Excitation Wave Propagation through Gap-Junction Channels between Synovial Cells

Oleg V. Kolomytkin[1], Ph.D., Andrew A. Marino[1], Ph.D., Dmitry Kolomytkin[2]

[1]Department of Orthopaedic Surgery, LSU Health Sciences Center, Shreveport, LA; [2]Moscow State University, Moscow, Russia

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Introduction

Cartilage degradation by matrix metalloproteinases (MMPs) is a major factor in the progression of osteoarthritis. We previously found that synovial cells of patients with osteoarthritis contained an increased concentration of gap-junction channels made of connexin 43, and that the channels were needed to maintain the interleukin-1β (IL-1β) signaling cascade that leads to increased MMP production. In the present study we sought evidence that the amplification mechanism by which pg/ml levels of IL-1β controlled MMP secretion involved propagation of a depolarization wave through the gap junctions in aggregates of synovial cells, resulting in the opening of membrane calcium channels, a known critical process in IL-1β signal transduction by synovial cells. Our specific aims were to experimentally detect the depolarization wave, and to provide a theoretical explanation of the wave propagation.

Materials and Methods

The nystatin double-patch method was used to study depolarization wave propagation in aggregates of ~20 HIG-82 cells (nontransformed rabbit synovial fibroblasts) grown in petri-dishes (Fig. 4). Two electrodes were connected to different cells in the aggregate, and cell resting potentials at zero current and ionic currents through the cell membranes at clamped voltages were measured using Axopatch 200B amplifiers. The nystatin permitted use of the whole-cell configuration while preventing diffusion of small signaling molecules from the cell into the electrode, thereby preserving intracellular regulation. Each measurement was done 5 times and the results were presented as mean ± SE.

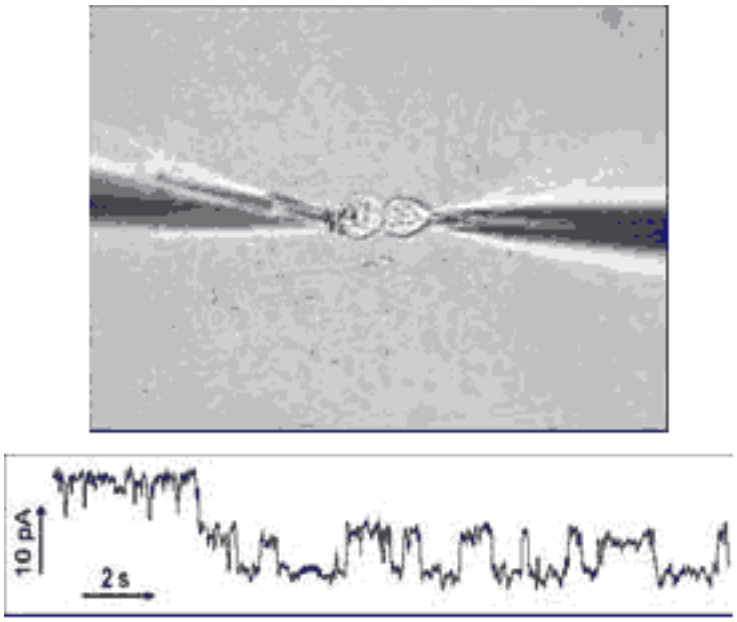

Fig. 1. Single gap junction channel recording between HIG-82 cells. Two electrodes were connected to two aggregated cells by the nystatin perforated-patch method. The current was measured at clamped 100 mV. Conductance of a single gap junction channel was 70±10 pS. Western blot and immunohistochemical analyses found that gap-junction channels between human and rabbit synovial cells were composed from connexin43.

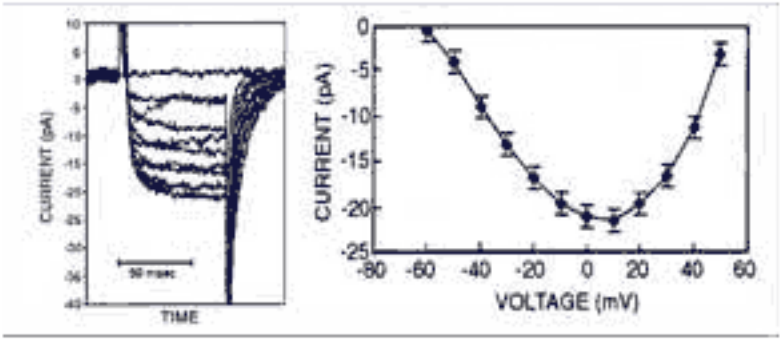

Fig. 2. Integral current-voltage curve for steady state currents through Ca^{2+}-channels in HIG-82 cells.

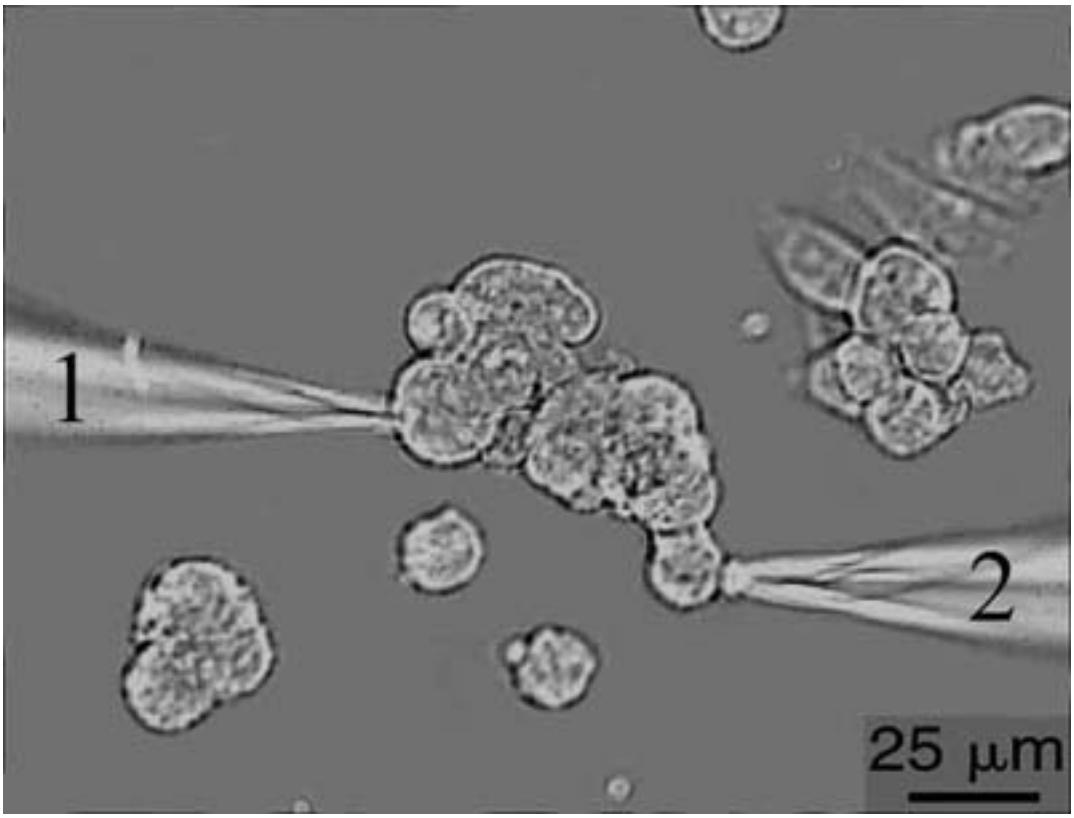

Fig. 4. Nystatin double-patch of an aggregate of HIG-82 cells. Electrode #1 (left) was used to clamp membrane voltage of cell #1 at −30 mV for 5 minutes while adding IL-1β. Electrode #2 (right) measured resting potential of cell #2.

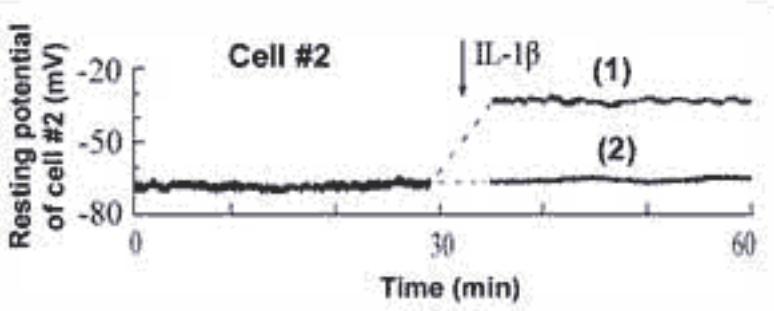

Fig. 5. Resting potential of cell #2 versus time in the aggregate shown in Fig. 4. Initially, both cells #1 and #2 had high resting potential –67 mV.
(1) Cell #1 was temporally pre-exposed to −30 mV and 100 pg/ml IL-1β was added to the bath solution. In 7±3 minutes, resting potential of cell #2 dropped to −33±10 mV.
(2) IL-1β was added without pre-exposition to low voltage.

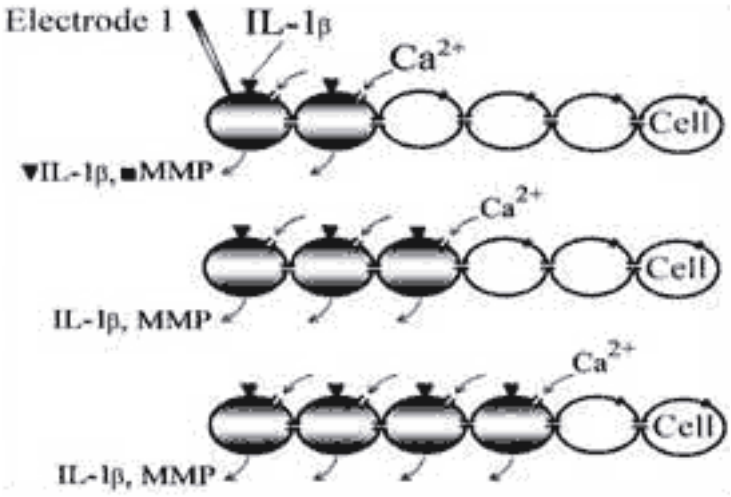

Fig. 6. Depolarization/Ca^{2+} wave propagation through gap-junction channels between synovial cells. Cells with high (–67 mV) and low (–33 mV) resting potential are shown by white and dark colors respectively. Opened and closed calcium and gap junction channels are shown.

Results

We proved the existence of gap-junction channels between neighbor HIG-82 cells by direct measurements of the channel (Fig. 1); the channel current was blocked by the gap-junction inhibitors 18alpha-glycyrrhetinic acid and octanol.

The voltage dependence of membrane L-type calcium channels was studied by measuring integral current with 10 mM Ba^{2+} in the bath solution as current carrier. Ca^{2+} channels were maximum opened at about zero membrane voltage, and closed at more negative voltages than –60 mV, and about half opened at –30 mV (Fig. 2).

Experiments using one electrode connected to one cell showed that the cells had a resting potential of −67±6 mV. Addition of 100 pg/ml IL-1β alone did not change the resting potentials. However, addition of the cytokine in the presence of a voltage clamp at −30 mV induced a permanent decrease in the resting potential of the patched cell to −33±10 mV. Experiments with Ca^{2+} channel blockers showed that pre-exposure of the cell to −30 mV was needed to open calcium channels, thereby increasing Ca^{2+} concentration in the cell.

Experiments with two electrodes attached to different cells in an aggregate (Fig. 4) revealed the following phenomenon. Pre-exposure to −30 mV of only one cell (#1) in an aggregate and subsequent application of IL-1β to the bath solution induced permanent depolarization of other cell (#2) in the aggregate, even though none of the other cells were pre-exposed directly to −30 mV (Fig. 5), indicating that the low voltage applied to one cell spread to other cells in the aggregate through gap-junction channels.

Control experiments showed that the clamped voltage (−30 mV) could propagate through gap junction channels without IL-1β only for one–two cells, which was significantly shorter in comparison with the distance between cells in the aggregate (Fig. 4). Therefore, passive propagation of low voltage cannot explain depolarization of cell #2 in Fig. 5. Only the special active wave initiated by cell #1 can explain depolarization of cell #2 in Figs. 4, 5.

We developed a mathematical model of the depolarization/Ca^{2+} excitation wave propagation in monolayer of synovial cells taking into account paracrine activation of the cells by IL-1β (Fig. 6). The equations predicted the observed rate of spread of the depolarization wave.

Discussion

The experimental evidence indicated that a −30 mV voltage clamp of a cell on one edge of the aggregate spread passively to adjacent cells through gap junction channels, thereby opening Ca^{2+} channels and resulting in increased Ca^{2+} concentration in the adjacent cells. Following addition of IL-1β, the signaling cascade worked synergistically with the elevated Ca^{2+} levels causing a decrease of resting potential in these cells which spread through gap junctions to previously unaffected cells (Fig. 6).

The obtained results demonstrated important principle that diffusion of signaling molecules like cytokines is not a simple passive process involving delivering the molecules to their receptors. In conjunction with nonlinear systems like gap junctions and other ion channels, diffusion can result in active signal propagation over relatively long distances. Such effects can play an important role in regulation in the organism, and in the development of disease such as osteoarthritis.

Conclusions

- **Gap-junction channels connected almost all synovial cell to each other in the aggregate**
- **Ca^{2+} channels were present in a cytoplasm membrane of synovial cells. The channels were opened at low (–30 mV) and completely closed at high voltage (–67 mV).**
- **Synovial cells had high –67 mV resting potential in norm. It was needed to open Ca^{2+} channels by applying –30 mV in order to provide IL–1ß signaling cascade with Ca^{2+} ions.**
- **Il-1ß/Ca^{2+} signaling cascade switched on MMPs synthesis and secretion.**
- **Il-1ß/Ca^{2+} signaling cascade self maintained ow resting potential at –33 mV.**
- **Applying IL-1ß and –30 mV to one of aggregated synovial cells initiated depolarization and Ca^{2+} wave propagation through gap-junction channels between synovial cells.**
- **The wave provides a great amplification of MMP production and can be one of important causes of osteoarthritis.**