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Rapid report

Membrane stretch activates a potassium channel in pig articular chondrocytes

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Abstract

Activity of stretch-activated potassium channels has been recorded in articular chondrocytes using patch-clamp technique. Pressure dependence is described by a sigmoidal function with a half-maximum effect at −20.5 mbar. Selectivity for potassium is demonstrated by agreement between the reversal potential measured at different $[K^+]_o$ and the prediction of Nernst equation and by block of these channels by caesium. © 1997 Elsevier Science B.V.

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Articular chondrocytes are endowed with the specific role of producing an extracellular matrix able to reduce friction at the joints and to resist mechanical stress. The turnover of matrix components, both in healthy animals and under pathological conditions, is conditioned by the metabolic state of chondrocytes, which in turn is sensitive to mechanical and chemical signals (compression [1,2], cell volume changes [3], intracellular pH [4] and ionic contents, growth factors [5], cytokines and mediators of inflammation [6], etc.). It is known that the mechanical loading of cartilage modifies the ionic composition of extracellular fluids [7] and that an intermittent stimulation of the tissue by physiological pressure levels [8] induces de novo synthesis of proteoglycans. However, very little is known about the effect of pressure on the activity of membrane ionic channels and transporters. For example, despite to the fact that some types of voltage activated potassium channels have been identified and characterized in chondrocytes of growth plate and articular cartilage [9,10], only indirect evidence is available [11,12] for the presence of mechanosensitive potassium channels in these cells.

Here we present the first electrophysiological evidence of the presence of high conductance, stretch-activated potassium channels in cultured articular chondrocytes.

Cells were isolated from the articular cartilage of the scapula-humerus joints of 150–200-kg pigs. The isolation procedure and the culture conditions were as described by Mozrzymas et al. [13].

Patch clamp recordings were carried out on cells cultured for 3 to 12 days. Currents were recorded

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either in the cell-attached or in the whole cell configuration of the patch clamp technique using borosilicate glass pipettes pulled on a vertical puller (List, Germany) and fire polished to obtain a tip resistance (in working solutions) of 3–4 MΩ for whole cell and 8–10 MΩ for single channel recording. The pipette solution contained (in mM) NaCl 135, KCl 3, Na-Hepes 10 (pH 7.3) for cell-attached and KCl 130, NaCl 3, K-Hepes 10, EGTA 5 (pH 7.3) for whole cell recordings. In some cell attached experiments the pipette solution contained high potassium (130 mM) and low sodium. The bath solution contained: NaCl 135, KCl 5, $CaCl_2$ 1.8, $MgCl_2$ 1, Na-Hepes 10 (pH 7.4). Solution around the cells could be exchanged within 400–500 ms through a multibarrel perfusion system controlled by electrovalves connected to an electronic timer. The currents were recorded with a standard patch clamp amplifier (EPC-7, List Medical Instruments, Germany). The current signal was sampled at 10 kHz for cell attached and at 1 kHz for whole cell recording, transferred to a microcomputer (Atari 1040 ST) and stored on disk. The same computer and an A/D D/A converter (ITC-16, Instrutech, USA) were used to control the pipette potential. Recordings were analyzed off line using the TAC and Rewiew programs (Instrutech). Pressure in the pipette was measured by a home made electronic transductor.

The cell-attached and whole cell configurations of the patch clamp technique were employed to study

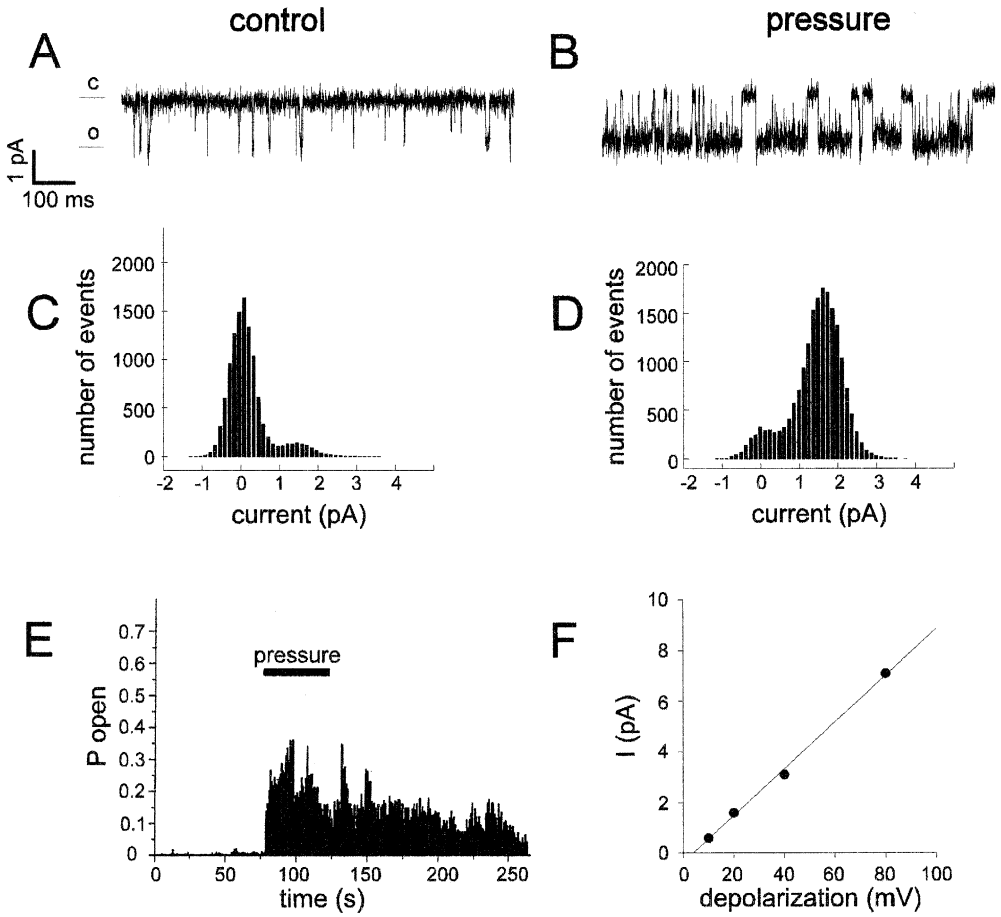

Fig. 1. Mechanical stress activates potassium channels in pig articular chondrocytes. Cell attached recordings (A and B) at $V_{pip} = -30$ mV in the absence (A) and in the presence (B) of the negative pressure of -80 mbar. B and C present all points histograms of single current signals shown in A and B, respectively. As seen in A and C, in the absence of mechanical stimulation most of time channel spend in the closed state whereas upon mechanical stress, in the open state (B,D). (E) shows the time course of the open probability during and after the mechanical stimulation (-80 mbar, indicated as the horizontal bar). Note a very slow return of P_{open} to the basal value after the stimulus is off. The slope conductance of the main class of channels (with 130 mM K^+ in the pipette) was 98.8 pS (F).

ionic channels in pig articular chondrocytes cultured for 2–8 days. In the cell-attached configuration we found voltage-gated potassium selective channels in all the cells tested ($n = 15$). These channels have already been described elsewhere [13,14]. In different preparations between 40 and 90% of the cells tested were also endowed with channels that could be activated upon mechanical stimulation (stretch) of the cell surface. As shown in the Fig. 1A,C when no pressure was applied the channel activity was low. In contrast, when under the same conditions, a negative pressure (5–90 mbar) was applied to the membrane, a large increase in the channel activity was observed (Fig. 1B,D). The occurrence of mechanosensitive channels seemed to be particularly frequent in cells cultured for 4–6 days. The channel activity did not show any decline during long lasting pressure stimulations and, after removal of stretch, returned to the same level as before the stimulus (Fig. 1E). Time required to return of the channel activity to the pre-stimulus values showed a substantial variability. In most cases this time course was slow (as e.g. in the Fig. 1E) but in some cells (3 out 8) this process was much faster lasting 10–30 seconds. The single channel conductance of the stretch activated channels, determined from the slope of the I–V relationship (see e.g. Fig. 1F), was 98.8 ± 10.5 pS ($n = 6$) when measured with 130 mM potassium in the pipette solution. In two cells an additional conductance state of 24 pS was present (data not shown). However, since this conductance was observed only in two cells it was not analyzed further. These channels were selective to potassium as shown by the fact that the reversal potential was shifted by ca. -85 mV ($n = 3$) when the intrapipette potassium concentration was changed from 5 mM to 130 mM, in agreement with the value predicted by the Nernst equation (-82 mV). Moreover, no channel activity was observed when caesium was substituted for potassium in the intrapipette solution.

The increase in the single channel activity (expressed in terms of N_p value) induced by mechanical stress showed a high degree of variability. However, when normalizing the activity of channels in each cell to its maximum value, the dependence of the N_p value on the pressure could be described by a sigmoidal function with the half-maximum activation at -20.5 mbar (Fig. 2). For instance, at $V_{pip} = -60$ mV and no pressure applied, the mean N_p value was 0.1 ± 0.03 ($n = 4$) while at -80 mbar $N_p = 0.65 \pm 0.20$

In the absence of a mechanical stimulation the channels showed a voltage-dependent gating, with half activation at $V_{pip} = 18$ mV. In cells that were responsive to stretch stimulation, the maximum activation obtained with depolarization ($N_p = 0.12 \pm 0.05$, $n = 3$ at $V_{pip} = -100$ mV) was, however, much smaller than that induced by pressure application ($N_p = 0.65 \pm 0.21$, $n = 6$ at $V_{pip} = -60$ mV, $p = -80$ mbar; Fig. 2). For instance in one of the cells tested the increase in the N_p value caused by a 100 mV depolarization, in the absence of mechanical stimulation, was only from 0.07 to 0.11 whereas, in the same cell, the application of a mechanical stress

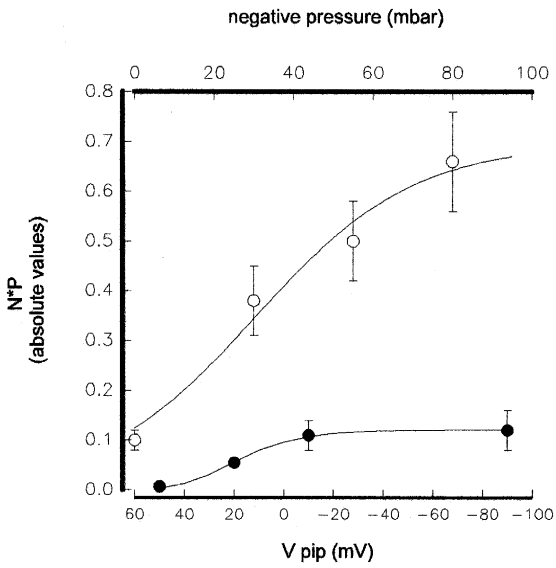

Fig. 2. The K^+ channels of pig articular chondrocytes are gated by voltage and pressure. Note the large difference in the maximum open probability attainable. Filled symbols: voltage dependence under control conditions (no pressure applied), $n = 5$. Open symbols: pressure dependence at constant $V_{pip} = -60$ mV, $n = 4$. The lines superimposed to the data points represent the best fit of a Boltzmann function in the form: $P = P_o + P_{max}/(1 + \exp(p - p_h)/s)$, where P is the open probability, P_o is the pressure-independent component of the open probability, P_{max} is the maximum value of the pressure-dependent open probability, p is pressure, p_h is half-maximum activating pressure, s is slope factor; the values of parameters in the fit are: $P_o = 0.05$, $P_{max} = 0.64$, $p_h = 35.5$, $s = 19.1$; for the voltage dependence: $P = P_V/(1 + \exp(V_h - V)/s)$, where P_V is the maximum voltage-dependent open probability, V_h is the half-activating voltage, V is voltage; the values of parameters in the fit are: $P_V = 0.12$, $V_h = 15.6$, $s = 44.2$.

(-30 mbar) induced an increase from $N_p = 0.11$ to 0.75 at a pipette potential of -60 mV.

The kinetic analysis of the single channel records revealed that both the open and the closed time distributions could be fitted well by the sum of two exponential functions (Fig. 3). The pressure stimulus significantly ($P < 0.05$) changed the duration (from 37.8 ± 8.7 to 18.5 ± 6.1 ms, $V_{pip} = -60$ mV, $P < 0.05$, $n = 5$) and the relative contribution (from 37 ± 11.1 to $18.5 \pm 6\%$, $P < 0.03$, $n = 5$) of the longer closed time component (see e.g. Fig. 3C,D). Also the duration of the longer open time component was significantly increased (from 3.02 ± 1.3 to 11.9 ± 5.1 ms at V_{pip} -60 mV, $P < 0.03$, $n = 5$, Fig. 3A,B). No significant changes were, however, induced either to the fast open (0.32 ± 0.13 ms in control and 0.75 ± 0.9 ms in the presence of -30 mbar applied pressure, $P > 0.4$, $n = 5$, see e.g. Fig. 3A,B) or to the fast closed time constant (0.22 ± 0.07 in control and 0.23 ± 0.08 ms at -30 mbar, $P > 0.4$, $n = 5$, see e.g. Fig. 3C,D).

We also tested the existence of stretch activated channels in whole cell configuration by applying a standard voltage pulse protocol in control conditions, and in the presence of an hyposmotic solution (NaCl in the bathing solution was reduced from 135 to 70 mM). In 4 out of 4 cells tested we found that in the hypotonic solutions the whole cell currents showed an evident increase in the amplitude with respect to the control conditions and no changes in reversal potential were observed (for the cell in the Fig. 4 the chord conductance increased from 7.4 to 25.7 nS and the reversal potential was -70 in control and -67 mV at -30 mbar). The fact that the reversal potential of the currents is close to the equilibrium potential for potassium (-81 mV) indicates that also the currents induced by the hypotonic shock were predominantly carried by potassium ions. A further evidence for potassium selectivity is given by the fact that these currents (both in the presence and in the absence of stress) were not observed when the cells were dialysed with intrapipette solution in which

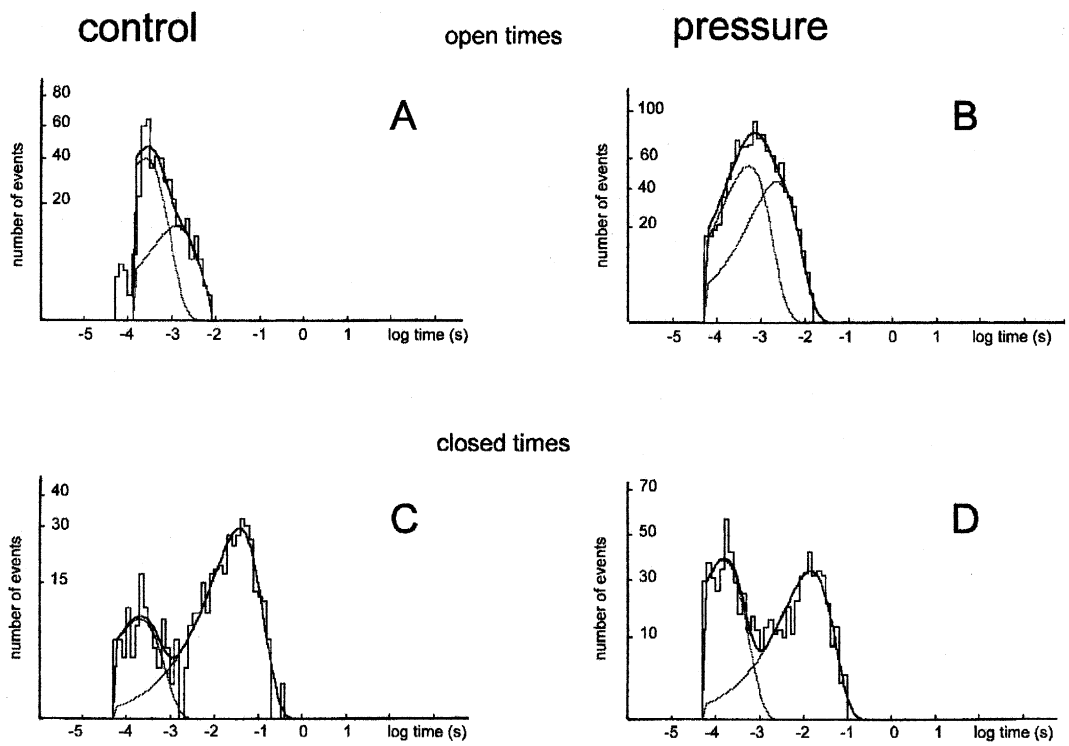

Fig. 3. Square root transformed histograms of open and closed state time distributions. The superimposed lines represent a maximum likelihood fitting. The best fitting was obtained assuming two open and two closed time constants. For the histograms in the figure the values were $\tau_{o1} = 0.28$ ms, $\tau_{o2} = 1.38$ ms, $\tau_{c1} = 0.22$ ms and $\tau_{c2} = 39.6$ ms under control conditions and $\tau_{o1} = 0.54$ ms, $\tau_{o2} = 2.4$ ms, $\tau_{c1} = 0.17$ ms and $\tau_{c2} = 15.5$ ms under stretch stimulus. Note the main effect on long closed time whereas the fast gating is not significantly affected.

caesium (a non-selective potassium channel blocker) was substituted for potassium ($n = 4$).

When the hypotonic stimulus was removed, the whole cell currents returned to the control values. Similarly to what observed at the single channel, the macroscopic currents elicited in the presence of the membrane stretch did not decrease even when applying pulses up to 500 ms (Fig. 4).

Chondrocytes are cells that in physiological conditions are exposed to mechanical stress. The major finding reported in this study is that articular chondrocytes are endowed with large-conductance potassium channels sensitive to mechanical stretch. This observation seems to be of particular interest as several lines of evidence have indicated that mechanical stimulation of chondrocytes can be an important modulatory factor in matrix biosynthesis [1,2]. However, only a few reports have so far provided indirect evidence that potassium conductance may be involved in stretch sensitivity [11,12].

The pressure-sensitive channel described in the present work shares common characteristics with the large-conductance voltage-dependent potassium channels described before in the same preparation [13–15]. It is thus likely that the same potassium channel may show both voltage-dependence and stretch-sensitivity. This view is further supported by the observation that upon pressure application, the conductance remains unaffected while the dwell time in the open state increases and in closed state decreases indicating that stretch increases the open probability of the same channel. Thus, as was observed for stretch activated potassium channels in other preparations [16,17], the increase in N_p value is a consequence of both an increase in the long open time constant and a decrease in the long closed time constant. This can be interpreted as a shift of the channel to a 'willing' mode, under the mechanical stress, similar to what has been previously described for calcium channels [18]. In any case, a more detailed kinetic analysis and, particularly, the comparison of the voltage-dependence of activation in the presence or in the absence of applied mechanical stimulation will be needed to further clarify the mechanism of the stretch-dependence.

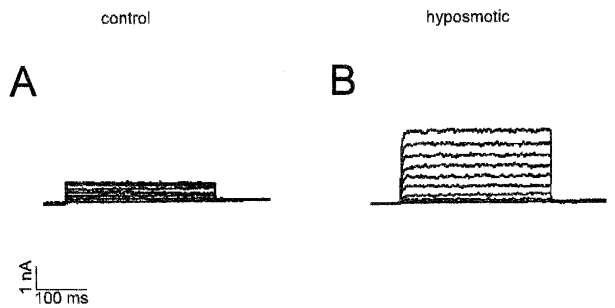

Fig. 4. Stretch activated channels are elicitable in whole cell recordings. A standard voltage protocol (holding potential $V_h = -80$ mV, increment 10 mV, the first step to $V = -60$ mV) to the same cell either in control (A) or in the presence of osmotic stress (B). The stretch activated currents did not show any decrease during a 500 ms pulse.

The increased channel activity, observed in the cell attached configuration when applying negative pressure, could theoretically be due also to induced differences in the patch geometry. For instance, upon application of mechanical pressure more membrane could be sucked inside the pipette increasing membrane patch area and the number of channels (the so called Ω patch effect). However, in two cases in which most likely only one channel within the patch was present, the effect of mechanical stretch caused a dramatic increase in the opening frequency with no sign of increase in the number of channels (no overlapping events). Moreover, also the increase in the potassium whole-cell conductance in response to the osmotic shock, clearly indicate that the open probability of potassium channels is sensitive to membrane deformation.

As the stretch activated channels were not present in all the cells tested and seemed to be related to the culture stage (mostly between 4 and 6 days) it could be suggested that the stretch sensitivity is related to some developmental processes. However, this issue would require further investigations.

It was reported that chondrocytes are endowed with volume regulation mechanisms [3]. Our data suggest that the potassium channels participate in this process mediating the cell response to the osmotic shock.

Although we cannot exclude that in the whole-cell configuration some channels other than those seen in the cell-attached patches could contribute to macroscopic current, the lack of decrease in the current intensity in the presence of stretch suggests that the channels seen in cell-attached patches are predominant also in whole-cell recordings.

Very recently D'Andrea et al. [19] found that a mechanical stimulation of cultured chondrocytes induces a Ca^{2+} influx which support the firing of a Ca^{2+} wave which is then communicated to the surrounding cells by involvement of gap junctions. Wright et al. [12] showed also that a cyclical pressurization is associated with an increase of cAMP concentration, of the rate of PG synthesis and with a hyperpolarization of chondrocyte cell membrane. According to these authors, during this hyperpolarization, Ca^{2+}-dependent K^+ ion channels and perhaps stretch-activated channels were activated. We suggest that the stretch activated K^+ channels described in this paper can be implicated both in Ca^{2+} wave generation and membrane hyperpolarization: one hypothesis is that the activation of stretch-activated potassium channels gives hyperpolarization of the plasma membrane, thus increasing the driving force for Ca^{2+} entry and generating the Ca^{2+} wave.

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