Functions of smooth muscle

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Objectives

- สิ่งที่ต้องการทำให้กล้ามเนื้อเรียบหดตัว
- สิ่งที่มีผลต่อการหดตัวของกล้ามเนื้อเรียบได้
  - extracellular Ca^{2+}
  - acetylcholine
  - adrenaline
  - อุณหภูมิ
  - Membrane depolarization with KCl and BaCl_2
Smooth muscle

Smooth Muscle
Contraction: Mechanism

1. Intracellular Ca²⁺ concentrations increase when Ca²⁺ enters cell and is released from sarcoplasmic reticulum.
2. Ca²⁺ binds to calmodulin (CaM).
4. MLCK phosphorylates light chains in myosin heads and increases myosin ATPase activity.
5. Active myosin crossbridges slide along actin and create muscle tension.
**Smooth Muscle Relaxation: Mechanism**

1. Free Ca²⁺ in cytosol decreases when Ca²⁺ is pumped out of the cell or back into the sarcoplasmic reticulum.
2. Ca²⁺ unbinds from calmodulin (CaM).
3. Myosin phosphatase removes phosphate from myosin, which decreases myosin ATPase activity.
4. Less myosin ATPase results in decreased muscle tension.

**Smooth Muscle Contraction**

1. cAMP (Relaxes Smooth Muscle)
2. cAMP-MLCK-MLCK-P (Contracts Smooth Muscle)
3. Ca²⁺ + Calmodulin (MLCK activation)
4. Ca²⁺-Calmodulin-MLCK activates
5. Actin + Myosin (Relaxed)
6. ATP
7. Cross Bridge Cycling
8. Actin-Myosin-LC₇-ADP-P
9. ADP + P_i
10. Power Stroke
11. Head Detachment
12. Re cock Head 90°
13. ATP
14. Myosin Light Chain Phosphatase
15. cAMP-MLCK-MLCK-P
16. Ca²⁺-Calmodulin-Inhibitory
17. Inactive MLCK
18. (Contracts Smooth Muscle)
Excitation-contraction coupling

Increase $[\text{Ca}^{2+}]_i$ can be accomplished by two methods

- Membrane depolarization
  - It is the major mode of stimulation.
  - Opening of voltage-gated $\text{Ca}^{2+}$ channels (L-type) and increase $\text{Ca}^{2+}$ influx

- Receptor-mediated $[\text{Ca}^{2+}]_i$ increase
  - This is a mechanism by which hormones, mediators, neurotransmitters and drugs act on smooth muscle.
  - Increase release from intracellular store

Control of intracellular $\text{Ca}^{2+}$

[Diagram showing the control of intracellular $\text{Ca}^{2+}$]
Duration of smooth muscle contraction

Slow wave potential

- Membrane potential of unitary smooth muscle consistently oscillates; *Slow wave potential*
- The depolarization by slow wave increase intracellular Ca\(^{2+}\) and causes constant and stable low level of contraction ( *Tonic contraction or tone* )
**Action potential**

- If the threshold is reached, there will be action potential (spike potential) on top of slow wave.
- This is due to the opening of voltage-gated Ca\(^{2+}\) channels (L-type).
- These action potential can propagate to other cells via gap junction.

**Smooth muscle contraction**

1. Slow wave potential
   - Determines the smooth muscle tone
   - Frequency of slow wave determines the frequency of contraction
2. Spike potential
   - Number of spikes indicates the amplitude of contraction

Action potentials and twitches can be superimposed on rhythmic activity.
Pacemaker

- The slow wave potential in smooth muscle is initiated in Pacemaker cell called interstitial cell of Cajal (ICC).
- The ICC are electrically coupled and also electrically coupled with smooth muscle via gap junction.

![Propagation of waves in ICC network](image)

Slow wave
Interstitial cell of Cajal

- ICC networks in pacemaker regions express the ionic mechanism to generate slow waves.
- No single ICC serves as a fixed, dominant pacemaker.
- Slow wave propagates at rate 5 mm/s
- Slow wave propagation in ICC network is regenerative
- Slow waves electrotonically conduct into smooth muscle cells, which are electrically coupled to the interstitial cells of Cajal (ICC).
Mechanism of pacemaker activity

- Nonselective cationic channels (NSCC)
  - Ca\(^{2+}\)-inhibit NSCC

  \[ \text{Low Ca}^{2+} \rightarrow \text{NSCC activation} \]
  \[ \text{Reduce Ca}^{2+}\text{ influx} \rightarrow \text{NSCC inhibition} \]
  \[ \text{Mitochondrial Ca}^{2+} \text{ Uptake} \]
  \[ \text{Localized release of Ca}^{2+}\text{ from IP3 receptors} \]

Enteric nervous system

- Neurotransmitters released from ENS can condition the electrical activity at both the interstitial cell of Cajal and the smooth muscle.
  - Release of excitatory transmitters activates nonselective cation channels and increases the effectiveness of slow waves to bring the muscle cells to threshold.
  - Release of inhibitory transmitters activates potassium channels, which decreases the probability of reaching threshold.
- Also, neurotransmitters can modulate contraction of smooth muscle
External modulators

- Autonomic nervous system and endocrine system can modify electrical activity in both ICC and smooth muscle and also affect smooth muscle contraction.

Specimen

- Frog’s pyloric ring
**Experimental setup**

- Monitor
- Force transducer
- Tissue chamber
- Smooth muscle
- Aquarium pump
- PowerLab

**Experiment**

- Characteristic of smooth muscle contraction
- Effects of
  - Extracellular Ca²⁺
    - 0.5% CaCl₂
    - Ca²⁺-free solution
  - Acetylcholine
  - Adrenaline
  - Cold
  - 0.5% KCl
  - 0.5% BaCl₂

Control: 5 minutes  Experiment: 5 minutes  Wash twice
Results

Chart Window

Frequency = contraction/minute

amplitude

report

Report

<table>
<thead>
<tr>
<th>Control 1</th>
<th>Experiment</th>
<th>Control 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude (mV)</td>
<td>Frequency (min)</td>
<td>Amplitude (mV)</td>
</tr>
<tr>
<td>0.5%CaCl₂</td>
<td></td>
<td></td>
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<tr>
<td>Ca²⁺ free (0 Ca²⁺)</td>
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</tr>
<tr>
<td>Adrenaline</td>
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</tr>
<tr>
<td>NaCl</td>
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<td></td>
</tr>
<tr>
<td>0.5%KCl</td>
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</tbody>
</table>
**Discussion**

**Effect of extracellular Ca\(^{2+}\):**
- **increase Ca\(^{2+}\)**

- **Effect on ICC**
  - The depolarization needs Ca\(^{2+}\) influx
  - increase Ca\(^{2+}\) ➔ increase slow wave amplitude

- **Effect on smooth muscle cell**
  - more Ca\(^{2+}\) entry when Ca\(^{2+}\) channels are opened

- **Effect on smooth muscle contraction**
  - Increase amplitude and tone
  - may increase frequency
Effect of extracellular Ca\(^{2+}\): increase Ca\(^{2+}\)

**Chart Window**

Effect of extracellular Ca\(^{2+}\): low Ca\(^{2+}\)

- Effect on ICC
  - Low Ca\(^{2+}\) ⇒ slow and small slow wave
- Effect on smooth muscle cell
  - Less Ca\(^{2+}\) entry
- Effect on smooth muscle contraction
  - Lower amplitude and tone
  - Lower frequency?
**Effect of extracellular Ca\(^{2+}\): low Ca\(^{2+}\)**

![Graph showing the effect of extracellular Ca\(^{2+}\): low Ca\(^{2+}\)]

**Effect of acetylcholine**

- **Effect of ACh on ICC**
  - Activation of non selective cationic conductance (M3: mediated by PKC)
  - Increase amplitude and frequency of slow wave
- **Effect of ACh on smooth muscle cell**
  - GI smooth muscle expresses M2 and M3 muscarinic cholinergic receptors
  - Increase intracellular Ca\(^{2+}\) (M3: mediated by IP\(_3\))
  - Reduce inactivation of MLCK (M2: decrease cAMP)
- **Effect of ACh on smooth muscle contraction**
  - Increase frequency, amplitude and tone
Effect of acetylcholine on ICC

Exterior

Interior

Voltage-gated Ca\(^{2+}\)-channel

NSCC

Na\(^+\)

ACh

M3

PLC

DAG

IP3

Sarcoplasmic reticulum

Protein kinase C

Effect of acetylcholine on smooth muscle cell

Exterior

Interior

Voltage-gated Ca\(^{2+}\)-channel

ACh

M3

PLC

DAG

IP3

Sarcoplasmic reticulum

Protein kinase C

Acetylcholine release

Calmodulin

MLCK

Sarcoplasmic reticulum

Smooth muscle contraction
**Effect of acetylcholine**

- Adrenergic receptors are expressed at cholinergic terminal
- Adrenaline inhibits acetylcholine release

**Effect of adrenaline**

- **Effect of adrenaline on ICC**
  - ICC do not express adrenergic receptor
  - Adrenergic receptors are expressed at cholinergic terminal
  - Adrenaline inhibits acetylcholine release

- **Effect of adrenaline on smooth muscle cell**
  - Smooth muscle cells express β-adrenergic receptors (increase cAMP)
  - Decrease intracellular Ca\(^{2+}\) (increase SR uptake)
  - Membrane hyperpolarization (increase K\(^{+}\) conductance)
  - Inactivate MLCK

- **Effect of adrenaline on smooth muscle contraction**
  - Decrease frequency, amplitude and tone
**Effect of adrenaline: smooth muscle cell**

Exterior

Voltage-gated Ca\(^{2+}\)-channel

Interior

β2

Adenylase cyclase

+ cAMP

Sarcoplasmic reticulum

Calmodulin

MLCK

 uptake

Smooth muscle contraction

**Effect of adrenaline**

Chart Window
Effect of cold

- Effect of cold on ICC and smooth muscle cell
  - Decrease cellular activity
  - Decrease ATP production
  - Decrease Na·K pump activity
  - Decrease Ca^{2+} pump activity

- Effect of cold on smooth muscle contraction
  - Decrease contraction
  - Initially, tone may increase due to residual ATP and Ca^{2+}
Effect of extracellular K⁺

- Effect of K⁺ on ICC and smooth muscle cell
  - membrane depolarization increase slow wave and action potential
  - increase intracellular Ca²⁺

- Effect of K⁺ on smooth muscle contraction
  - Increase amplitude, tone and frequency

![Chart Window](chart.png)
Effect of extracellular Ba$^{2+}$

- Effect of Ba$^{2+}$ on ICC and smooth muscle cell
  - Ba$^{2+}$ blocks some K$^+$ channels (K$_v$)
    - Membrane depolarization
    - Increase intracellular Ca$^{2+}$
  - Ba$^{2+}$ enters Ca$^{2+}$ channels and acts as Ca$^{2+}$

- Effect of Ba$^{2+}$ on smooth muscle contraction
  - Increase amplitude, tone and frequency
Conclusion

- Smooth muscle can contract without nerve supply.
- The contraction is initiated by intrinsic pacemaker.
- The contraction is slow (duration over 200 ms).
- Extracellular Ca\(^{2+}\) is important for the contraction. (Ca\(^{2+}\) free solution)
- Neurotransmitters modulate smooth muscle contraction.