Vol. 202, No. 1, 1994 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 15, 1994 Pages 31-37

POTASSIUM CHANNELS OF PIG ARTICULAR CHONDROCYTES ARE BLOCKED BY PROPOFOL

Jerzy W. Mozrzymas[1]*, Massimo Visintin[1], Franco Vittur[2] and Fabio Ruzzier[1#]

[1] Istituto di Fisiologia, Università degli Studi di Trieste, Via A. Fleming 22, I-34127 Trieste, Italy

[2] Dipartimento di Biochimica, Biofisica e Chimica delle Macromolecole, Università degli Studi di Trieste, Via L. Giorgieri 1, I-34127 Trieste, Italy

Received May 2, 1994

SUMMARY: The effect of propofol on the voltage-activated potassium channels in pig articular chondrocytes was investigated. Propofol was found to reversibly block the potassium channels in a dose-dependent manner. The blocking effect was voltage-independent and the Hill coefficient was 1.85 $\pm$ 0.18. No changes either in the slope conductance or in the single channel kinetics were observed. The half-blocking concentration (*Ec*50) was 6.0 $\pm$ 0.49 μM which is much lower than the concentrations used to observe the scavenging effect of the drug in an artificial synovial fluid. Interestingly, *Ec*50 found in our experiments is also smaller than the blood concentration of propofol used in anaesthesia. These results show that propofol may strongly affect the potassium channels in some non-excitable cells.

Oxygen derived free radicals are implicated in cartilage damage associated with some inflammatory joint diseases. Recently, Kvam et al. (1) have reported that these radicals cause a depolimerization of hyaluronic acid, a fundamental component of cartilage matrix and, that propofol (2,6-diisopropylphenol; PR), a widely used general anaesthetic, exerts a scavenging effect against free radicals (·OH) in artificial synovial fluid. It was thus proposed that PR could be used as a radical scavenger in joints of patients affected by arthritis.

On the other hand, PR is known to act on various membrane channels. For instance, PR, at micromolar concentrations, potentiates inhibitory transmission by strongly enhancing the activity of $GABA_A$-activated chloride channels (2, 3). PR also affects the membrane currents underlying the action potential (4, 5). Moreover, Magnelli et al. (6) have reported that PR blocks potassium channels in PC12 cells.

* Permanent address: Department of Biophysics, Academy of Medicine, ul. Chalubinskiego 10, PL 50-368 Wroclaw, Poland.

To whom correspondence should be addressed (Fax:+39 40 567862).

0006-291X/94 $5.00

Taking into account both the antioxidant effect of PR and a growing evidence that potassium channels may play an important role in non-excitable cells (see e.g. 7, 8), it is of interest to study the effect of PR on the potassium channels in articular chondrocytes. The properties of the voltage-dependent potassium channels in pig articular chondrocytes have been recently studied (9). In the present work we describe a direct effect of PR on the potassium channel activity of the same cells.

MATERIALS AND METHODS

Cell cultures. Articular cartilage was obtained from the scapula-humerus joints of 150-200 kg pigs killed in an abattoir. The tissue was rinsed in phosphate buffered saline (PBS; in mM: 137 NaCl, 2.7 KCl, 8.1 Na_2PO_4, 1.5 KH_2PO_4, 0.9 $CaCl_2$, 0.49 $MgCl_2$; pH 7.4), minced and treated for 2-3 h with hyaluronidase (Miles, Naperville, U.S.A.; 0.1%, w/v), in PBS in the presence of penicillin (500 U/ml) and streptomycin (500 μg/ml). The solution was then removed and replaced by PBS containing 250 U/ml of collagenase (CLC type II, Biochrom, Berlin, Germany) and the antibiotics. After digestion at 37 °C for 14-18 h under mild agitation, the digest was filtered and centrifuged for 10 min at 700 *g*. By this procedure 20-40·10^6 cells were obtained from 7-10 g of cartilage tissue. The viability of the cells recovered after collagenase digestion was in the range of 90-95%. Chondrocytes were plated at density of 1.6·10^6 cells/cm^2 in DMEM (Dulbecco's minimum essential medium; Biochrom) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine (Biochrom), and the same concentration of antibiotics as described above. The culture medium was changed every 2 days. The experiments were performed on cells cultured for at least 6 days.

Propofol. Propofol, technical grade, was obtained from Lancaster (Strasbourg, France) and purified by distillation at reduced pressure (3 times) and crystallization from hexane. The purity was assessed by Nuclear Magnetic Resonance. Propofol was then dissolved in dimethyl sulfoxide (DMSO) at a concentration of 100 mM and stored at 4 °C.

Single channel recordings. Electrophysiological experiments were performed in a bathing saline (in mM: 130 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES; pH 7.4) at room temperature (21 - 24 °C) using a Zeiss IM 35 phase contrast inverted microscope. Patch pipettes were filled with an internal solution (in mM: 130 KCl, 10 EGTA, 1 $CaCl_2$, 2 $MgCl_2$, 10 HEPES; pH 7.3). Single-channel activity was recorded in the outside-out configuration of the patch clamp technique (10). The current signals were stored on a VHS video-recorder after pulse-code modulation (modified 501ES, Sony) for subsequent analysis. Signals were low-pass filtered at 5 or 6.3 kHz (-3 dB, 8-pole Bessel filter, Ithaco 4302, Ithaca, U.S.A.), connected to the analog-to-digital converter CED 1401 (Cambridge, U.K.), sampled at 40-50 kHz and stored on the computer hard disk. Single channel currents were analysed as already described (11) and an algorithm with two threshold levels (30 and 70% of the mean elementary current amplitude) was used for channel transition detection.

The amount of channel activity was expressed as Np, where N is the number of channels within the patch, and p is the single channel open probability. The Np value was calculated from the ratio $<I>/I_S$, where $<I>$ stands for the mean current during the entire record and I_S is the unitary current. The mean value of current during the record ($<I>$) was calculated from the open channel current amplitude histogram (constructed for single and overlapping channel events) employing the formula:

$$<I> = \frac{1}{100\%} \sum_{i=1}^{512} p_i I_i$$

where p_i is the percentage of time (bin area) during which the recorded channel intensity fell into the interval (I_i - $\Delta I/2$, I_i + $\Delta I/2$) and i is the summation factor. ΔI is the bin width in the

histogram. In practice, with the number of bins equal to 512, ΔI was in the range 0.065-0.195 pA. In some experiments, $\langle I \rangle$ was determined from the integral of the current signal divided by the time duration of the record. The difference between the $\langle I \rangle$ values calculated using the two approaches was always smaller than 3%.
Unless otherwise stated, data are given as mean $\pm$ standard error (SE).

RESULTS

The single-channel activity was recorded in the outside-out configuration of the patch clamp technique. In control conditions, the activity of channels characterized by a slope conductance of 131 $\pm$ 7.5 pS (n = 6; Fig. 1B) was observed in all the outside-out patches done. In two patches, a small percentage (less than 4%) of activity of channels characterized by lower conductance (ca. 20 pS) was also observed. Due to the very low opening frequency of these events, they were not further analysed. The following evidence indicates that the activity of the major class was due to potassium channels: (i) in the outside-out configuration the extrapolated reversal potential was ca. -60 mV (Fig. 1B), a value which after taking into account the composition of the solutions used, is very near to the equilibrium potential of potassium ions; (ii) performing the experiments in the

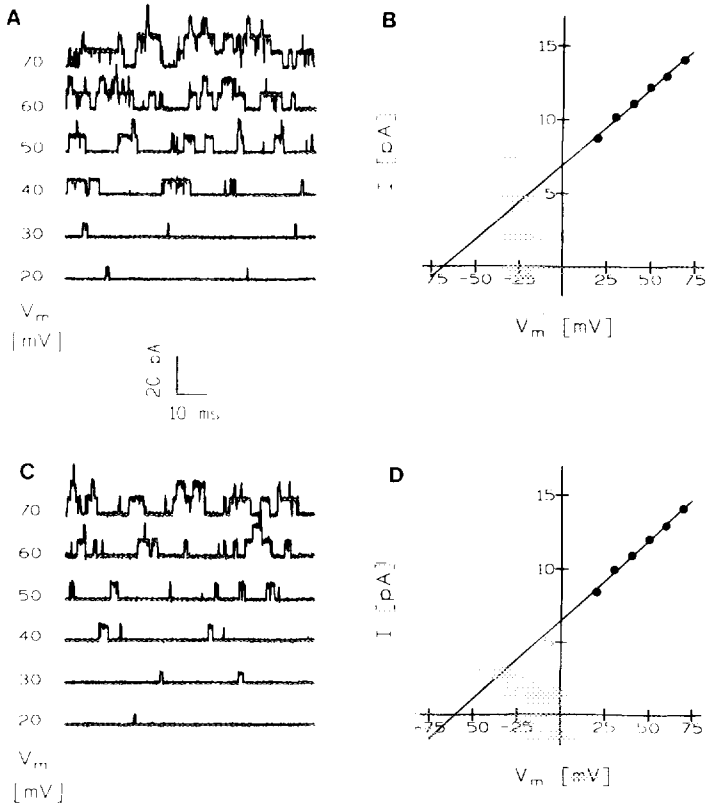

Fig. 1. Representative single potassium channel currents recorded at various membrane potentials (V_m) in control conditions (**A**) and in the presence of 5 μM propofol (**C**). **B** and **D** show the unitary currents *vs.* membrane voltage plots for control (**B**) and after addition of PR (**D**). Lines were drawn by linear regression and the slope conductances were 121 (**B**) and 125 pS (**D**).

cell-attached configuration with high-potassium (130 mM) solution in the pipette, the single-channel currents reversed at more negative pipette potentials when compared with analogous experiments performed with low-potassium (5 mM) pipette solution (9).

The amount of single-channel activity was expressed in terms of *Np* (see Methods). At membrane potential of 20 mV, very few channel openings were recorded. However, upon membrane depolarization, a dramatic increase in channel opening frequency was observed (Fig. 1A, Fig. 3A), indicating its voltage-dependence. The *Np* value varied substantially from one patch to another, probably due to the different number of channels in the patches (e.g. at V_m=60 mV, *Np* was ranging from 0.07 to 0.59 with mean=0.25 and SD=0.29, n=6). The apparent single channel mean open time showed a tendency to increase with membrane voltage, varying from 0.49 ms at 20 mV to 0.94 ms at membrane potential 60 mV. At 70 and 80 mV the channel activity was so high that very few single (not overlapping) channel events were found and so, at these voltages, the open time analysis was not performed.

The outside-out patches were very stable, allowing for long (even several hours) recordings. It was thus possible to monitor the effect of PR while changing several times the drug concentration or the pipette potential of the same patch. Taking into account the variability of *Np* determined in different patches, such a stability was of great advantage when quantifying the effect of the drug. In three most complete experiments, we measured the channel activity at eight different PR concentrations.

In order to study the effect of PR on potassium channels, the drug was gently added to the bath at final concentrations ranging from 2.5 to 80 μM. No significant changes either in the mean apparent open time (at each membrane voltage tested: 20, 30, 40, 50 and 60 mV, $p > 0.8$, n=3, one way analysis of variance, one way ANOVA; see Fig. 1A, C) or in the slope conductance (138 $\pm$ 7.2 pS, n=6, $p > 0.5$, t-Student test, Fig. 1D) were observed at any PR concentrations used. In 2 out of 6 outside-out patches, after addition of PR, an additional conductance level (ca. 85 pS) appeared in the single channel recordings (Fig. 2). In the control recordings no such conductance state was detected (Fig. 1A). This additional conductance level was observed not only as separated single-channel events (Fig. 2A) but also within bursts (Fig. 2B, C, D) or even during a single channel opening (Fig. 2E). The percentage of events with 85 pS-conductance was always smaller than 3% and they were not further analysed.

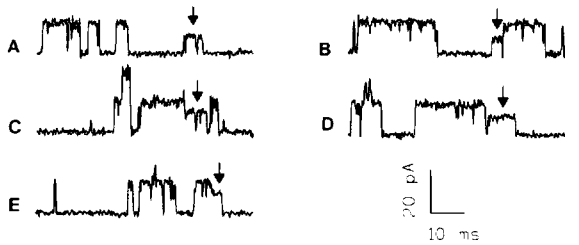

Fig. 2. Examples of single channel currents, recorded in the presence of 5 μM PR at V_m = 60 mV, in which an additional conductance level (ca. 85 pS, arrows) was observed. In **A** the 85 pS-conductance state appears as a separate single channel event, in **B**, **C**, **D** is a part of burst and in **E** the low conductance level is seen during an apparent single-channel opening.

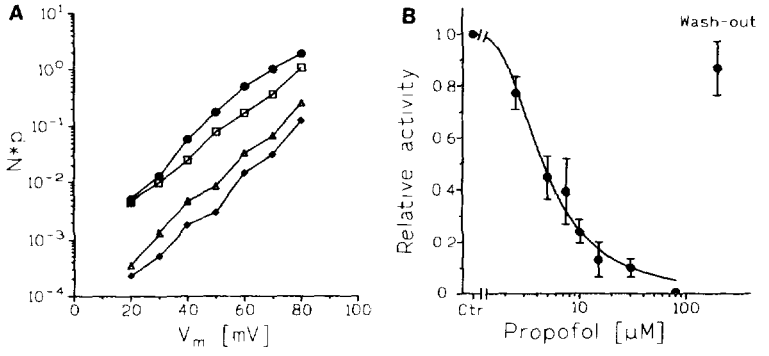

Fig. 3. In **A** the single channel activity, expressed as *Np*, *vs.* membrane potential in control conditions (filled circles) and in the presence of 5 μM (open squares), 15 μM (open triangles) and 30 μM PR (open diamonds). **B** shows the dose-response relation between normalized *Np* values (mean ± SE, n=3) and PR concentrations at V_m = 70 mV. The curve was drawn to the Hill equation with *Ec*50 = 6.2 μM and Hill coefficient equal to 1.68.

PR caused a decrease of *Np* value in a dose-dependent manner (Fig. 3). The recordings were performed at least 10 min after addition, allowing for equilibration of the drug concentration. After this period of time, the *Np* value remained stable, indicating that 10 min was sufficient for *Np* to reach a plateau. In order to quantify the effect of PR, dose-response relationships between the normalysed *Np* values and the drug concentration (Fig. 3B) were constructed for different membrane potentials, and fitted with the Hill equation. The half-blocking concentration of PR was found to be voltage-independent (Fig. 4A; F=0.55, n=3, p > 0.75, one way ANOVA). The mean *Ec*50 value (for all voltages) was 6.0 ± 0.49 μM. The Hill coefficient apparently showed a tendency to decrease with the membrane potential (Fig. 4B) but this effect was not statistically significant (F=0.73, n=3, p > 0.6, one way ANOVA). The mean value of the Hill coefficient was 1.85 ± 0.18.

After the drug was washed out, the channel activity (measured 10 min after changing the solution) was restored almost completely (Fig. 3B).

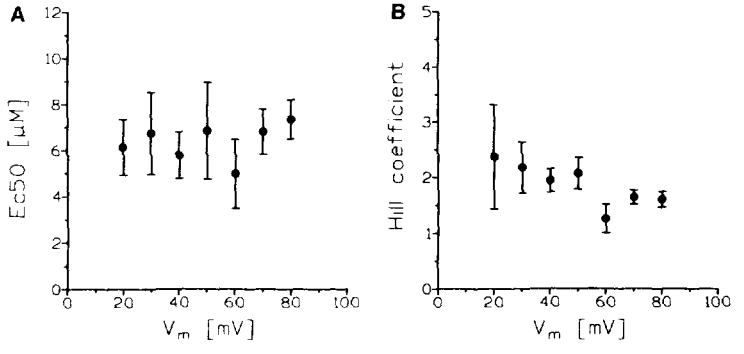

Fig. 4. Half-blocking concentration (*Ec*50) of PR (**A**) and Hill coefficient (**B**) at different membrane potentials. The data are presented as mean ± SE (n=3).

In order to examine the possibility of an open-channel block a different experimental protocol was performed. The membrane patch was stimulated with a train of 50 depolarizing pulses (150 ms-long, 0.2-1 Hz, from the holding potential V_h=-60 mV to 50 mV). The half-blocking concentration of PR determined in these experiments was not significantly different from that obtained in the steady-state recordings and no changes in the channel opening frequency were observed from one record to another. The ensemble-averaging of records before and after addition of PR showed the lack of inactivation (data not shown).

DISCUSSION

The biophysical properties of voltage-activated potassium channels in pig articular chondrocytes are similar to those observed in other types of chondrocytes (12, 13).

Our data demonstrate that PR blocks these potassium channels in pig articular chondrocytes in a dose-dependent manner. No changes either in the single channel conductance or in the single channel kinetics were observed. The lack of effect of PR on the apparent single channel open times suggests that the kinetics of drug interaction with the channel is much slower than that of channel openings. It must be pointed out, however, that PR could induce a very fast flickering block beyond the resolution of our recordings. As shown in Results, in one third of patches, some occasional channel events characterised by a lower conductance level appeared after addition of PR. Since this conductance level appeared even during the single channel openings, it seems evident that it was due to the activity of the same potassium channels. This may suggest a possibility of a "partial block" of the channel by PR.

The half-blocking concentration of PR was around 6 μM and no voltage-dependence of the block was observed. A similar concentration of PR was found to affect the $GABA_A$ channels (2, 3). The value of *Ec*50 obtained in our experiments is smaller than the blood concentration of PR employed for maintenance of general anaesthesia (10-55 μM; see 14). It must be pointed out, however, that PR we used was highly purified (see Methods) at variance with the commercial PR used in other studies. In a series of experiments in which we used PR prepared according to the method described by Eriksson (15), the half-blocking concentration was significantly higher (ca. 30-50 μM) indicating that the way of purification of the drug could affect its action on membrane channels. Interestingly, a similar concentration of PR (50 μM) was found to reduce the potassium channel open probability in PC12 cells from 44% to 12% at 0 mV (6). However, these Authors have reported that the blocking effect of PR was clearly voltage-dependent and that the drug affected also the single channel mean open time. These facts indicate thus that the mechanisms of potassium channel block in PC12 cells and in chondrocytes are different.

Applying the protocol of depolarising voltage stimuli, no evidence for an open channel block mechanism was observed. In fact, in the case of the open-channel block one would expect an "inactivation-like" decay of the ensemble-averaged currents (16, 17). In the control experiments no channel inactivation was observed and the presence of PR caused a reduction in the frequency of channel openings without affecting the kinetics of the ensemble-averaged currents.

The Hill coefficient was found to be significantly higher than 1, suggesting a cooperative block of channel by PR.

However, the data discussed above are not sufficient to describe completely the mechanism of the observed channel block. More studies are needed to elucidate this problem.

Kvam et al. (1) proposed to use PR as a radical scavenger in joints of patients affected by arthritis. However, in their experiments, a marked effect of the drug was observed only at millimolar concentrations. In the light of our results, such high PR concentrations would cause practically a complete block of potassium channels in the articular chondrocytes. Since several lines of evidence indicate an important role of potassium channels in the non-excitable cells (7, 8), it seems relevant to study the possible effects of PR on the chondrocyte cell functions.

In conclusion, these data show that PR may strongly affect the potassium channels in some non-excitable cells, and suggest the need for further studies before using this drug as a radical scavenger in a number of diseases.

ACKNOWLEDGMENTS. We wish to thank Professors Andrea Nistri and Ugo Traversa for valuable comments and suggestions, and Dr Emanuela Fragonas for preparing the cell cultures. This work was financed by MURST, 40 - 60 % grants to F.R and F.V. J.W.M. was partly supported by grants from the University of Trieste (Legge Aree di Confine) and E.E.C. (Action 1227).

REFERENCES

1. Kvam, C., Granese, D., Flaibani, A., Pollesello, P., and Paoletti, S. (1993) Biochem. Biophys. Res. Commun., 193, 927-933.
2. Hales, T.G., and Lambert, J.J. (1991) Br. J. Pharmacol., 104, 619-628.
3. Hara, M., Kai, Y., and Ikemoto, Y. (1993) Anaesthesiology, 79, 781-788.
4. Puttick, R.M., and Terrar, D.A. (1992) Br. J. Pharmacol., 107, 559-565.
5. Veintemilla, F., Elinder, F., and Århem, P. (1992) Eur. J. Pharmacol., 218, 59-68.
6. Magnelli, V., Nobile, M., and Maestrone, E. (1992) Pflügers Arch., 420, 393-398.
7. DeCoursey, T., Chandy, K.G., Gupta, S., and Cahalan, M.D. (1984) Nature, 307, 465-468.
8. Dubois, J.M., and Rouzaire-Dubois, B. (1993) Prog. Biophys. molec. Biol., 59, 1-21.
9. Mozrzymas, J.W., Martina, M., Starc, T., and Ruzzier, F. (1992) Acta Pharm., 42, 345-346.
10. Hamill, O.P., Marty, A., Neher, E., Sakmann, B., and Sigworth, F.J. (1981) Pflügers Arch., 391, 462-464.
11. Dempster, J. (1989) In Microcomputers in Physiology: A Practical Approach. pp. 53-93. IRL Press, Oxford.
12. Grandolfo, M., Martina, M., Ruzzier, F., and Vittur, F. (1990) Calcif. Tissue Int., 47, 302-307.
13. Vittur, F., Grandolfo, M., Fragonas, E., Godeas, C., Paoletti, S., Pollesello, P., Kvam, B.J., Ruzzier, F., Starc, T., Mozrzymas, J.W., Martina, M., and de Bernard, B. (1994) Exp. Cell Res., 210, 130-136.
14. Shafer, A., Doze, V.A, Shafer, S.L., and White, P.F. (1988) Anaesthesiology, 69, 348-356.
15. Eriksson, O. (1991) FEBS Letters, 279, 45-48.
16. Armstrong, C.M. (1966) J. Gen. Physiol., 50, 491-503.
17. Armstrong, C.M. (1969) J. Gen. Physiol., 54, 553-575.