special communication

Interleukin-1β switches electrophysiological states of synovial fibroblasts

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[1]*Department of Orthopaedic Surgery and* [3]*Department of Medicine, Section of Rheumatology, Louisiana State University Medical Center, Shreveport, Louisiana 71130–3932; and* [2]*Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino, Moscow Region, 142292 Russia*

Kolomytkin, Oleg V., Andrew A. Marino, Kalia K. Sadasivan, Robert E. Wolf, and James A. Albright. Interleukin-1β switches electrophysiological states of synovial fibroblasts. *Am. J. Physiol.* 273 (*Regulatory Integrative Comp. Physiol.* 42): R1822–R1828, 1997.—The role of electrophysiological events in signal transduction of interleukin-1β (IL-1β) was investigated in rabbit synovial fibroblasts using the perforated-patch method. Aggregated synovial fibroblasts occurred in two different electrophysiological states having membrane potentials (V_m) of -63 ± 4 ($n = 71$) and -27 ± 10 mV ($n = 55$) (high and low V_m, respectively). IL-1β affected the cells with high V_m; it switched the state of the cell from high to low V_m. This effect was strongly dependent on the external potential applied to the cell membrane. Low V_m (−30 mV) alone without IL-1β did not switch the state of the cells. Thus a synergistic effect involving the cytokine and cell V_m in switching the electrophysiological state of the cell was shown, indicating that electrophysiological changes are involved in signal transduction. Gap junctions between aggregated cells were necessary for the cells to have a high V_m and to respond to IL-1β. Gap junction resistance between adjacent cells was estimated as 300 ± 100 MΩ. Our findings suggest that the electrophysiological behavior of synovial fibroblasts is tightly connected to a signaling or intracellular mediator system that is triggered by IL-1β.

cytokine; immunomodulator; patch clamp; membrane potential; arthritis

CYTOKINES PLAY an important role in cell regulation, and have been studied intensively in recent years (11, 19). Cytokine interactions with synovial cells can affect their production of matrix proteases, and abnormal regulation of cytokine-induced pathways is associated with rheumatoid arthritis and osteoarthritis (2, 30). Interleukin-1β (IL-1β) is particularly important in this regard because elevated levels occur in arthritic patients (22, 27), and IL-1β triggers production of proteases and prostaglandins by synovial fibroblasts (10, 28) and regulates their synthesis of collagens (4, 14). Despite significant advances in the molecular biology of IL-1β and its receptors and antagonists (1), the immediate posttransduction pathways are not well understood, particularly with regard to the possible importance of electrophysiological changes (11, 31, 33).

Our findings show directly a synergistic effect of IL-1β and the membrane electric potential in switching the electrophysiological state of HIG-82 cells, a well-studied rabbit synovial cell line (7, 8, 12, 13, 29). Thus the results suggest that the electrophysiological behavior of the cell must be considered in evaluating the signal transduction pathway of IL-1β.

METHODS

Cells. The cells were grown at 37°C, 5% CO_2, without antibiotics in 25-ml polystyrene flasks containing F-12 medium (GIBCO BRL) with 10% fetal bovine serum. For passage, confluent cultures were trypsinized (1 ml, 0.08%) for 3–5 min, after which 4 ml of medium was added and the suspended cells were centrifuged, resuspended, and then seeded (10^6 cells) into 4 ml of medium. For electrophysiological measurements, 10^5 cells were added to 35-mm petri dishes and incubated at 37°C for 24 h, after which the cells were treated for 2 min with 1 ml of 0.01% collagenase and 0.01% hyaluronidase; this step was necessary to obtain stable gigaseals. The specific consequences of our enzyme treatment, although mild by comparison with routine trypsinization of the cells, have not been conclusively established. The cells were incubated in medium for 40–60 min at 37°C to allow recovery from the enzyme treatment; the medium was then replaced with bath solution, and all measurements were made in bath solution at 25°C, employing the cells (10–20 μm in diameter) that remained adhered to the bottom of the petri dish (most cells remained adherent after enzyme treatment).

The dish containing the clamped cell was rapidly perfused with bath solution containing human recombinant IL-1β (Sigma no. I-4019) and 0.1% bovine albumin (carrier protein). Control experiments showed that 0.1% bovine albumin did not influence the current-voltage (*I-V*) characteristic of the cells.

Electrodes. The nystatin perforated-patch method (17) 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) 125 K-aspartate (monopotassium salt), 30 KCl, 4 NaCl, 10 *N*-2-hydroxyethylpiperazine-*N*′-

2-ethanesulfonic acid (HEPES)-KOH, pH 7.2; 318 mosM (calculated). The composition of the bath solution was (in mM) 145 NaCl, 5.4 KCl, 1.5 $CaCl_2$, 1.0 $MgCl_2$, 5.0 HEPES-NaOH, 5.0 glucose, pH 7.3; 328 mosM (calculated). Because nystatin interfered with gigaseal formation, the tip of the pipette was filled with a nystatin-free solution before the addition of pipette solution containing 0.3 μg/ml of nystatin. The gigaseal was formed during the time needed for the nystatin to diffuse to the tip of the micropipette (17).

Measurements. Gigaseals (≥10 GΩ) were formed under negative pressure (5–10 cmH_2O), typically within 0.5–5 min; the success rate was >50%. After gigaseal formation the negative pressure was removed and the nystatin channels formed within 5–15 min; the resistance of the perforated-patch membrane was 40 ± 20 MΩ. Gigaseals and nystatin pores usually remained stable for hours.

Membrane potential (V_m; measured as the reversal potential at zero-current clamp), *I-V* characteristics, and transient currents 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. The systematic error of the measurement of V_m due to the electrode potential did not exceed 2 mV. Variation of current and reversal potential at the same conditions was determined by variability of different cells. The error values presented are SE of the mean.

In patients, IL-1β was detected in the marrow plasma at 0.2–0.3 ng/ml and in synovial fluid at 0.15 ng/ml (20, 24). Previous in vitro studies using HIG-82 cells were typically performed using 0.1–10 ng/ml (18, 34). In the studies reported here, we used 0.1–10 ng/ml, with most studies being performed with 1 ng/ml.

RESULTS

V_m. V_m was initially measured in single HIG-82 cells by the perforated-patch-clamp method (17), and in most instances a low V_m was found immediately after the nystatin channels were formed in the patch membrane; the mean value was −3 ± 3 mV (n = 25). Five additional cells initially exhibited higher values (−37 ± 12 mV, n = 3, and −63 ± 6 mV, n = 2), but in each case V_m dropped to about −3 mV within 15 min.

The results for small cell aggregates (≤3 cells) were similar to those for single cells, but the potentials measured from cells in larger aggregates were higher and more stable. V_m measured in 126 cells in aggregates of 5–15 cells (1 measured cell/aggregate) exhibited a bimodal distribution with peaks around −65 mV and −30 mV (Fig. 1). The potentials were typically stable for >0.5 h. When the distribution was divided at the point of minimum potential (−45 mV), the means of the two groups were −63 ± 1 (n = 71) and −27 ± 2 mV (n = 55). Thus aggregated cells occurred in two different electrophysiological states, with mean V_m of −63 and −27 mV (high and low V_m, respectively).

The resistance of the pipette seal was measured in each experiment just after gigaseal formation and was ~10 GΩ. The resistance of the membrane of single cells was ~1 GΩ, as estimated from their *I-V* curves. On the basis of Ohm's law, therefore, the low-V_m state in the aggregated cells and the −3-mV state exhibited by single cells were real and were not artifacts due to current leakage through the pipette seal.

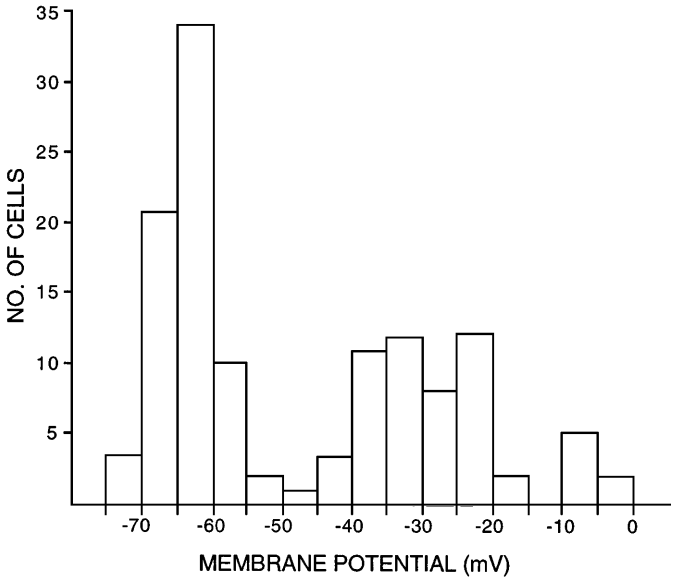

Fig. 1. Histogram of membrane potential of rabbit synovial fibroblasts (HIG-82, ATCC). Membrane potential was averaged for 1 min immediately after perforated patch formation (n = 126 cells).

Because V_m depends on position in the cell cycle (6, 21, 32), it was possible that the distribution of V_m resulted from sampling of cells in different positions in the cycle. To explore this possibility, cells were prepared as they would have been for electrophysiological study, but instead were used to evaluate the distribution of the cells in the cycle. After the collagenase-hyaluronidase treatment of a sufficiently large number of petri dishes, the adhering cells were trypsinized, permeabilized, reacted with propidum iodide, and then analyzed by flow cytometry to assess DNA content (25). On the basis of cellular DNA analysis by flow cytometry, 81% of the cells were in G_0/G_1, 9% in S-phase, and 10% in the G_2+M phase. With −45 mV as the dividing line, the cell states were in a proportion of 56 to 44% (Fig. 1), which did not correlate with the results of the cell cycle analysis showing that 81% of the cells were in G_0/G_1.

Gap junction resistance. The necessity of cell aggregation for high-V_m measurements (V_m < −45 mV) led us to consider whether aggregated cells were in electrochemical communication, and we therefore developed a method to investigate the possibility of gap junctions. After formation of a perforated patch, a 2-mV step was applied and the transient current passing through the electrode was measured in aggregates of different sizes. Analysis (see APPENDIX) showed that the time constant of the transient current decay depended strongly on the resistance between adjacent cells in an aggregate. If cells in an aggregate were electrically separated from each other, the resistance would be high and the time constant would be low regardless of the number of cells in the aggregate, because the voltage step would charge the membrane capacitance of only one cell. However, if the cells were connected to each other by gap junctions, the resistance would be relatively low and the time

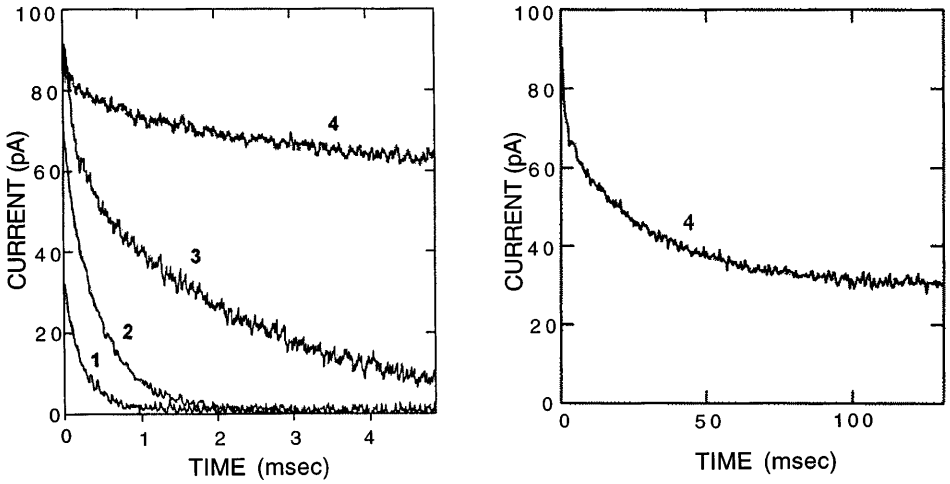

Fig. 2. Transient current responses to a voltage step. After perforated patch formation, a 2-mV step was applied to the electrode at *time 0*. *Curve 1*, patch formed on a single cell; *curves 2* and *3*, patch formed in a cell that was aggregated with 1 and 2 cells, respectively; *curve 4*, aggregate of >10 cells. Membrane potential was −20 mV for *curves 1*, *2*, and *3* and −65 mV for *curve 4*. Individual records were filtered with a 10-kHz, low-pass, 4-pole Bessel filter; each curve was produced by averaging transient current for 10 voltage steps. Variation of the transient current measured for different single cells of the same size did not exceed the amplifier noise seen in *curve 1*. Transient current variation for different aggregates of 2 or 3 cells was larger. This variation (not shown for clarity) was used to estimate the SE of gap junction resistance (300 ± 100 MΩ).

constant of the current decay would be greater with an increasing number of cells in the aggregate because the voltage step would charge the membrane capacitance of all cells in the aggregate.

The time constant for a typical cell in a large aggregate was more than three orders of magnitude greater in comparison with that from a single cell (Fig. 2, *curves 1* and *4*), leading to the conclusion that the aggregated synovial fibroblasts were connected to each other by gap junctions. The gap junction resistance between adjacent cells was found by the method described in the APPENDIX to be 300 ± 100 MΩ in measurements for seven different aggregates of two or three cells.

Gap junction resistance was not ascertained for larger aggregates because the calculations would have required specification of the two-dimensional geometry of the aggregate. Large aggregates can have many different geometries, so there was little practical reason to extend the calculation to large aggregates.

The assumption that gap junction resistance (r) is constant for all pairs of adjacent cells (Fig. 2) in an aggregate of given size is supported by the observation that elementary gap junctions can be disassembled into half pores that can laterally diffuse in the membrane and assemble into new elementary gap junctions with other cells having half pores (23). This suggests that the equilibrium distribution of elementary gap junctions between all pairs of cells in an aggregate is uniform (under the assumption that the total number of half pores for each cell is equal) and thus that r is constant for all pairs of adjacent cells in the aggregate.

Effect of IL-1β. Cells displaying a high V_m were exposed to IL-1β (0.1–10 ng/ml; most experiments were done at 1 ng/ml) and the time dependence of the V_m was observed. Application of IL-1β did not produce a consistent effect on V_m; 19 of 27 cells did not respond to the addition of 1 ng/ml IL-1β, but eight cells showed a decrease to −30 ± 12 mV within 30–40 min. Experimental records for three of these cells are shown in Fig. 3. When the effect of IL-1β on V_m occurred (Fig. 3) it was reversible, but only when interrupted before completion of the depolarization process (by removal of the IL-1β from the bath solution). Reversibility was confirmed for five different cells.

Cells with a low V_m ($V_m > -45$ mV) did not respond to the presence of IL-1β. The last result was confirmed in measurements from five different cells in which the cell was initially exposed to 0.1 ng/ml for 15 min and then to 1 and 10 ng/ml (for 15 min in each case).

In an attempt to understand why only some cells responded to IL-1β, we tried to maximize the effect. It was suggested that the intracellular signaling cascade triggered by the interaction of IL-1β with its receptor may involve influx of Ca^{2+} (3, 11, 31). Because some Ca^{2+} channels are voltage sensitive and have a high probability of being open at membrane voltages such that $V_m \geq -30$ mV (9, 15), we hypothesized that the probability for IL-1β to cause particular cells to transition from high to low V_m would be enhanced if voltage-gated Ca^{2+} channels were opened simultaneously with those linked to the IL-1β receptors. We therefore measured the IL-1β effect on cells under −30-mV voltage clamp.

A typical *I-V* curve from a cell in the high-V_m state is shown in Fig. 4. The cell exhibited a V_m of about −70 mV and a region of negative slope commencing around −30 mV. The effect of IL-1β on the *I-V* curve of a high-V_m cell is shown in Fig. 5. After the initial measurement (Fig. 5*A*, *curve 1*) the cell was held at −30 mV for 15 min to promote entry of Ca^{2+} before the addition of IL-1β. A change in the *I-V* curves was seen, but the cell did not transition to the low-V_m state (Fig. 5*A*, *curve 2*).

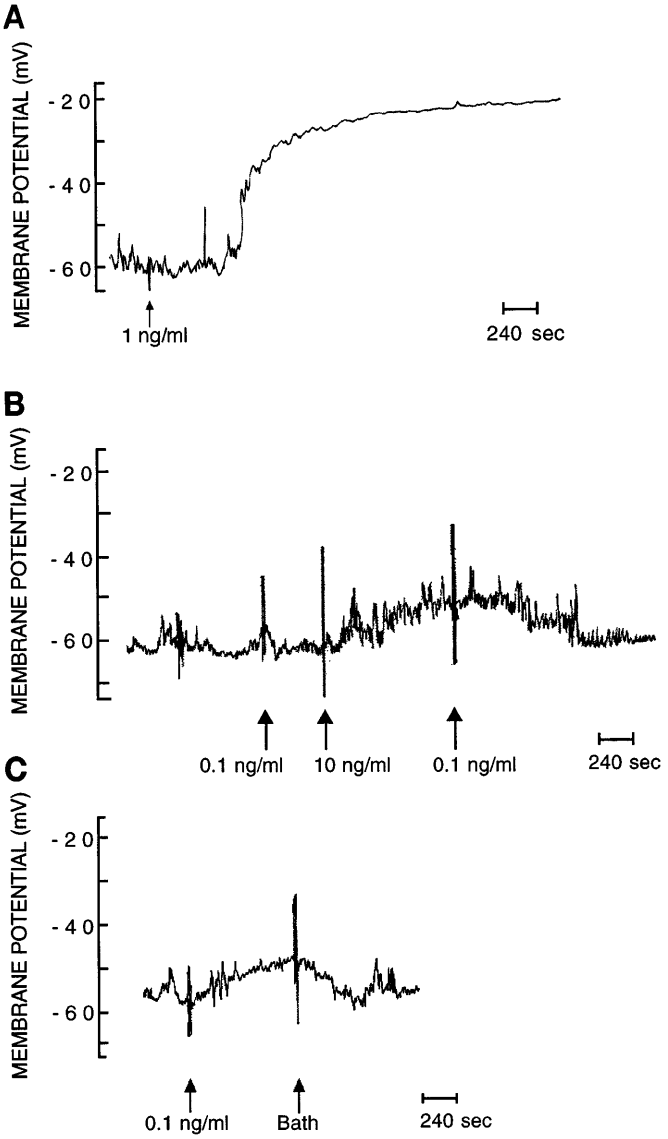

Fig. 3. Reversibility of interleukin-1β (IL-1β)-induced effect on membrane potential. *A*: addition of 1 ng/ml of IL-1β resulted in a decrease in the membrane potential. *B*: addition of 100 pg/ml of IL-1β had no immediate effect, but addition of 100× greater amount initiated depolarization that could be reversed by returning the bath solution to the lower concentration. *C*: Similar observation but from a cell that was responsive at the lower concentration. Effect of IL-1β was studied by exchanging the bath solution for bath solution containing 0.1% albumin and known concentrations of IL-1β. Control experiments showed that 0.1% bovine albumin did not produce any significant effects.

When 1 ng/ml IL-1β was added to the bath solution, the *I-V* curve changed significantly within 3–5 min and the V_m shifted from −63 mV to about −40 mV (Fig. 5*B*). As shown in Fig. 5, addition of IL-1β caused an increase in inward current. When the IL-1β was removed and the cell was held at −70 mV (to permit the voltage-gated Ca^{2+} channels to return to their resting condition), the cell substantially returned to its initial condition (Fig. 5*C*). *I-V* curves of one cell at different IL-1β concentrations are presented in Fig. 5 as continuous lines. Mean values (±SE) of the current for five different cells are also shown in Fig. 5.

IL-1β had no discernible effect on the *I-V* characteristic of cells with low V_m (Fig. 6) (5 cells were measured at 1 ng/ml and 2 cells at 10 ng/ml).

DISCUSSION

Addition of IL-1β produced a rapid (5–15 min) depolarization only in cells having a high V_m. IL-1β switched the electrophysiological state of the cell from the high- to the low-V_m state. This effect was strongly dependent on the V_m applied to the cell membrane from an external source. If IL-1β was added at zero-current clamp mode (meaning that the average initial V_m was about −63 mV), most of the cells (70%) did not exhibit a change to the state with low potential. But if the cells with high V_m were clamped at a low potential (−30 mV), then 100% of cells exhibited a change to the state with low potential after addition of IL-1β. Low V_m (−30 mV) alone without IL-1β did not cause the cells to switch from the high- to the low-V_m state. These observations indicated a synergistic effect of IL-1β and membrane electric potential in the cells. Thus the electrophysiological behavior of the cell must be considered in evaluating the signal transduction pathway of

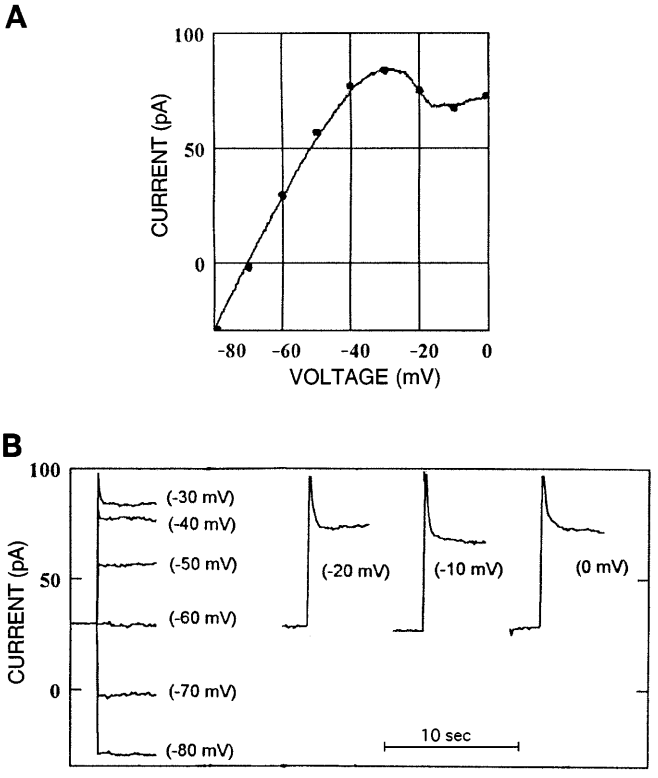

Fig. 4. Representative current-voltage (*I-V*) curve of an HIG-82 cell in a cell aggregate. *A*: continuous line, *I-V* curve obtained using voltage ramp (1.67 mV/s). *B*: transmembrane current due to a series of membrane voltage pulses. Initial voltage value for each pulse was −60 mV; final voltage was from −80 to 0 mV in 10-mV increments. As indicated in *A*, the results obtained by measuring steady-state currents (shown as points) were identical to those obtained by applying a voltage ramp.

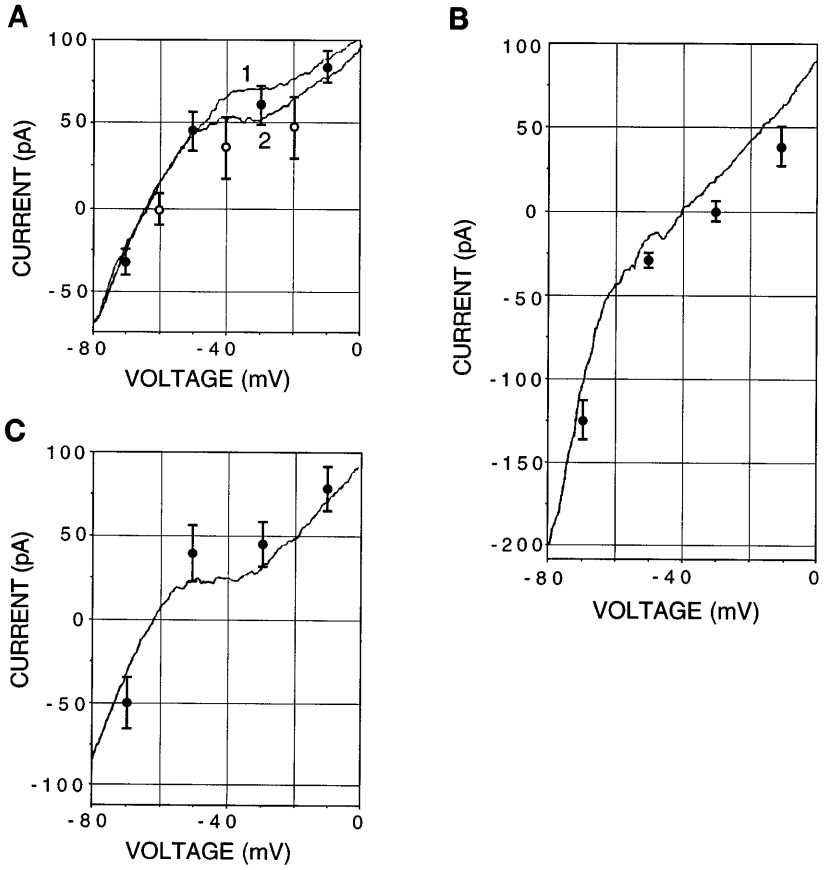

Fig. 5. Effect of IL-1β on *I-V* characteristic of an aggregated HIG-82 cell with high membrane potential. *A*, *curve 1*:(●), initial *I-V* curve; *A*, *curve 2*:(○), after 15 min while voltage clamped at −30 mV. *B*: 5 min after addition of 1 ng/ml IL-1β. *C*: after removal of IL-1β and maintenance at −70 mV for 15 min. *I-V* curves were obtained by measuring the current while applying a voltage ramp (1.67 mV/s). *I-V* curves for one of the cells are presented as continuous lines. Mean value and SE of current for 5 different cells are shown at different voltages.

IL-1β. The perforated patch-clamp method is ideal for studying electrophysiological effects connected with second-messenger systems because this method permits use of the whole cell configuration for measuring electrical properties of the cell and does not permit small signaling molecules to diffuse into the electrode.

The preparation of IL-1β had a purity >97% and it produced the effects at 1 ng/ml. Therefore we can assume with high probability that the observed effects were caused by IL-1β itself. Nevertheless, we cannot exclude the possibility that an unknown substance in the IL-1β preparation produced the observed effects at <0.03 ng/ml.

We found that gap junctions between aggregated synovial cells were necessary to have the high V_m to permit the cells to respond to IL-1β. Gap junctions have been reported in normal human synovium, and they appear to occur more frequently in osteoarthritis (26).

Because the synovial cells were connected by gap junctions, it is possible that the consequences of a ligand-receptor interaction in one or a few cells in an aggregate could spread either actively or passively through an ensemble of cells, effectively propagating the ability of cells to transition from the high- to the low-V_m state (5, 16). If such a mechanism operated in vivo, it would permit a response by large regions of the synovium (in either normal or pathological cases) after ligand-receptor interactions in localized regions. The effective IL-1β concentrations in these studies (1 ng/ml) were reasonably comparable to levels measured in patients (20, 24), and gap junctions have been described in synovial tissue (26). This evidence suggests, therefore, that the observations reported here are relevant to the clinical situation.

Our findings suggest that the electrophysiological behavior of synovial fibroblasts is tightly connected to a signaling or intracellular mediator system that is modulated by IL-1β.

Perspectives

Attempts to understand the pathophysiological mechanisms of arthritis usually focus on identifying chemical pathways that link cytokine signals with protein synthesis. Our studies suggest that electrophysiological changes may occur early in the signal detection process before any changes in gene transcription or protein synthesis. Thus the mechanisms that mediate the electrical changes are potential targets for rational therapy aimed at altering the subsequent signal transduction cascade. Furthermore, our observation that the synovial fibroblasts formed gap junctions raises the possibility that electrical coupling between the synoviocytes might be an important aspect of synovial physiology. If functional gap junctions are observed in cultures of human synovial fibroblasts and in human synovial tissue, then studies of the properties of such gap junctions might lead to significant advances in under-

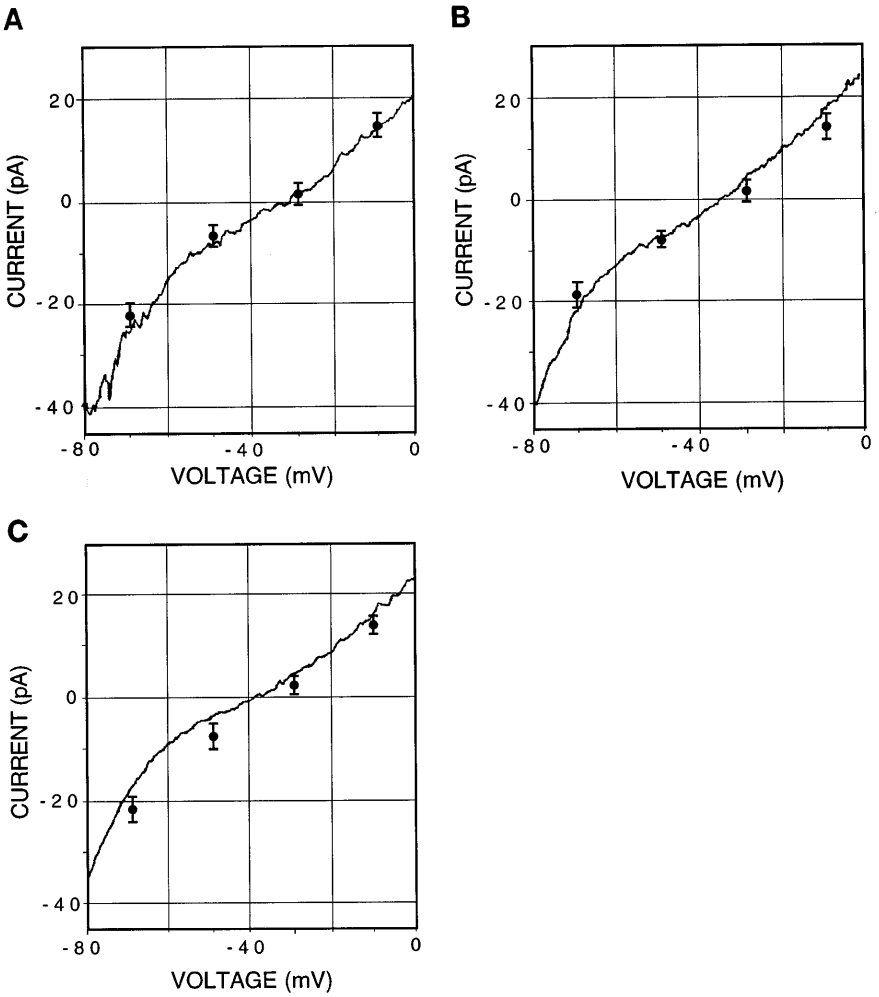

Fig. 6. Effect of IL-1β on *I-V* characteristic of an aggregated HIG-82 cell with low membrane potential. *A*: initial *I-V* curve. *B* and *C*: 3 and 30 min after addition of 1 ng/ml IL-1β, respectively. *I-V* curves were obtained by measuring the current while applying a voltage ramp (1.67 mV/s). *I-V* curves for one of the cells are presented as continuous lines. Mean value and SE of current for 5 different cells are shown at different voltages.

standing both normal and abnormal synovial tissue. For example, on the basis of morphological evidence, it appears that the number of synovial gap junctions is altered in arthritic patients (26). Thus it is possible that changes in gap junctions might play a causative role in development of arthritis.

APPENDIX: TRANSIENT CURRENT FOR AN AGGREGATE OF CELLS CONNECTED TO EACH OTHER BY GAP JUNCTIONS

Assume that the measured cell in an aggregate is surrounded by n identical adjacent cells and that gap junctions are present between adjacent cells. If a voltage step (V) is applied to the electrode, the resulting current (I) satisfies

$$RC\ddot{I} + \{[n + 1 + (r/R_c)](R/r) + 1\}\dot{I} + [1/(rC)][(r/R_c) + 1]\, I = [1/(rC)][n + 1 + (r/R_c)](V/R_c)$$

where C is the capacitance of the membrane of each cell, R is the resistance of the perforated patch, r is the resistance of the gap junctions between two adjacent cells in the aggregate, and R_c is the resistance of the cell membrane.

The solution is

$$I(t) = C_1 \exp[k_1 t] + C_2 \exp[k_2 t] + I_0$$

where

$$k_{1,2} = \left\{ -[n + 1 + (r/R_c)](R/r) - 1 \pm \sqrt{[[n + 1 + (r/R_c)](R/r) + 1]^2 - 4(R/r)[1 + (r/R_c)]} \right\} \Big/ 2RC$$

C_1 and C_2 are constants and t is time.

$$I_0 = V[n + 1 + (r/R_c)]/R_c[1 + r/R_c]$$

I_0 is the current for $t \rightarrow \infty$.

For the case of a single cell ($n = 0$, $r \rightarrow \infty$), $k_1 = k_2 = 1/RC$. R was found from the initial condition, $R = V/I(0)$, where $V = 2$ mV; C was obtained by fitting the solution $[I(t) = I(0) \exp[-t/RC]]$ to the experimental curve (Fig. 2, *curve 1*). The result was C = 3.47 pF. Measurements were made on cells that were oblate ellipsoids having major and minor axes of 12 ± 1 and 6 ± 1 μm, respectively. On the assumption that the plasma membrane capacity was 1 μF/cm^2, it can be shown that C = 3.2 ± 0.6 pF, in good agreement with the results obtained by curve fitting.

Employing the value of C ascertained from measurements on single cells, gap junction resistance (r) was estimated by fitting the theoretical solution for $I(t)$ to experimental *curves 2* and *3* in Fig. 2 (which correspond to $n = 1$ and $n = 2$, respectively). Fitting was done by the method of least squares, with fractional error of 0.001.

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

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Received 29 May 1997; accepted in final form 7 August 1997.

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