Interleukin-1ß is Transduced by Synovial Fibroblasts Via a Pathway Involving Protein Kinase C and Ca^{2+} Influx

K. Sadasivan, O. Kolomytkin, A. Marino, R. Wolf* and J. Albright

Department of Orthopaedic Surgery
*Department of Medicine

Louisiana State University Medical Center, Shreveport, Louisiana

Abstract

Interleukin-1 B was shown previously to cause membrane depolarization in synovial fibroblasts. The hypothesized role of protein kinase C (PKC) and Ca^{2+} influx in mediating the electrophysiological effect was tested in this study, using inhibitor and activator analysis. The effect of IL-lß was blocked by bisindolymaleimide I (an inhibitor of PKC) and by the Ca^{2+} channel blockers nifedipine and verapamil. In other experiments, PKC was activated using phorbol-12-myristate-13-acetate (PMA) and Ca^{2+} influx was increased by means of a Ca^{2+} ionophore. Simultaneous application of PMA and Ca^{2+} ionophore in the absence of IL-lß mimicked the depolarization caused by the cytokine. The results proved that, under the conditions studied, activation of PKC and Ca^{2+} influx were necessary and sufficient processes in the early transduction pathway of IL-l ß by the synovial cells leading to membrane depolarization. The essential role of protein phosphorylation and Ca^{2+} influx in the early electrophysiological response of synovial fibroblasts to IL-lß was therefore established.

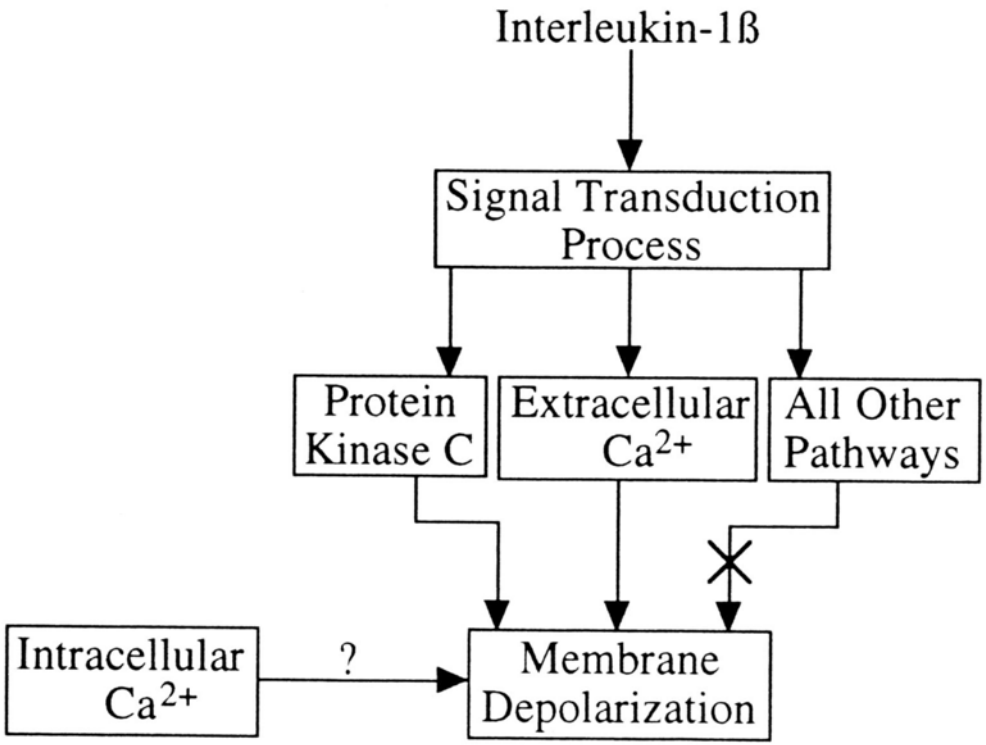
Introduction

Interleukin-1ß (IL-lß) is an important regulator of synovial cells. Binding of the cytokine by a cell surface receptor results in signal transduction leading to expression of many different proteins. Dysregulation of this response can lead to the destruction of joint cartilage and other adverse changes.

A spectrum of second messengers, enzymes, G proteins, and transcription factors have been implicated as components of IL-l signal transduction in various cell types. But contemporary ideas regarding the effect of IL-lß in inexcitable cells do not attribute a significant role to electrophysiological factors. Nevertheless, using the perforated-patch method, we observed changes in the voltage-current characteristics of rabbit synovial fibroblasts during transduction of IL-lß. When aggregated, the cells exhibited two states having high and low membrane potentials, respectively. IL-lß caused cells to transition from the high to the low state, particularly when the cell was voltage-clamped under conditions that favored Ca^{2+} influx.

The aim of this study was to determine the mechanism responsible for the previously observed joint effect of IL-1ß and voltage clamp on the membrane potential of synovial cells. Based on preliminary data, we hypothesized that activation of protein kinase C and Ca^{2+} influx mediated the effect of IL-1ß. In this study, we used activator/inhibitor analysis and showed that these processes were necessary and sufficient to cause synovial cells to transition from the high to the low state of membrane potential.

Hypothesis


Interleukin-1ß
Signal Transduction Process
Protein Kinase C
Extracellular Ca^{2+}
All Other Pathways
Intracellular Ca^{2+}
?
Membrane Depolarization

Materials and Methods

Cells

Rabbit synovial fibroblasts (HIG-82, ATCC) were studied.

Electrical Measurements

The nystatin perforated-patch method was used to measure the transmembrane current at voltage clamp. This method was used because it permits use of the whole-cell configuration for measuring electrical properties of the cell while preventing diffusion of small signaling molecules from the cell into the electrode. The nystatin method therefore preserves intracellular regulation.

Glass capillaries 1.0 mm in diameter were pulled in two steps (PB-7, Narishige) and fire- polished in a microforge (MF-9, Narishige). The resistance of the electrodes was 7–9 MΩ in bath solution. The pipette salt solution was (in mM): K-aspartate (monopotassium salt), 125; KCl, 30; NaCl, 4; HEPES-KOH, 10; pH, 7.2, 318 milliosmoles/liter. The composition of the bath solution was (in mM): NaCl, 145; KCl, 5.4; $CaCl_2$, 1.5; $MgCl_2$, 1.0; HEPES-NaOH, 5.0; glucose, 5.0; pH, 7.3, 328 milliosmoles/liter. Because nystatin interfered with giga-seal formation, the tip of the pipette was filled with a nystatin-free solution prior to the addition of pipette solution containing 0.3 μg/ml of nystatin. The giga-seal was formed during the time needed for the nystatin to diffuse to the tip of the micropipette.

Giga-seals (≈10 GΩ) were formed under negative pressure (5–10 cm, H_2O), typically within 0.5-5 minutes; the success rate was greater than 50%. After giga-seal formation the negative pressure was removed and the nystatin channels formed within 5–15 minutes; the resistance of the perforated-patch membrane was 40±20 MQ. Giga-seals and nystatin pores usually remained stable for hours.

Membrane potentials were recorded using a patch-clamp amplifier (Axopatch 200B, Axon Instruments). The amplifier was connected to a computer (TL-1 DMA Interface, Axon Instruments), and commercial software (pCLAMP 6, Axon Instruments) was used to control the amplifier, and to collect and analyze the experimental data.

Activators and Inhibitors

The dish containing the clamped cell was perfused with bath solution containing the agent under study. The agents used were: human recombinant interleukin-1ß (IL-1ß); phorbol-12-myristate-13-acetate (PMA) (activator of protein kinase C); bisindolymaleimide I (BIS) (inhibitor of protein kinase C); A23187 Ca^{2+} ionophore; and nifedipine and verapamil (blockers of Ca^{2+} channels).

IL-1 B was added into solution in the presence of 0.1% bovine albumin as carrier protein. Control experiments showed that 0.1% bovine albumin did not influence the current-voltage (I-V) characteristics of the cells. PMA, BIS, A23187 Ca^{2+} ionophore, verapamil and nifedipine were dissolved in dimethyl sulfoxide and then added to the bath solution. Final concentration of dimethyl sulfoxide did not exceed 0.3%; control experiments showed that it did not influence the I-V characteristics of the cells.

Results

Effect of IL-lß

The effect of IL-1ß on membrane potential and on the I-V curves is shown in the Figures. Following the control measurements in bath solution (Figure A), the cells were held at -30mV for 15 minutes prior to the addition of IL-lßB (1 ng/ml of bath solution), whereupon the I-V curves were measured again after 5 minutes (Figure D). The results of normalizing each control curve to the average, and the average ± SEM of these curves are shown in Figures B and C, respectively. The corresponding results following addition of IL-lß are shown in Figures E and F. IL-lß caused an increase in inward current, and a shift in the reversal potential (Table).

Effect of IL-lß in the Presence of PMA

Control measurements showed that 1.5 μM PMA did not change the membrane potential (Table). Thus, activation of PKC by PMA did not produce membrane depolarization.

PMA (1.5 μM) was added to the bath solution and the cell was held at -30 mV for 15 minutes. IL-lß was then added, and the I-V curve changed significantly within 5 minutes (Table). Thus, activation of protein kinase C by PMA did not eliminate the effect of IL-lß on membrane depolarization.

Effect of IL-1ß in the Presence of BIS

Addition of 3 μM BIS alone to the bath solution did not change the membrane potential of the cells (Table).

BIS (3 μM) was added to the bath solution and the cells were held at -30 mV for 15 minutes prior to the addition of 1 ng/ml IL-1ß. The I-V curves did not change (Table), indicating that inhibition of protein

kinase C eliminated the effect of IL-1ß on the I-V curves. Because depolarization did not occur after addition of IL-lß in the presence of an inhibitor of protein kinase C, it can be concluded that activation of protein kinase C by IL-1ß is necessary to produce membrane depolarization. Since activation of protein kinase C alone (by PMA) did not cause membrane depolarization, it follows that IL-1ß activates not only protein kinase C but other systems as well.

Effect of PMA in the Presence of Ca^{2+} Ionophore

Addition of 10 μM Ca^{2+} ionophore did not alter the membrane potential (Table).

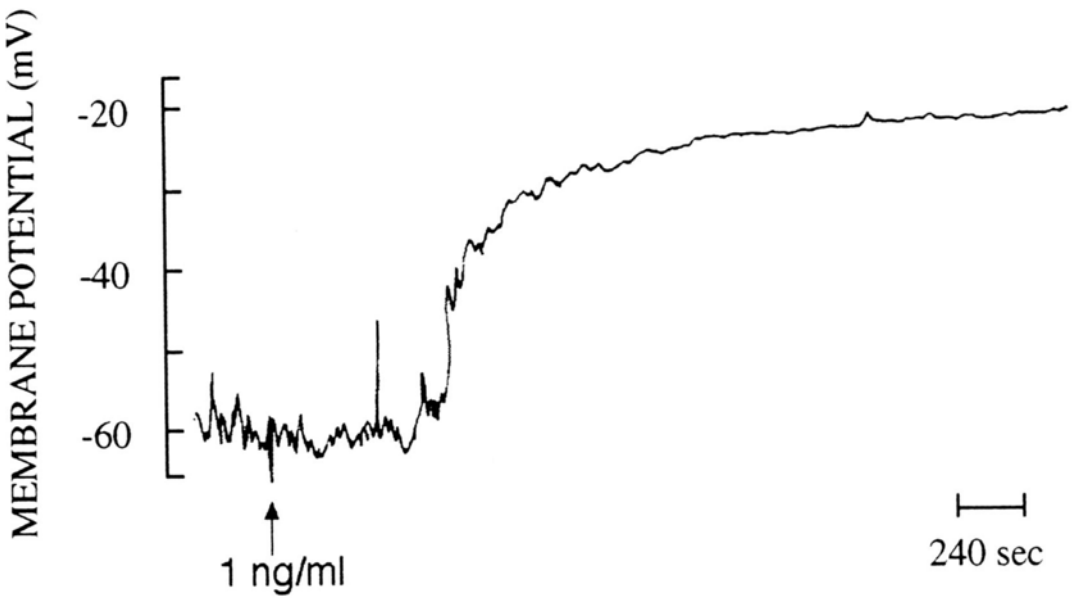
PMA (1.5 μM) and 5 μM Ca^{2+} ionophore were added, and an increase in inward current was observed, accompanied by a shift in reversal potential (Table). Thus, activation of protein kinase C and increased Ca^{2+} concentration resulted in a membrane depolarization similar to that produced by IL-1ß.

Effect of IL-1ß in the Presence of Nifedipine and Verapamil

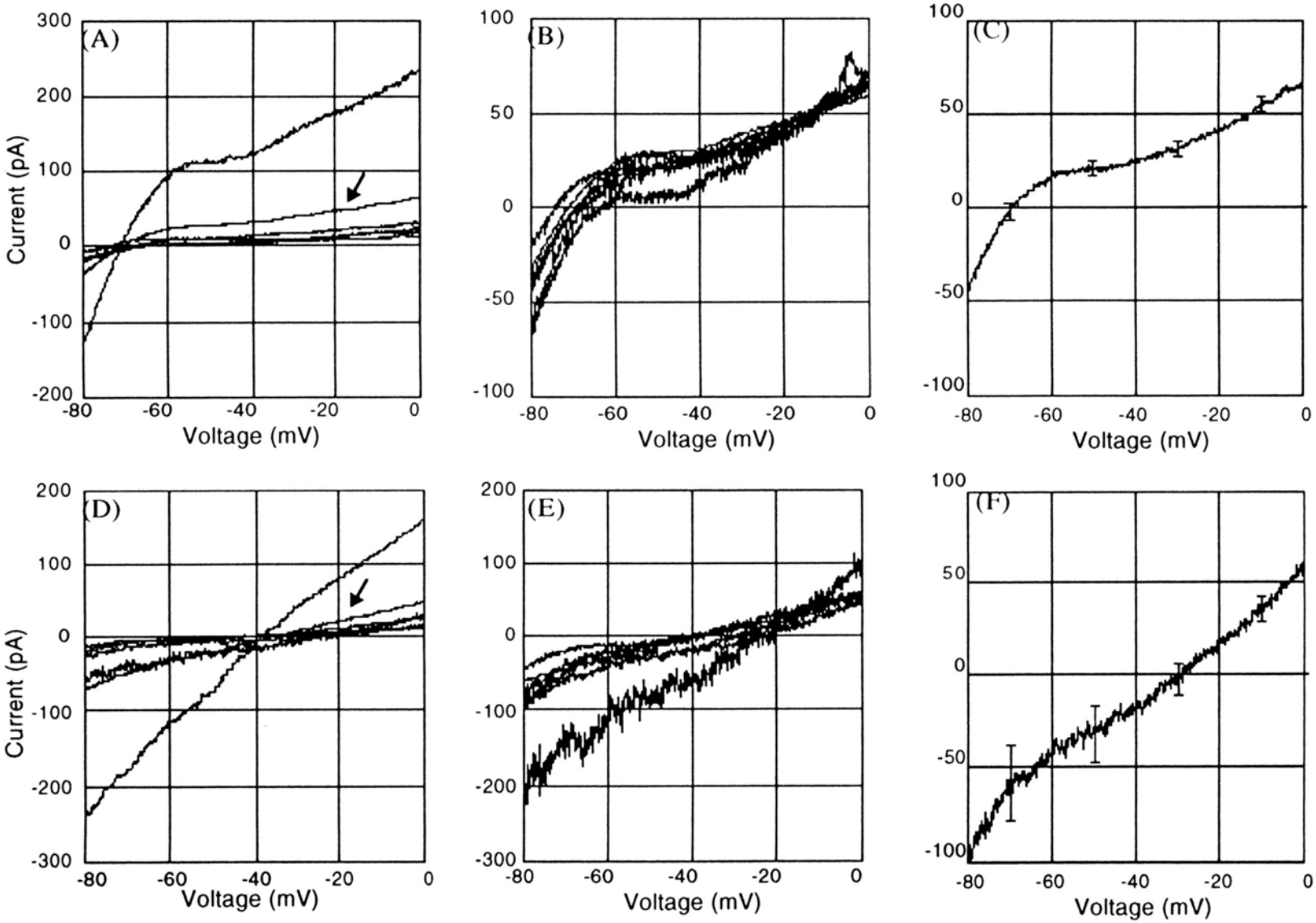
Neither 50 μM nifedipine nor 10 μM verapamil altered the membrane potential.

Nifedipine or verapamil were added to the bath solution, and the cells were held at -30 mV for 15 minutes prior to the addition of 1 ng/ml IL-1ß. The I-V curves did not change significantly within 5 minutes, indicating that blocking of Ca^{2+} channels in the cytoplasmic membrane eliminated the effect of IL-1ß (Table). Thus, opening of membrane Ca^{2+} channels were essential for membrane depolarization caused by IL-l B.

Effect of IL-1ß on Membrane Potential of a Synovial Fibroblast

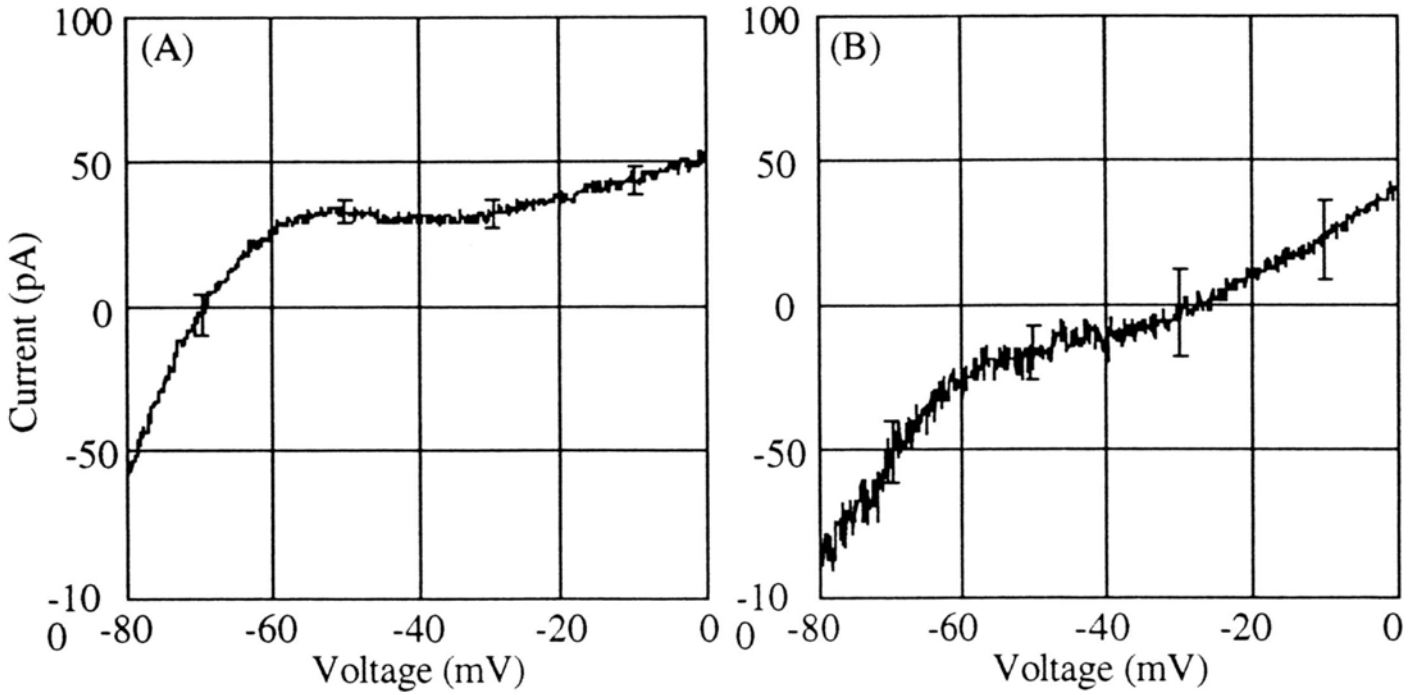


Effect of IL-1ß on the I-V Curves of Synovial Fibroblasts



The effect of IL-1ß (1 ng/ml) on the I-V characteristics of 5 different synovial fibroblasts measured in different aggregates. (A) Curves obtained in bath solution, and the mean (arrow). (B) Results of fitting each curve to the mean by multiplying the current for each point on the curve by a constant coefficient determined using the method of least squares. (C) Mean ± SEM of fitted curves. (D) Curves for the same cells after 15 minutes of voltage clamp at 30 mV followed by 5 minutes' exposure to IL-1ß. (E) Results of multiplying the current for each point on the curve in D by the previously determined constant coefficient for each cell. (F) Mean ± SEM of the curves in E. The effect of IL-1ß was evaluated by comparing the curves in C and F.

Effect of PMA and Ca^{2+} Ionophore on the I-V Curves of Synovial Fibroblasts



(A) In bath solution. (B) After exposure to PMA (1.5 μM) and Ca^{2+} ionophore (5 μM) for 15 minutes. The mean ± SEM of the normalized curves are presented for 5 different synovial fibroblasts.

Influence of Different Substances on Reversal Potential of Synovial Fibroblasts

Substances	Reversal Potential (mV)	
	Before	After
1 ng/ml IL-1ß	-69± 2.0	-29* ± 3.5
1.5 µM PMA	-66 ± 2.3	-68 ± 1.0
1 ng/ml IL-1ß and 1.5 µM PMA	-65 ± 2.4	-39* ± 7.0
3 µM BIS	-70± 1.0	-71 ± 1.0
1 ng/ml IL-1ß and 1.5 µM BIS	-69 ± 1.0	-67 ± 3.2
10 µM Ca^{2+} ionophore	-67 ± 1.6	-66 ± 1.9
0.25 µM PMA and 5 µM Ca^{2+} ionophore	-70 ± 1.3	-30* ± 8.2
50 µM nifedipine	-68.5 ± 1.6	-70.5 ± 1.0
10 µM verapamil	-65 ± 4.8	-63 ± 5.3
1 ng/ml IL-1ß and 50 µM nifedipine	-68 ± 1.6	-65.5 ± 4.1
1 ng/ml IL-1ß and 10 µM verapamil	-68 ± 1.3	-60 ± 4.8

*P< 0.05

Reversal potential is shown before and 5–15 minutes after addition of the substances. Mean ± SEM from measurements on 5 different cells for each experiment.

Discussion

Prior to our work, the initial steps of IL-1ß transduction in synoviocytes were unstudied. We found that IL-1ß induced relatively fast (about 5 minutes) switching of electrophysiological states of synovial fibroblasts, leading to transitions to a state of low membrane potential (O.V. Kolomytkin, A.A. Marino, K.K. Sadasivan, R.E. Wolf and J.A. Albright. Interleukin-1ß switches electrophysiological states of synovial fibroblasts. *Am. J. Physiol*. 273 (Regulatory Integrative Comp. Physiol. 42):Rl822-R1828, 1997).

IL-1ß activates different signaling pathways in different cell types, and only some of the pathways involve PKC and Ca^{2+}. The hypothesis of this study was that one particular pathway out of the large number of theoretically possible pathways was responsible for the early electrophysiological response of synovial fibroblasts to IL-1ß.

Membrane depolarization occurred soon after ligation of IL-1ß by its receptor; within 5 minutes after addition of IL-1ß, the mean reversal potential of the cells decreased from -69 to -29 mV (Table). BIS alone had no effect on V_m, indicating that activation of protein kinase C was not necessary to maintain a high resting potential. However, the IL-1ß-induced decrease in V_m was blocked by BIS (Table), thereby implicating participation of protein kinase C in the effect of IL-1ß.

The Ca^{2+} channel blockers nifedipine and verapamil had no effect on membrane potential, indicating that passage of Ca^{2+} from the bath solution into the cell through voltage-gated ion channels was not necessary to maintenance of the cell resting potential. But nifedipine and verapamil blocked the action of IL-1ß, indicating that Ca^{2+} influx was an essential step in the signal pathway of IL-1ß leading to membrane depolarization.

The question whether PKC activation and Ca^{2+} influx were sufficient to produce depolarization was studied by exposing the cells to PMA and Ca^{2+} ionophore in the absence of IL-1ß. Neither agent alone affected the membrane potential or I-V characteristics of the cells. Thus, neither activation of PKC alone, nor Ca^{2+} influx alone were sufficient to mimic the effect of IL-1ß on membrane potential. Jointly, however, the agents produced a membrane depolarization that was indistinguishable from the effect of IL-1ß (Table and Figure).

We conclude that the depolarization that occurs as an early consequence of transduction of IL-1ß by voltage-clamped HIG-82 cells is caused by activation of PKC and Ca^{2+} influx. The role of the IL-1ß induced depolarization in regulating protein expression by the cells remains to be determined.

Perspectives

Rational therapy can be based on attempts to interact with specific steps in the process that leads to clinical manifestation of disease. In the case of arthritis, the need for improved treatments led to ongoing clinical evaluations of agents designed to antagonize excessive production of metalloproteinases, which is believed to play an important role in the pathophysiology of joint disease. It may be more effective, however, to inhibit earlier stages in the signaling pathway that leads to metalloproteinase over-production in response to cytokine stimulation. The results reported here show that changes in membrane electrical potential in metalloproteinase-producing cells occur within 5 minutes of interaction of IL-1ß with its receptor. Identification of the membrane channels that are responsible for the electrical changes and knowledge of their gating characteristics may provide new possibilities for therapies aimed at preventing the consequences of abnormal cytokine stimulation of synovial cells. Such an approach would have the advantage of blocking the pathophysiological process early in its development.

Acknowledgment

This work was supported by Louisiana State University Medical Center, Center for Excellence in Arthritis and Rheumatology.