Mechanistic Basis of Osteoarthritis

Andrew A. Marino, Ph.D.
Oleg Kolomytkin, Ph.D., D.Sc.

Department of Orthopaedic Surgery
LSUHSC - Shreveport
Summary

• Arthritis is …
• Gap junctions are …
• Approach
• Results
• Evaluation
Generic Synovial Joint

- Cartilage
- Synovial fluid
- Synovium
Organization of Synovium

- Synovial Lining Cells
- No Basement Membrane
- Sub-synovial Tissue:
  - Fat
  - Loose connective tissue
  - Dense connective tissue
Hypothetical Role of Membrane Potential ($V_m$) in Regulation of Synovial Cells

- IL-1β
- TNFα

$\Delta V_m$

- Proteases
- Protease Inhibitors

- Proteases
- Protease Inhibitors
Why $V_m$?

1. Analogy with Excitable Cells

- Input
- Input
- Input

Dendritic Tree

- $\sum$

Output
Why $V_m$?

2. Fractal Dimension

![Graph showing membrane potential fluctuations over time.]
Nystatin Patch-Clamp
Initial Measurements
Cell Membrane Potential

- Aggregated HIG-82 cells (rabbit synovial cells)

- In isolated cells, Membrane Potential $\sim 0$
Effect of Cytokines on Current-Voltage Curves

- **IL-1β (1 ng/ml)**
  - Initial
  - 10 minutes after addition of cytokine

- **TNFα (1 nM)**
  - Initial
  - 10 minutes after addition of cytokine
## Mechanism of IL-1β Effect

<table>
<thead>
<tr>
<th>Substances</th>
<th>Reversal Potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ng/ml IL-1β</td>
<td>-69 ± 2.0</td>
</tr>
<tr>
<td>1.5 µM PMA</td>
<td>-66 ± 2.3</td>
</tr>
<tr>
<td>1 ng/ml IL-1β and 1.5 µM PMA</td>
<td>-65 ± 2.4</td>
</tr>
<tr>
<td>3 µM BIS</td>
<td>-70 ± 1.0</td>
</tr>
<tr>
<td>1 ng/ml IL-1β and 3 µM BIS</td>
<td>-69 ± 1.0</td>
</tr>
<tr>
<td>10 µM Ca²⁺ ionophore</td>
<td>-67 ± 1.6</td>
</tr>
<tr>
<td>0.25 µM PMA and 5 µM Ca²⁺ ionophore</td>
<td>-70 ± 1.3</td>
</tr>
<tr>
<td>50 µM nifedipine</td>
<td>-68.5 ± 1.6</td>
</tr>
<tr>
<td>10 µM verapamil</td>
<td>-65 ± 4.8</td>
</tr>
<tr>
<td>1 ng/ml IL-1β and 50 µM nifedipine</td>
<td>-68 ± 1.6</td>
</tr>
<tr>
<td>1 ng/ml IL-1β and 10 µM verapamil</td>
<td>-68 ± 1.3</td>
</tr>
</tbody>
</table>

*P < 0.05

Activation of PKC and Ca²⁺ Influx necessary and sufficient

Discovery of Gap Junctions

Current Through Gap Junctions

Circuit for Measurement of Gap Junctions

Current

\[ V_{\text{gap}} \]

\[ V = 0 \]
Organization of Gap Junctions
## Mammalian Connexins

<table>
<thead>
<tr>
<th>CONNEXIN</th>
<th>TISSUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cx26</td>
<td>Liver, Pancreas, Endometrium</td>
</tr>
<tr>
<td>Cx30</td>
<td>Brain, Skin</td>
</tr>
<tr>
<td>Cx30.3</td>
<td>Skin</td>
</tr>
<tr>
<td>Cx31</td>
<td>Skin, Placenta</td>
</tr>
<tr>
<td>Cx31.1</td>
<td>Skin</td>
</tr>
<tr>
<td>Cx32</td>
<td>Liver, Kidney, Pancreas</td>
</tr>
<tr>
<td>Cx33</td>
<td>Testes</td>
</tr>
<tr>
<td>Cx37</td>
<td>Endothelium, Lung, Ovary</td>
</tr>
<tr>
<td>Cx40</td>
<td>Endothelium, Smooth muscle, Myocardium, Lung</td>
</tr>
<tr>
<td>Cx43</td>
<td>Epithelium, Heart, Uterus, Connective tissue, Brain</td>
</tr>
<tr>
<td>Cx45</td>
<td>Kidney, Skin</td>
</tr>
<tr>
<td>Cx46</td>
<td>Lens</td>
</tr>
<tr>
<td>Cx50</td>
<td>Lens</td>
</tr>
</tbody>
</table>
**Connexon Complexity**

- **Connexins**
- **Connexon**
  - Cell No. 1
  - Cell No. 2

12 Subunits x 13 Proteins
= $10^{10}$ combinations
or  = $10^{50}$ permutations
Relation between the Time Constant for Current Decay in HIG-82 Cells and Aggregate Size in Culture
Transient Current Response of a Synovial Lining Cell in Tissue
Western Blot Analysis

RAT Cx43  HUMAN CELLS

125 - 78 - 44 - 31.9 - 17.5 -
Immunohistochemical Detection in Cultured Human Synovial Cells

Connexin43
Immunohistochemical Detection in Cryosections of Human Synovial Tissue

Connexin43
Dye Transfer Through Gap Junctions
Gap Junctions in Human Synovium and between HIG-82 Cells

Normal Synovium

HIG-82 Cells

OA Synovium

RA Synovium
# Gap-Junction Plaques in Normal and Arthritic Human Synovia

<table>
<thead>
<tr>
<th>SPECIMEN No.</th>
<th>DIAGNOSIS</th>
<th>No. PLAQUES per 100 CELLS</th>
<th>MEAN ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>1.43</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>**</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>**</td>
<td>1.11</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>**</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>**</td>
<td>0.62</td>
<td>0.71 ± 0.57</td>
</tr>
<tr>
<td>1</td>
<td>Osteoarthritis</td>
<td>3.72</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>**</td>
<td>1.26</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>**</td>
<td>3.61</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>**</td>
<td>3.65</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>**</td>
<td>7.52</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>**</td>
<td>6.35</td>
<td>* 4.35 ± 2.24</td>
</tr>
<tr>
<td>1</td>
<td>Rheumatoid Arthritis</td>
<td>3.98</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>**</td>
<td>2.04</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>**</td>
<td>9.56</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>**</td>
<td>1.30</td>
<td>* 4.22 ± 3.74</td>
</tr>
</tbody>
</table>

* P < 0.05
Metalloproteinase Assay

Collagen Solution → Change pH → Collagen Film → Stained Film

Addition of MMPs → Degraded Film → OD Measurement Of Solubilized Film → Collagen Concentration
Protease Activity of IL-1β Treated Synovial Cells

![Graph showing the relationship between reaction time (hours) and collagen degraded (mg/m²). The data points are plotted with error bars, indicating variability. The graph shows a linear increase in collagen degradation over time.](image-url)
Effect of IL-1β on Protease Activity

MMP Activity (mg/hr·m²)

Incubation Time (hours)
Effect of 18α-glycyrrhetinic acid (GR) on Protease Production by Synovial Cells in the Presence of IL-1β

MMP Activity (mg/hr·m²)

Incubation Time (hours)

- Control
- 1 µM GR
- 5 µM GR
- 10 µM GR
Effect of Octanol on Protease Production by Synovial Cells in the Presence of IL-1β

MMP Activity (mg/hr·m²)

Incubation Time (hours)
Effect of Brief Application of Gap Junction Inhibitors on Protease Production

**Graph:**
- **MMP Activity (mg/hr·m²)**
- **Incubation Time (hours)**
- Lines represent:
  - IL-1β
  - IL-1β + Octanol
  - IL-1β + GR

The graph shows a significant increase in MMP activity over time for all conditions, with IL-1β + GR showing the least activity compared to the other conditions.
Effect of Gap Junction Inhibitors on Gap Junction Intercellular Communication

18α-GA

Current (pA)

Time (ms)

Octanol

Control

10 minutes after application of inhibitor

10 minutes after removal of inhibitor
(70 minutes after initial application)
Summary (Know)

IL-1 → PKC↑ → Ca++↑ → ΔV_m

Rabbit Cells
Rabbit Cells
Human Cells
Rabbit Cells
Tissue
Human Cells
Synovial Tissue

N_{ARTHRITIS} > N_{NORMAL}

Cx43

IL-1 → GJIC → Protease Production

Rabbit Cells
Summary (Think)

IL-1 → Δ $V_m$ → GJIC → Protease Production

(Sub-type of Na$^+$ Channel) (PKC Ca$^{2+}$)
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