Indian J Physiol Pharmacol 2010; 54 (4) : 361–365

ANALYSIS OF DEPOLARIZATION-INDUCED OUTWARD CURRENTS IN GOAT CHONDROCYTES USING THE PATCH CLAMP TECHNIQUE

VINAY OOMMEN AND SATHYA SUBRAMANI

Department of Physiology,
Christian Medical College,
Vellore – 632 004

(Received on December 5, 2009)

Abstract : This study analyzes outward currents in freshly isolated goat chondrocytes patched in the whole cell mode. Capacitance tracings were recorded from the cells by the time domain method. The average capacitance was 6.33 pF ±2.15pF (Mean±SD, n=60). The range was 2.7 pF to 11.2 pF. A family of outward currents was seen when the cell was depolarized from –70 mV to +70 mV in 10 mV increments. The current density at +60 mV varied from 125 pA/pF to 2410 pA/pF. The currents were inhibited by 10 mM tetraethylammonium chloride (TEA) and the current-voltage profile suggests that these are voltage gated K^+ channels. The currents were also recordable in a chloride-free external solution, thereby proving that these currents are not chloride currents. There was no evidence of voltage-gated sodium channels in these cells.

Key words : chondrocyte patch clamp potassium currents

INTRODUCTION

Chondrocytes are the cellular elements of cartilage. They are responsible for the maintenance of the constitution of the extracellular matrix by a combination of matrix synthesis and breakdown (1). The matrix has several unique features. It is avascular and nutrients and oxygen pass through by diffusion across large distances. The matrix is also hyperosmolar as compared to normal plasma (2). Mechanical loading as during walking causes changes in both the osmolarity and the hydrostatic pressure of articular cartilage. The extracellular pH is usually about 6.9 (2). A number of channels and other volume regulating mechanisms ensure the survival and optimum function of Chondrocytes in this hyperosmolar, avascular, acidic environment (1).

A wide variety of channels have been described on the chondrocyte membrane. Voltage gated K^+ channels have been described in rabbit, canine, porcine and rat Chondrocytes (3–6). Stretch activated K^+ channels have also been described on the chondrocyte membrane. Sugimoto et al have also found tetrodotoxin (TTX) sensitive Na^+ channels and voltage gated Cl^- channels in rabbit Chondrocytes (3). Voltage gated H^+ channels have been described in bovine Chondrocytes (7). Indirect evidence based on the effect of pharmacological blockers has

*Corresponding Author : Vinay Oommen, Email : vinayoommen@cmcvellore.ac.in; Phone : 0416 2284268; Fax : 0416 2282788

suggested the presence of T-type and L-type Ca^{++} channels (8) and stretch activated K^+ channels (9). Calcium dependent K^+ channels and aquaporins have also been described on the chondrocyte membrane (10, 11).

Electrophysiological studies have been performed in Chondrocytes from various species such as the rat (4), the horse (12), the elephant (12), the dog (5), the pig (6), the chicken and also in bovine (13) and human cartilage (14). While goat chondrocytes have been studied in different culture conditions, there is little information on its electrophysiological characteristics. This study provides base-line electrophysiological data on goat chondrocytes.

MATERIALS AND METHODS

Isolation procedure4

Goat legs were obtained from the slaughter house within 2 to 4 hours from the time of slaughter. They were immediately treated with 70% ethanol, de-skinned and refrigerated until the time of harvest. The joint cavity was opened under aseptic conditions. Cartilage shavings were taken from the metatarsophalangeal joint. For the samples from the iliac crest, the iliac bone was obtained with its attached muscle. This was dissected to reveal the iliac crest, from which cartilage shavings were taken under sterile conditions. The cartilage shavings were washed well with Dulbecco's Modified Eagle's medium with Ham's Nutrient Mixture F12. They were stored in the same medium until used. The medium was supplemented with Penicillin/ Streptomycin 100 U/ml and Amphotericin B 2.5 µl/ml. The cartilage shavings were digested using Sigma Collagenase Type IA (1.45 mg/ml) for a period of 16 hours. Digestion and storage of the cartilage shavings was done in culture flasks in a CO_2 incubator at 37 degrees centigrade, 85% humidity and 5% CO_2. The digested cells were allowed to pass through a 40 µM filter which removed any undigested particles. The cells were centrifuged at 200 rpm for 10 minutes, washed with culture medium and then plated on cover slips for patch clamp studies. Viability of the cells using this protocol was checked and was found to be more than 90% using the trypan blue dye exclusion test.

Recordings

Patch clamp recordings were made using the Axopatch 200B. Recordings were made in whole cell mode. Patch pipettes were fashioned from Harvard capillary glass using a Narishige PP-830 vertical pipette puller. Pipettes used had a resistance of less than 4 MΩ. The pipettes were polished using a GlasswoRx 500 Fine Pont microforge before use. Cells were bathed in a bath of volume 1 ml. The control external (bath) solution consisted of (in mM) NaCl 140, MgCl2 1, $CaCl_2$ 1, HEPES 10, Glucose 11, pH 7.4. The chloride-free external solution had the following composition (in mM) : NaGlutamate 140, $MgSO_4$ 1, $CaSO_4$ 1, Glucose 11, HEPES 10, pH 7.4. The internal (pipette) solution for both contained (in mM) KC1 140, $MgCl_2$ 4, HEPES 10, Glucose 11, pH 7.3. The osmolarity of both the external solution and the internal solution was kept between 280-300 mOsm. After obtaining giga-seals the cells were voltage clamped from a holding voltage of –80 mV through a range of voltages from –70 mV to +70 mV with 10 mV increments, each pulse lasting for 180 ms and currents were recorded. Data was sampled at 10 KHz. The waveforms were analyzed using Clampfit 9.2 (Axon Instruments) and IGOR Pro Version 5.0.4.8 (Wavemetrics Inc.).

Membrane capacitance of cells was recorded using the membrane test feature in the Axopatch 200B immediately after going into the whole cell mode. This records capacitances using repeated square pulses. The values of 50 capacitance transients were averaged and 20 such values were recorded each second.

Tetra Ethyl Ammonium Chloride, a known K^+ channel blocker (15), was added into the bath solution to make a final concentration of 10 mM. Recordings were made before and after the addition of TEA.

Recordings were also made in a chloride-free external solution.

A cell with a large surface area will have a greater number of channels on its surface and thus record a larger current. Thus for analysis of currents, the current density or current per unit surface area is used. The capacitance of the cell membrane is directly proportional to its surface area and hence current density is represented as current per unit capacitance.

Statistical analysis was done using the repeated measures ANOVA. $P<0.05$ was considered significant. SPSS 12.0 was used for data analysis.

RESULTS

The cells studied varied widely in their size (as viewed microscopically) and in their capacitances. The capacitances recorded varied from 2.7 pF to 11.2 pF averaging at 6.33±2.15pF (Mean±SD, n=60). This data corresponds with the wide variation in the size of cells patched.

Subjecting the cell to a voltage protocol from –70 mV to +70 mV demonstrated a family of outward currents (Fig. 1). These

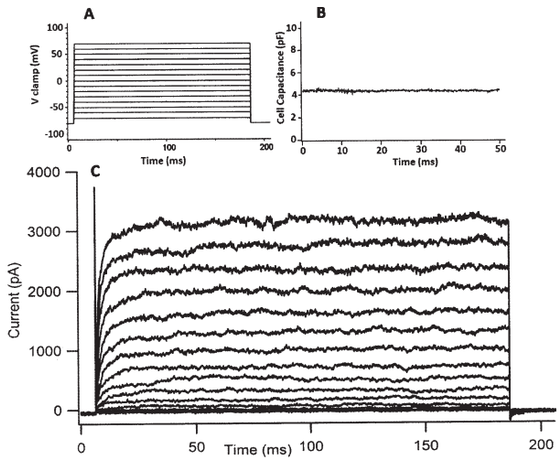

Fig. 1A : Voltage pulse protocol used for experiments. The cell was kept at a holding voltage of –80 mV. It was depolarized by 10 mV steps from –70 mV to +70 mV, each step lasting for 180 ms.
B : Capacitance tracing taken immediately after going whole cell.
C : Family of ward currents seen on applying the above mentioned pulse protocol to goat chondrocytes.

currents did not run down over time and could be recorded for up to 10 min after sealing. The currents seen were of a large magnitude, even reaching 10,000 pA at +70 mV.

Addition of TEA

A drop in current densities after the addition of 10 mM TEA was seen in five of the six cells tested (Fig. 2). There was no change in one cell. The IV curve of mean current density under control conditions was found to differ significantly from that of the IV curve after the addition of TEA ($P=0.001$)

Recordings in Cl^--free extracellular fluid

In the six cells studied in the Cl^--free external solution, large outward currents were recorded. Although the currents the Cl^--free external solution were larger in amplitude (Fig. 3), this difference was not found to be significant ($P=0.15$).

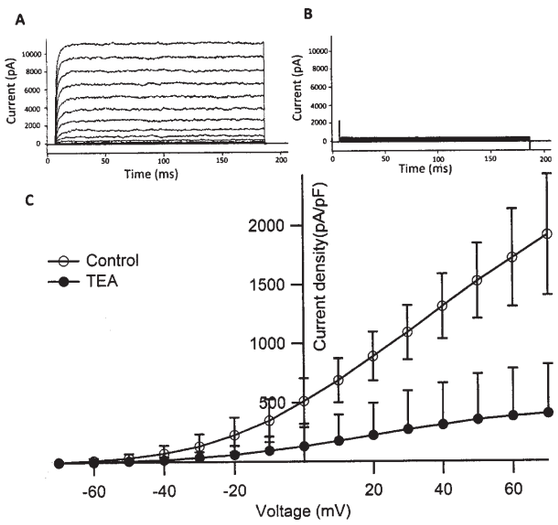

Fig. 2A : Currents recorded from a goat chondrocyte with a depolarizing pulse protocol. Currents in the same cell after the addition of 10 mM TEA showing a large reduction in current amplitude.

C : Current density-voltage curve of 5 cells studied before and after the addition of 10 mM TEA. A drop in the magnitude of currents is seen at every voltage after the addition of TEA. (Values : Mean±SD, n=5).

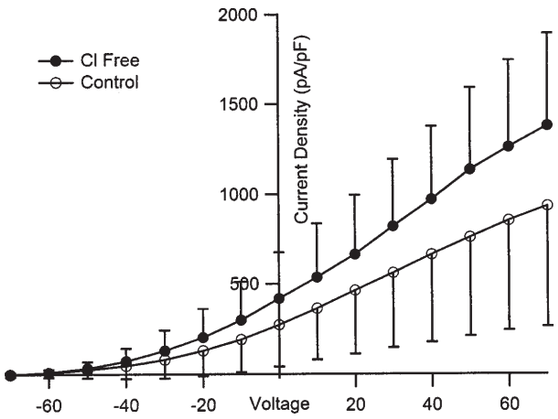

Fig. 3 : Comparison of current densities in goat chondrocyte controls with normal external solution (Mean±SD, n=19) and cells in Cl^--free solution (Mean±SD, n=6).

DISCUSSION

An outward current by convention indicates the flow of a positive ion out of the cell or the flow of a negative ion into the cell. In the control solutions, the only positive ion that could carry these currents from the internal solution was potassium, and the only negative ion that could cause these currents is a chloride movement from the external solution. As the outward currents are blocked by 10 mM TEA which is a known K^+ channel blocker (3, 4) they must be K^+ currents. No change was seen in one cell. This remains to be investigated if such a phenomenon in seen repeatedly.

If the currents were due to an inward flow of Cl^- ions they should have been absent when Cl^- was removed from the extracellular solution. This was not the case. The current densities in cells in a chloride-free external solution were comparable and in fact larger than those seen in cells in the control external solution. This difference was not significant. However this difference needs to be investigated further.

The current densities (which reflect the density of ion channels) also varied widely among the cells. This indicates that there seems to be a variation in the potassium channel density among chondrocytes.

The IV curves of the cells are suggestive of voltage gated K^+ channels. They show voltage dependence and outward rectification. The currents did not inactivate with time.

The depolarizing protocol and the solutions that have been used would have revealed voltage gated Na^+ channel currents if present. These will be seen as inward currents with a fast activation and inactivation. There was no evidence of such a current in any of our cells which shows that the goat chondrocyte does not have sodium currents. This is unlike the finding reported by Sugimoto et al in rabbit chondrocytes (3).

In summary goat chondrocytes show a high density of voltage gated potassium channels. The presence of large currents even in the absence of chloride in the external solution suggest that

voltage gated chloride channels may not be present on the goat chondrocyte membrane. Although voltage gated Na^+ Channels have been suggested in chondrocytes of other species we have not found evidence for the same in goat chondrocytes.

ACKNOWLEDGEMENTS

The authors thank Christian Medical College, Vellore for funding and Dr. Robert Chow for training one of the authors in electrophysiology.

REFERENCES

1. Wilkins R, Browning J, Ellory J. Surviving in a Matrix: Membrane Transport in Articular Chondrocytes. *J Memb Biol* 2000; 177: 95–108.
2. Hall AC, Horwitz ER, Wilkins RJ. The cellular physiology of articular cartilage. *Exp Physiol* 1996; 81: 535–545.
3. Sugimoto T, Yoshino M, Nagao M, Ishii S, Yabu H. Voltage-gated ionic channels in cultured rabbit articular chondrocytes. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol* 1996; 115: 223-232.
4. Ponce A. Expression of voltage dependent potassium currents in freshly dissociated rat articular chondrocytes. *Cell Physiol Biochem* 2006; 18: 35–46.
5. Wilson JR, Duncan NA, Giles WR, Clark RB. A voltage-dependent K^+ current contributes to membrane potential of acutely isolated canine articular chondrocytes. *J Physiol* 2004; 557: 93–104.
6. Mozrzymas JW, Martina M, Ruzzier F. A large-conductance voltage-dependent potassium channel in cultured pig articular chondrocytes. *Pflügers Arch* 1997; 433: 413–427.
7. Sánchez JC, Powell T, Staines HM, Wilkins RJ. Electrophysiological demonstration of voltage-activated H^+ channels in bovine articular chondrocytes. *Cell Physio Biochem* 2006; 18: 85–90.
8. Shao Y, Alicknavitch M, Farach-Carson MC. Expression of voltage sensitive calcium channel (VSCC) L-type Cav1.2 (alphalC) and T-type Cav3.2 (alpha1H) subunits during mouse bone development. *Dev Dyn* 2005; 234: 54–62.
9. Martina M, Mozrzymas JW, Vittur F. Membrane stretch activates a potassium channel in pig articular chondrocytes. *Biochim Biophys Act* 1997; 1329: 205–210.
10. Grandolfo M, D'Andrea P, Martina M, Ruzzier F, Vittur F. Calcium-activated potassium channels in chondrocytes. *Biochem Biophys Res Commun* 1992; 182: 1429–1434.
11. Mobasheri A, Trujillo E, Bell S, Carter SD, Clegg PD, Martin-Vasallo P, Marples D. Aquaporin water channels AQP1 and AQP3, are expressed in equine articular chondrocytes. *Vet J* 2004; 168: 143–150.
12. Mobasheri A, Gent TC, Womack MD, Carter SD, Clegg PD, Barrett-Jolley R. Quantitative analysis of voltage-gated potassium currents from primary equine (Equus caballus) and elephant (Loxodonta africana) articular chondrocytes. *Am J Physiol Regul Integr Comp Physiol* 2005; 289: R172–R180.
13. Sánchez JC, Powell T, Staines HM, Wilkins RJ. Electrophysiological demonstration of Na^+/Ca_2^+ exchange in bovine articular chondrocytes. *Biorheology* 2006; 43: 83–94.
14. Wohlrab D, Lebek S, Krüger T, Reichel H. Influence of ion channels on the proliferation of human chondrocytes. *Biorheology* 2002; 39: 55–61.
15. Gutman GA. Chandy KG. Grissmer S et al. (International Union of Pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels. *Pharmacol Rev* 2005; 57: 473–508.