with a rounded profile, barely attached. Under these circumstances, changes in the properties and magnitudes of the currents recorded, due to dedifferentiation or attachment to glass cannot be discarded but given the short times employed, the chances are minimized. Some related works have found changes in the electrophysiological properties of chondrocytes after several days of culture. For instance, the conductance of potassium channels of preosseous cartilage changes after three days of culture at confluence [33]. Similarly, chondrocytes change its ability to produce matrix proteins such as collagens and proteoglycans after days of culture [25, 26].

Voltage dependent potassium channels are mostly expressed in excitable tissues [30, 31], but there are increasing evidence of their presence in non-excitable cells where their physiological role is not well understood [5, 32]. The results reported herein support the hypothesis

that Kv channels contribute in the regulation of the resting membrane potential of articular chondrocytes, although they may participate in other cellular processes yet to be proved.

A molecular and immunohistochemical approach would help to identify the nature of the potassium channels expressed by articular chondrocytes. In this respect, the study of rat chondrocytes as a model has the advantages, over other species, that molecular probes can be reliably designed, based in the knowledge of potassium channels already identified in other tissues of this species and the availability of a variety of antibodies against potassium

channels designed from rat cDNA sequences. The study of this model would help to understand the role of ion channels in the physiology of articular cartilage, under normal and pathological conditions.

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