

Intracellular signaling mechanisms of interleukin-1 β in synovial fibroblasts

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Intracellular signaling mechanisms of interleukin-1 β in synovial fibroblasts. *Am. J. Physiol.* 276 (*Cell Physiol.* 45): C9–C15, 1999.—The possibility that membrane depolarization of synovial fibroblasts caused by interleukin-1 β (IL-1 β) was mediated by protein kinase C (PKC) and Ca²⁺ influx was studied using inhibitor and activator analysis. The effect of IL-1 β was blocked by bisindolylmaleimide I, an inhibitor of PKC, and by the Ca²⁺ channel blockers nifedipine and verapamil. In other experiments, PKC was activated using phorbol 12-myristate 13-acetate, and Ca²⁺ influx was increased by means of a Ca²⁺ ionophore. Simultaneous application of phorbol ester and Ca²⁺ ionophore in the absence of IL-1 β mimicked the depolarization caused by IL-1 β . The results were consistent with the hypothesis that, under the conditions studied, activation of PKC and Ca²⁺ influx are necessary and sufficient processes in the transduction of IL-1 β by synovial cells leading to membrane depolarization. The essential role of protein phosphorylation and Ca²⁺ influx in the early electrophysiological response of synovial fibroblasts to IL-1 β was therefore established. The role of IL-1 β -induced depolarization in regulating protein expression by the cells remains to be determined, but the results reported here, taken together with observations that protein phosphorylation and Ca²⁺ influx also mediate the effect of IL-1 β on protease production (1, 2), suggest that electrophysiological changes are actually part of the pathway for expression of proteases in response to IL-1 β .

membrane potential; nystatin; voltage clamp

THE CYTOKINE interleukin-1 β (IL-1 β) is an important regulator of synovial cells. Binding of IL-1 β by a cell surface receptor results in signal transduction leading to the expression of many different proteins, including metalloproteinases and IL-1 β itself (5, 8, 19). Dysregulation of this response, especially chronic overproduction of metalloproteinases, can lead to the destruction of joint cartilage and other adverse changes (17, 23).

A spectrum of second messengers, enzymes, G proteins, and transcription factors have been implicated as components of IL-1 signal transduction in various cell types (7, 16). The wide range of reported processes and mechanisms suggests that the consequences of ligation of IL-1 β with its receptor vary with cell type, and

perhaps even within a cell type under different study conditions.

Contemporary ideas regarding the effect of IL-1 β in inexcitable cells do not attribute a significant role to electrophysiological factors (7, 16). Nevertheless, using the perforated-patch method, we observed changes in the current-voltage (*I-V*) characteristics of rabbit synovial fibroblasts during transduction of IL-1 β . When aggregated, the cells exhibited two states having high and low membrane potentials (V_m), respectively. IL-1 β caused cells to transit from the high to the low state, particularly when the cell was voltage clamped under conditions that favored Ca²⁺ influx (14). Thus it is possible that IL-1 β can affect important cellular processes known to be influenced by V_m , such as transport of amino acids and exocytosis.

The aim of this study was to determine the mechanism responsible for the previously observed joint effect of IL-1 β and voltage clamp on the V_m of synovial cells. We hypothesized that activation of protein kinase C (PKC) and Ca²⁺ influx were necessary and sufficient processes for the effect of IL-1 β , and this was established using inhibitor and activator analysis.

MATERIALS AND METHODS

Cells. Rabbit synovial fibroblasts (HIG-82, American Type Culture Collection) were grown at 37°C with 5% CO₂ in 25-ml polystyrene flasks containing F-12 medium (GIBCO BRL) with 10% fetal bovine serum and without antibiotics. For passage, confluent cultures were trypsinized (1 ml, 0.08%) for 3–5 min, after which 4 ml of medium were added and the suspended cells were centrifuged, resuspended, and then seeded (10⁶ cells) into 4 ml of medium. For electrophysiological measurements, 10⁵ 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, which was mild by comparison with routine trypsinization of the cells, was necessary to obtain stable gigaseals. 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 μ m in diameter) that remained adherent to the bottom of the petri dish (most cells remained adherent following enzyme treatment).

Electrodes. The nystatin perforated-patch method (9) was used to measure the transmembrane current under voltage clamp. The perforated-patch method was employed because it permitted use of the whole cell configuration to measure electrical properties of the cell while preventing diffusion of small signaling molecules from the cell into the electrode, thereby preserving intracellular regulation. Glass capillaries 1.0 mm in diameter were pulled in two steps (PB-7, Narish-

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ige) and fire polished in a microforge (MF-9, Narishige). The resistance of the electrodes was 7–9 M Ω in bath solution. The pipette salt solution was (in mM) 125 monopotassium aspartate, 30 KCl, 4 NaCl, and 10 HEPES-KOH (pH 7.2; 318 mosmol/l, calculated). The composition of the bath solution was (in mM) 145 NaCl, 5.4 KCl, 1.5 CaCl₂, 1.0 MgCl₂, 5.0 HEPES-NaOH, and 5.0 glucose (pH 7.3; 328 mosmol/l, 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 μ g/ml nystatin. The gigaseal was formed during the time needed for the nystatin to diffuse to the tip of the micropipette (9).

Inhibitors and activators. The dish containing the clamped cell was perfused with bath solution containing the agent under study. The agents used were human recombinant IL-1 β (Sigma no. I 4019), phorbol 12-myristate 13-acetate (PMA; Sigma no. P 8139), bisindolylmaleimide I (Calbiochem no. 203290), A 23187 Ca²⁺ ionophore (Sigma no. C 7522), verapamil (Sigma no. V 4629), and nifedipine (Sigma no. N 7634). IL-1 β was added into solution in the presence of 0.1% bovine albumin (Sigma no. A 2153) as carrier protein. Control experiments showed that 0.1% bovine albumin did not influence the *I-V* characteristics of the cells. PMA, bisindolylmaleimide I, A-23187 Ca²⁺ ionophore, verapamil, and nifedipine were dissolved in DMSO (Sigma no. D 5879) and then added to the bath solution. Final concentration of DMSO did not

exceed 0.3%; control experiments showed that it did not influence the *I-V* characteristics of the cells.

The inhibitors and activators were used at concentrations typically used in previous studies to cause relatively specific reactions and thereby permit inferences regarding particular mechanisms involved in processes under study.

Electrical measurements. Gigaseals (\sim 10 G Ω) were formed under negative pressure (5–10 cm H₂O), 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 \pm 20 M Ω . Gigaseals and nystatin pores usually remained stable for hours.

I-V curves and V_m (measured as the reversal potential at zero-current clamp) were recorded using a patch-clamp amplifier (Axopatch 200B, Axon Instruments) as described earlier (14). 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 collect and analyze the experimental data. For measurement of *I-V* curves, a voltage ramp was applied to the electrode from –80 to 0 mV at 1.67 mV/s, and the current was sampled at 6 kHz. It was shown that *I-V* curves thus obtained represented steady-state *I-V* characteristics of HIG-82 cells (14).

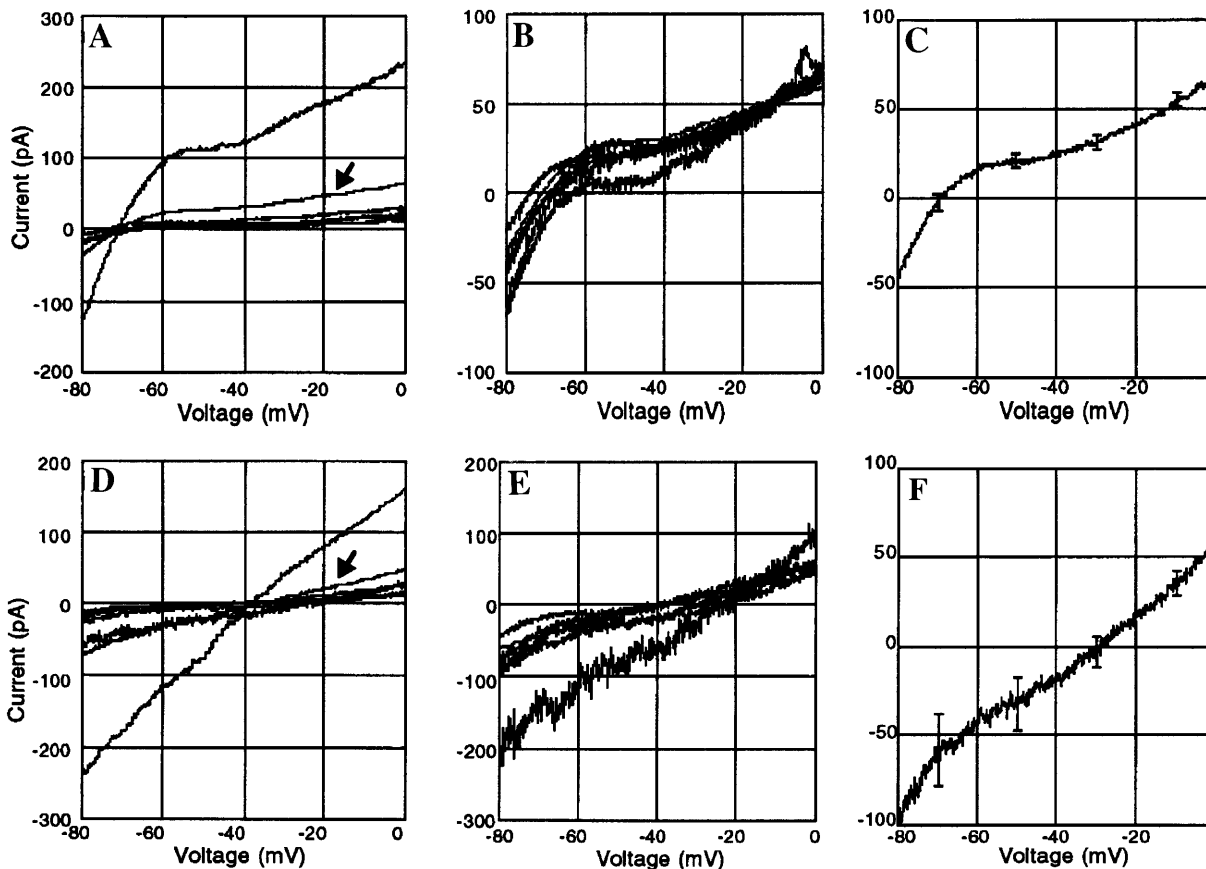


Fig. 1. Effect of IL-1 β (1 ng/ml) on current-voltage (*I-V*) characteristics of 5 different synovial fibroblasts measured in different aggregates. *A*: curves obtained in bath solution. *B*: results of fitting each curve to mean by multiplying current for each point on curve by a constant coefficient determined using method of least squares. *C*: means \pm SE of fitted curves. *D*: curves for same cells after 15 min of voltage clamp at –30 mV followed by 5 min of exposure to interleukin-1 β (IL-1 β). *E*: results of multiplying current for each point on curve in *D* by previously determined constant coefficient for each cell. *F*: means \pm SE of curves in *E*. Effect of IL-1 β was evaluated by comparing curves in *C* and *F*. Arrows indicate average curves.

Table 1. Influence of different substances on reversal potential of synovial fibroblasts

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

Values are means \pm SE of measurements on 5 different cells for each experiment, obtained from current-voltage curves. Reversal potentials are shown before and 5–15 min after addition of substances. IL-1 β , interleukin-1 β ; PMA, phorbol 12-myristate 13-acetate; BIS, bisindolylmaleimide I. * $P < 0.05$ vs. value before addition.

Procedure. The influence of different agents on the IL-1 β -induced changes in the I - V curves of synovial fibroblasts was studied, and the responsible mechanisms were evaluated using substances having known physicochemical properties. In each experiment, the I - V curve was measured initially in bath solution and then measured again after perfusion of the dish with bath solution containing the agent or combination of agents under study. I - V curves obtained before and after addition of the test agent were compared to determine the influence of the agent. Each measurement was repeated five times using five cells in five different dishes.

We showed earlier that HIG-82 cells exhibit a normal V_m only when aggregated with each other (14). Therefore, the measurements reported here were carried out on a cell aggregated with other cells. The aggregates contained different numbers of cells (5–20 cells) because it was not convenient to restrict the study to aggregates with the same number of cells. Because the cells were connected electrically to each other by gap junctions (14), however, the I - V curve depended on the number and configuration of the cells in the aggregate. The current was higher for aggregates containing a larger number of cells. Consequently, a procedure was needed to standardize the curves so that the results from cells in aggregates of different sizes recorded under the same experimental conditions could be combined. It turned out that, by following the procedure described below, we could fit I - V curves from different aggregates to each other simply by

changing the scale of the current (Fig. 1, *A* and *B*). Our ability to do so indicated that the varying number of cells in the aggregate only changed the magnitude of the current and did not change the shape of the I - V curves. We used the property of constancy of shape to compare the results obtained on cells in different aggregates.

For each experiment, I - V curves for five cells in different aggregates in bath solution were averaged (Fig. 1*A*). We used the average I - V curve to fit I - V curves for different cells to each other. The I - V curve for each cell was fitted to the average I - V curve by multiplying the current by a certain coefficient. The value of the coefficient for each cell was found by fitting its I - V curve to the average I - V curve, employing the method of least squares. The fitted I - V curves (Fig. 1*B*) were found to coincide well with each other. It is seen in Fig. 1, *A* and *B*, that even an I - V curve for a cell in a relatively large aggregate (with large current) coincides well with other I - V curves after fitting. Thus I - V curves for all cells were normalized to the I - V curve of the cell aggregate of average size. The means \pm SE for the fitted I - V curves were used to display the behavior of the cells in bath solution (Fig. 1*C*).

The effect of IL-1 β (or of the other substances studied) on the I - V curve was obtained as follows. The I - V curve was measured for each cell after addition of IL-1 β (Fig. 1*D*; or other substances). Then, the current in the I - V curve was multiplied by the same coefficient found for the cell in bath solution (Fig. 1*E*). The mean \pm SE of these curves (Fig. 1*F*) was then compared statistically with the results obtained in bath solution (Fig. 1*C*) to ascertain the effect of IL-1 β (or other substances). The procedure followed permitted comparison of results obtained from cells in aggregates of different sizes subjected to the same conditions.

The reversal potential (voltage corresponding to zero current) was found for each I - V curve, and reversal potentials of the cells in bath solution and those exposed to the substance under study were compared using the Wilcoxon signed rank test at a significance level of $P < 0.05$. The reversal potentials for the original and fitted (multiplied by a coefficient) I - V curves coincided with each other.

The systematic error of the measurement of V_m due to the electrode potential did not exceed 2 mV.

RESULTS

Effect of IL-1 β . The effect of IL-1 β on the I - V curve of cells having a high V_m is shown in Fig. 1. After the initial (control) measurements in bath solution (Fig. 1*A*), the cells were held at -30 mV for 15 min before the addition of IL-1 β (1 ng/ml of bath solution), whereupon the I - V curves were measured again after 5 min

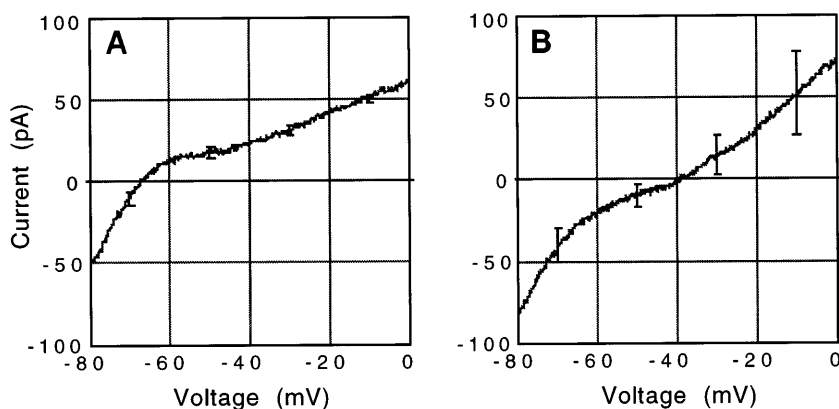
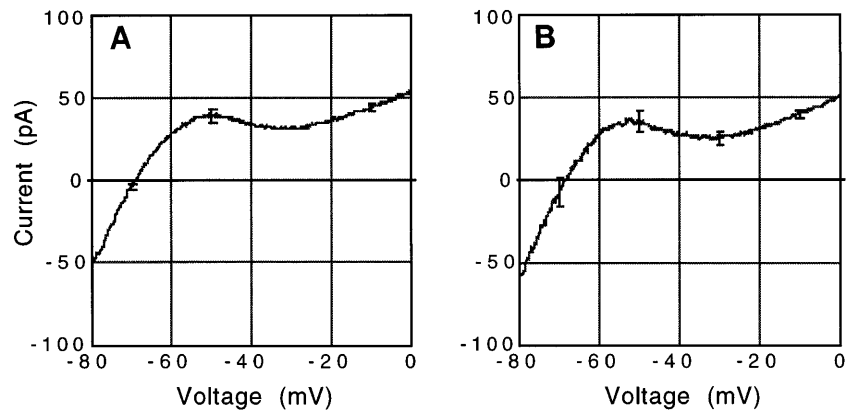


Fig. 2. Effect of IL-1 β (1 ng/ml) on I - V characteristics (means \pm SE of normalized curves) of 5 different synovial fibroblasts measured in different aggregates in presence of phorbol 12-myristate 13-acetate (PMA; 1.5 μ M). *A*: I - V curves in bath solution. *B*: I - V curves after 15 min of voltage clamp at -30 mV and PMA treatment followed by exposure to IL-1 β for 5 min.

Fig. 3. Effect of IL-1 β (1 ng/ml) on I - V characteristics (means \pm SE of normalized curves) of 5 different synovial fibroblasts measured in different aggregates in presence of bisindolylmaleimide I (3 μ M). *A*: I - V curves in bath solution. *B*: I - V curves after 15 min of voltage clamp at -30 mV and bisindolylmaleimide I treatment followed by exposure to IL-1 β for 5 min.



(Fig. 1D). The results of normalizing each control curve to the mean and the means \pm SE of these curves are shown in Fig. 1, *B* and *C*, respectively. The corresponding results following addition of IL-1 β are shown in Fig. 1, *E* and *F*. IL-1 β caused an increase in inward current and a shift in the reversal potential (Table 1), confirming our previous results (14).

Effect of IL-1 β in presence of PMA. PMA, an activator of PKC, mimicked the effect of IL-1 β on induction of metalloproteinase synthesis by HIG-82 cells at 0.15 μ M (1), possibly indicating that protein phosphorylation by PKC was responsible for the membrane depolarization caused by IL-1 β . To test this hypothesis, we studied the effect of PMA on V_m using the same protocol as for IL-1 β . Control measurements showed that 1.5 μ M PMA did not change the I - V curve or the V_m (Table 1). Thus activation of PKC by PMA did not produce membrane depolarization.

After the control measurements (Fig. 2A), 1.5 μ M PMA was added to the bath solution and the cell was held at -30 mV for 15 min. IL-1 β was then added, and the I - V curve changed significantly within 5 min (Fig. 2B, Table 1). Thus activation of PKC by PMA did not eliminate the effect of IL-1 β on membrane depolarization.

Effect of IL-1 β in presence of bisindolylmaleimide I. Although PKC alone was not responsible for changing the I - V curves following addition of IL-1 β , PKC could have functioned with other cell systems to produce membrane depolarization. To test this hypothesis, we studied the effect of IL-1 β in the presence of bisindolyl-

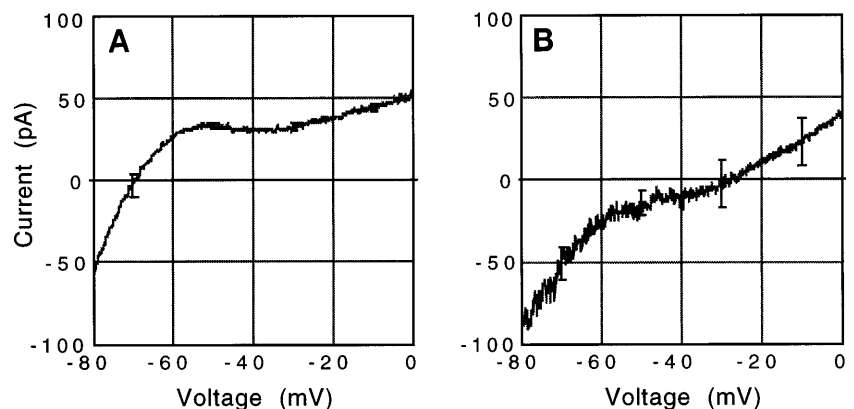
maleimide I, a protein kinase inhibitor that is specific for PKC and that inhibits the activity of all four PKC subtypes with similar potency (21). Addition of 3 μ M bisindolylmaleimide I alone to the bath solution did not change the I - V curve or the V_m of the cells (Table 1).

After the control measurements (Fig. 3A), 3 μ M bisindolylmaleimide I was added to the bath solution and the cells were held at -30 mV for 15 min before the addition of 1 ng/ml IL-1 β . The I - V curves did not change (Fig. 3B, Table 1), indicating that inhibition of PKC eliminated the effect of IL-1 β on the I - V curves. Because depolarization did not occur after addition of IL-1 β in the presence of an inhibitor of PKC, it can be concluded that activation of PKC by IL-1 β is necessary to produce membrane depolarization. Because activation of PKC alone (by PMA) did not cause membrane depolarization, it follows that IL-1 β activates not only PKC but other systems as well.

Effect of PMA in presence of Ca $^{2+}$ ionophore. Previous evidence suggested that the system responsible for intracellular Ca $^{2+}$ homeostasis was the system that worked with PKC to mediate the effect of IL-1 β (7, 18). If so, then addition of PMA and Ca $^{2+}$ ionophore together should produce membrane depolarization. Addition of 10 μ M Ca $^{2+}$ ionophore did not alter the V_m (Table 1).

After the control measurements (Fig. 4A), 1.5 μ M PMA and 5 μ M Ca $^{2+}$ ionophore were added. After 15 min, an increase in inward current was observed (Fig. 4B), accompanied by a shift in reversal potential (Table 1). Thus activation of PKC and increased Ca $^{2+}$ concen-

Fig. 4. Effect of PMA (1.5 μ M) and Ca $^{2+}$ ionophore (5 μ M) on I - V characteristics (means \pm SE of normalized curves) of 5 different synovial fibroblasts measured in different aggregates. *A*: I - V curves in bath solution. *B*: I - V curves after exposure to PMA and Ca $^{2+}$ ionophore for 15 min.



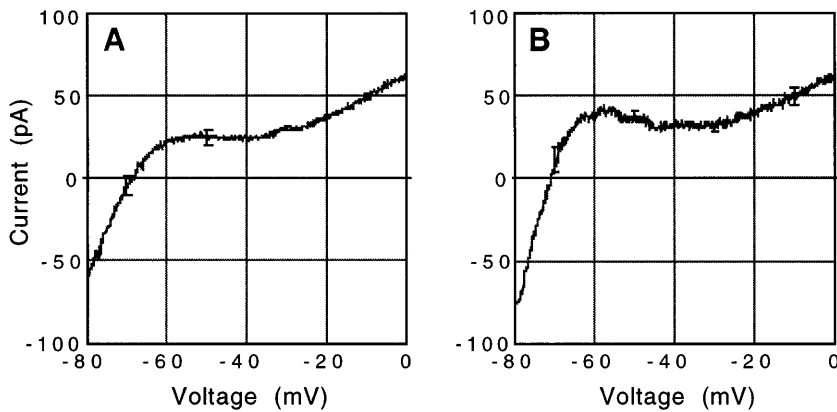


Fig. 5. Effect of IL-1 β (1 ng/ml) on I - V characteristics (means \pm SE of normalized curves) of 5 different synovial fibroblasts measured in different aggregates in presence of nifedipine (50 μ M). *A*: I - V curves in bath solution. *B*: I - V curves after 15 min of voltage clamp at -30 mV and nifedipine treatment followed by exposure to IL-1 β for 5 min.

tration resulted in a membrane depolarization similar to that produced by IL-1 β .

Effect of IL-1 β in presence of nifedipine and verapamil. To clarify the role of Ca²⁺ in the effect of IL-1 β on V_m , we used the Ca²⁺ channel blockers nifedipine and verapamil. Neither 50 μ M nifedipine nor 10 μ M verapamil altered the V_m .

After the control measurements (Figs. 5*A* and 6*A*), 50 μ M nifedipine or 10 μ M verapamil was added to the bath solution and the cells were held at -30 mV for 15 min before the addition of 1 ng/ml IL-1 β . The I - V curves did not change significantly within 5 min (Figs. 5*B* and 6*B*, respectively), indicating that blocking of Ca²⁺ channels in the cytoplasmic membrane eliminated the effect of IL-1 β . Thus opening of membrane Ca²⁺ channels was essential for membrane depolarization caused by IL-1 β .

DISCUSSION

Protein expression by synovial cells in culture in response to IL-1 β typically occurs after exposure for 1–2 days (5, 8, 19), and there is some evidence suggesting that PKC and Ca²⁺ are involved somewhere in the signaling pathway (1). Before our work (14), the initial steps of IL-1 β transduction in synoviocytes were unstudied. We found that IL-1 β induced relatively fast (\sim 5 min) switching of electrophysiological states of synovial fibroblasts, leading to transitions to a state of low V_m .

IL-1 β activates different signaling pathways in different cell types, and only some of the pathways involve PKC and Ca²⁺ (7, 16). The hypothesis of this study was

that one particular pathway of the large number of theoretically possible pathways was responsible for the early electrophysiological response of synovial fibroblasts to IL-1 β . As hypothesized, we found that activation of PKC and Ca²⁺ influx were necessary and sufficient processes in the transduction of IL-1 β by synovial cells, under the conditions studied, to cause membrane depolarization.

Membrane depolarization occurred soon after ligation of IL-1 β by its receptor; within 5 min after addition of IL-1 β , the mean reversal potential of the cells decreased from -69 to -29 mV (Fig. 1, Table 1). Bisindolylmaleimide I alone had no effect on V_m , indicating that activation of PKC was not necessary to maintain a high resting potential. However, the IL-1 β -induced decrease in V_m was blocked by bisindolylmaleimide I (Fig. 3), thereby implicating participation of PKC in the effect of IL-1 β .

The Ca²⁺ channel blockers nifedipine and verapamil had no effect on V_m , indicating that passage of Ca²⁺ from the bath solution into the cell through voltage-gated ion channels was not necessary for maintenance of the cell resting potential. However, nifedipine (Fig. 5) and verapamil (Fig. 6) blocked the action of IL-1 β , indicating that Ca²⁺ influx was an essential step in the signal pathway of IL-1 β leading to membrane depolarization.

The question of whether PKC activation and Ca²⁺ influx were sufficient to produce depolarization was studied by exposing the cells to PMA and Ca²⁺ iono-

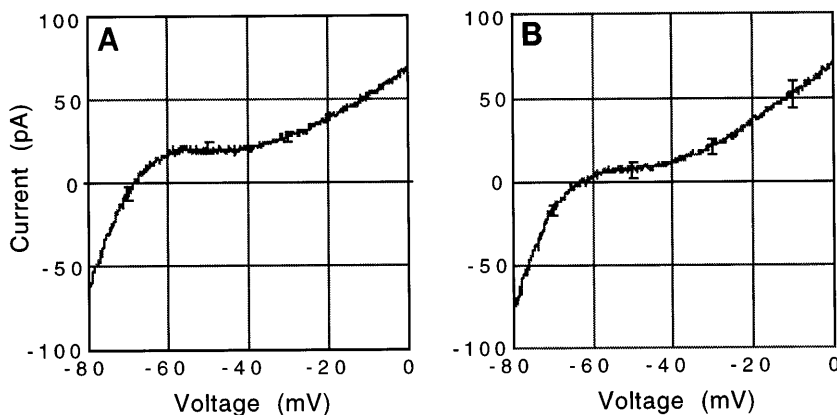


Fig. 6. Effect of IL-1 β (1 ng/ml) on I - V characteristics (means \pm SE of normalized curves) of 5 different synovial fibroblasts measured in different aggregates in presence of verapamil (10 μ M). *A*: I - V curves in bath solution. *B*: I - V curves after 15 min of voltage clamp at -30 mV and verapamil treatment followed by exposure to IL-1 β for 5 min.

phore in the absence of IL-1 β . Neither agent alone affected the V_m or I - V characteristics of the cells. Thus neither activation of PKC alone nor Ca^{2+} influx alone was sufficient to mimic the effect of IL-1 β on V_m . Jointly, however, the agents produced a membrane depolarization that was indistinguishable from the effect of IL-1 β (Fig. 4).

It could be argued that PKC in the synovial cells was self-activating and that the basic role of IL-1 β was to increase Ca^{2+} influx, which then permitted the already activated PKC to catalyze processes that led to depolarization. However, this view is inconsistent with the observation that Ca^{2+} ionophore alone did not mimic the effect of IL-1 β (Table 1). A similar argument in favor of an effect of IL-1 β on PKC activation with spontaneously open Ca^{2+} channels is inconsistent with the evidence that PMA alone did not mimic the effect of IL-1 β (Table 1). It can be concluded, therefore, that the PKC was not self-activating and that Ca^{2+} channels were not spontaneously open but rather that both processes were activated by IL-1 β .

Changes in Ca^{2+} influx and in intracellular Ca^{2+} have previously been implicated in the regulation of protease production by synovial cells (10, 22), but a clear picture of the role of PKC in the IL-1 signaling pathway in synovial cells has not emerged from previous PKC inhibitor studies. IL-1-induced expression of proteases (3), protease inhibitor (6), IL-1 (24), and arachidonic acid (4) was suppressed by PKC inhibitors. In other studies, however, different effects were seen. The inhibitors were associated with increased expression of PGE₂ (20), a biphasic effect on IL-8 production (12), and no effect on proteases (11). One possible explanation for the range of observed responses is the occurrence of nonspecific reactions induced by the various PKC inhibitors. For example, IL-1 can induce expression of PGE₂ (5), which is an agonist of cAMP (15); cAMP can downregulate stromelysin transcription (13). Thus the observation that staurosporine antagonized stromelysin production (3) could possibly have been due to a PKC-independent mechanism (up-regulation by staurosporine of IL-1-induced PGE₂, leading to increased levels of cAMP that inhibited stromelysin). The point is that inhibitor analysis is best in relatively simple cases in which the system has few different intermediate states in its signaling pathway. It is logically difficult to identify pathways when observations are made many hours after addition of the inhibitor because alternative and indirect signaling pathways potentially could increase the probability of an unspecified interaction with the inhibitor. This consideration underscores the importance of electrophysiological measurements in the study of IL-1 transduction because the short time scale of the measurements tends to exclude long-term processes, thereby making inhibitor analysis a more probative instrument.

IL-1 is a potent inducer of metalloproteinases by synovial cells, and this process probably plays an important role in the pathophysiology of joints (5, 8, 17,

19). Attempts at therapy based on reducing the levels of metalloproteinases in diseased joints do not appear to be successful (17), suggesting that blocking the disease earlier in its development might be a more effective strategy. This consideration provided the impetus for our study of the early electrical events associated with binding of IL-1 β by synovial cells.

We conclude that the depolarization that occurs as an early consequence of transduction of IL-1 β by voltage-clamped HIG-82 cells is caused by activation of PKC and Ca^{2+} influx. The role of the IL-1 β -induced depolarization in regulating protein expression by the cells remains to be determined, but the results reported here, taken together with observations that activation of PKC and Ca^{2+} influx also mediate the effect of IL-1 β on protease production (1, 2), suggest that electrophysiological changes are actually part of the pathway for expression of proteases in response to IL-1 β .

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