Research Updates in Periodic Paralysis

Steve Cannon, M.D., Ph.D.
Department of Physiology
HYPOKALÆMIC PERIODIC PARALYSIS
STUDIED IN VITRO

BY

W. W. HOFMANN AND R. A. SMITH

(From the Department of Neurology, Veterans Administration Hospital, and Division of Neurology, Stanford University School of Medicine, Palo Alto, California)

The second, G. W., was a 28-year-old man who was well until age 15, when he began to have periodic episodes of generalized muscular weakness, usually most severe on awakening in the morning. Distal muscles of all four extremities were flaccid and totally paralysed in some attacks, the longest of which was sixteen hours. No difficulty was ever experienced in swallowing or breathing, but during a severe attack the patient could not lift his head. Low serum potassium
Familial hypokalemic periodic paralysis
Clinical, diagnostic and therapeutic aspects

Thera P. Links a,*, Andries J. Smit b, Willemina M. Molenaar c, Machiel J. Zwarts d and Hans J.G.H. Oosterhuis d

Departments of a Endocrinology, b Internal Medicine, c Pathology, and d Neurology, University Hospital Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands

Dutch family of 120 individuals, 64 with HypoPP of which 38 had symptomatic attacks of acute weakness

Subsequently found to have CACNA1S R528H mutation
Characterization of hyperkalemic periodic paralysis: a survey of genetically diagnosed individuals

G. Charles · C. Zheng · F. Lehmann-Horn · K. Jurkat-Rott · J. Levitt
• **What’s wrong with my muscles during an attack of periodic paralysis?**

• Leaky channels in HypoKPP: insights on disease mechanism and a possible opportunity to develop new drugs

• Mouse models of periodic paralysis; what are we learning?
Channelopathies of Skeletal Muscle

Myotonia

Periodic Paralysis

asymptomatic

weakness / paralysis

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Channelopathies of Skeletal Muscle

Myotonia

Periodic Paralysis

- asymptomatic
- weakness / paralysis

Voltage (millivolts)

Time (milliseconds)
Channelopathies of Skeletal Muscle

Myotonia
- Myotonia Congenita
- Sodium Channel Myotonia

Paramyotonia Congenita

Hyperkalemic Periodic Paralysis

Hypokalemic Periodic Paralysis
- Andersen-Tawil Syndrome
- Thyrotoxic Periodic Paralysis

Sodium Channel
- Sodium Channel

Potassium Channel

Chloride Channel
Channelopathies of Skeletal Muscle

**Myotonia**
- Myotonia Congenita
  - Chloride Channel
    - CIC-1
    - CLCN1
  - Sodium Channel
    - Myotonia Congenita
    - SCN4A
  - Calcium Channel
    - Kir2.6 (Kir2.1, Kir3.4 (?))

**Periodic Paralysis**
- Hyperkalemic Periodic Paralysis
  - Sodium Channel
    - Na\textsubscript{v}1.4
  - Calcium Channel
    - Ca\textsubscript{v}1.1
  - Potassium Channel
    - KCNJ2, KCNJ5
- Hypokalemic Periodic Paralysis
  - Sodium Channel
    - Na\textsubscript{v}1.4
  - Potassium Channel
    - Kir2.6
- Andersen-Tawil Syndrome
  - Sodium Channel
    - Na\textsubscript{v}1.4
  - Calcium Channel
    - Ca\textsubscript{v}1.1
  - Potassium Channel
    - KCNJ18
• What’s wrong with my muscles during an attack of periodic paralysis?

• **Leaky channels in HypoKPP: insights on disease mechanism and a possible opportunity to develop new drugs**

• Mouse models of periodic paralysis; what are we learning?
Leaky Channels in Hypokalemic Periodic Paralysis

All 11 HypoPP mutations of the sodium channel are substitutions that replace an arginine “R”

8 of 9 HypoPP mutations of the calcium channel are substitutions that replace an arginine “R”
Leaky Channels in Hypokalemic Periodic Paralysis

HypoPP Mutations in Na\textsubscript{v}1.4
- R1135H/C
- R1132Q
- R222G/W
- R669H/G
- R672H/G/S/C

HypoPP Mutations in Ca\textsubscript{v}1.1
- R528H/G/C
- R897S
- V876E
- R1239H/G

Frog eggs injected with messenger RNA for Na\textsubscript{v}1.4
Measure currents conducted by HypoPP mutant channels
HypoPP Mutations in Na\(_v\)1.4

- R1135H/C
- R1132Q
- R222G/W
- R669H/G
- R672H/G/S/C

HypoPP Mutations in Ca\(_v\)1.1

- R528H/G/C
- R900S/G
- R897S
- V876E
- R1239H/G

Leaky Channels in Hypokalemic Periodic Paralysis
All 11 HypoPP mutations of the sodium channel are substitutions that replace an arginine “R”

All 11 HypoPP mutations have been studied and published. All 11 (100%) cause a “gating pore” leak

8 of 9 HypoPP mutations of the calcium channel are substitutions that replace an arginine “R”

7 of 9 HypoPP mutations have been studied. All 4 published had a “gating pore” leak, including the atypical V876E.
“Seeing the Leak” Atomic structure of the Sodium Channel

top view

side view

Na\(^+\) in the pore

“S4” helix

At risk for leak

Jiang et al. (Catterall Lab)  Nature May 16, 2018
**“Seeing the Leak” Atomic structure of the Sodium Channel**

**“HypoPP” R/G mutation Model for R672G**

- “Depolarized” to 0 mV (available data)
- “Resting” at -90 mV (model)

**Normal (WT)**

- “Resting” at -90 mV (model)

- Purple blob = water access

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Jiang et al. (Catterall Lab) Nature May 16, 2018
Spider toxin blocks the leak in a HypoKPP mutant sodium channel

Hm-3 toxin 10 μM

Spider toxin inhibits gating pore currents underlying periodic paralysis

Rooge Männikõ, Zakhar O. Shenkarev, Michael G. Thor, Antonina A. Berkut, Mikhail Yu Myshkin, Alexander S. Paramonov, Dmitrii S. Kukatski, Dmitry A. Kuzmin, Marisol Sampedo Castaneda, Louise King, Emma R. Wilson, Ekaterina N. Lyukmanova, Mikhail P. Kirpichnikov, Stephanie Schorge, Frank Bosmans, Michael G. Hanna, Dimitri M. Kullmann, and Alexander A. Vassilievski

PNAS 115:4495, 2018 April 24
Spider toxin blocks the leak in a HypoKPP mutant sodium channel

Hm-3 toxin
10 μM

Na⁺ current (α current) blocked by Hm-3

But… the toxin also reduces the normal Na⁺ current.
• What’s wrong with my muscles during an attack of periodic paralysis?

• Leaky channels in HypoKPP: insights on disease mechanism and a possible opportunity to develop new drugs

• **Mouse models of periodic paralysis; what are we learning?**
  • *first accurate measure of potassium sensitivity in periodic paralysis*
Mouse Models of Periodic Paralysis
Mouse Models of Hypokalemic Periodic Paralysis

**HypoPP Mutations in Na\_v1.4**
- R222G/W
- R669H/G
- R669H/S/C
- R1132Q
- R1135H/C

**N\_a\_v1.4 - R669H**

**HypoPP Mutations in Ca\_v1.1**
- R528H/G/C
- R897S
- R900S/G
- R1239H/G
- V876E

**A**
- (+/+)
- (+/m)
- (m/m)
Mouse Models of Hypokalemic Periodic Paralysis

**Na\textsubscript{v}1.4 - R669H**

**HypoPP Mutations in Na\textsubscript{v}1.4**
- R222G/W
- R689H/G
- R672H/G/S/C
- R1132Q
- R1135H/C

**HypoPP Mutations in Ca\textsubscript{v}1.1**
- R528H/G/C
- R897S
- R900S/G
- R1239H/G

**NH\textsubscript{3}{\textsuperscript{+}}**

**COO\textsuperscript{-}**

**Relative Force**

**Time (min)**

**[K\textsuperscript{+}] (mM)**

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• What’s wrong with my muscles during an attack of periodic paralysis?

• Leaky channels in HypoKPP: insights on disease mechanism and a possible opportunity to develop new drugs

• **Mouse models of periodic paralysis; what are we learning?**
  • first accurate measure of potassium sensitivity in periodic paralysis
  • **For HypoKPP, chloride is important too**
Why does the gating pore “leak” cause depolarization in low $K^+$?

Wildtype muscle will depolarize, if the $[K^+]$ is low enough.

Model of Channels and Pumps in Muscle

- Na/K-ATPase
- NKCC
- $2\text{K}^+$
- $3\text{Na}^+$
- $\text{K}^+$
- $\text{K}^+$
- $\text{Cl}^-$

$V_{rest}$, mV

$[K^+]_o$, mM
Why does the gating pore “leak” cause depolarization in low $K^+$?

Wildtype muscle will depolarize, if the $[K^+]$ is low enough.
Why does the gating pore “leak” cause depolarization in low $K^+$?

**Chloride Gradient Impacts Bistability of $V_{\text{rest}}$ in Muscle**

Wildtype muscle will depolarize, if the $[K^+]$ is low enough.

**Model of Channels and Pumps in Muscle**

- **Na/K-ATPase**
- **NKCC**
- **muscle fiber**
- **gating pore**
- **Kir**
- **$K_{\text{DR}}$**
- **CIC-1**
- HypoPP
  - $Na_v1.4$, $Ca_v1.1$

High $Cl_{\text{in}}$

Low $Cl_{\text{in}}$

WT

HypoPP

- $V_{\text{rest}}$, mV
- $[K^+]_o$, mM

WT

Gating pore leak
Why does the gating pore “leak” cause depolarization in low $K^+$?

Chloride Gradient Impacts Bistability of $V_{\text{rest}}$ in Muscle

Wildtype muscle will depolarize, if the $[K^+]$ is low enough

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Model of Channels and Pumps in Muscle

- Na/K-ATPase
- NKCC
- $2K^+$
- $3Na^+$
- $Na^+K^+2Cl^-$
- Muscle fiber
- Gating pore
- Kir, $K_{\text{DR}}$, CIC-1
- HypoPP $Na_v1.4, Ca_v1.1$
Inhibition of NKCC with bumetanide ameliorates attacks of HypoPP

**Na$_V$1.4 – R669H$^+/m$**

- **Control**
- **2 K$^+$**
- **2 K$^+$ + BMT

**Ca$_V$1.1 – R528H$^+/m$**

- **Control**
- **2 K$^+$**
- **2K$^+$ + BMT**
- **Wash**

(Wu et al. Neurology 2013; Wu et al. Brain 2013)
Why does the gating pore “leak” cause depolarization in low K⁺?

Chloride Gradient Impacts Bistability of V_{rest} in Muscle

Wildtype muscle will depolarize, if the [K⁺] is low enough

Model of Channels and Pumps in Muscle

- Na/K-ATPase
- NKCC
- Na⁺ K⁺ 2Cl⁻
- 2 K⁺
- 3 Na⁺
- [K⁺]o, mM
- muscle fiber
- K⁺
- K⁺
- Cl⁻
- gating pore
- Na⁺
- Kir
- K_{DR}
- CIC-1
- HypoPP
- Naᵥ1.4, Caᵥ1.1

Chloride Gradient Impacts

Bistability of V_{rest} in Muscle

Why does the gating pore “leak” cause depolarization in low K⁺?

Hypertonic (dehydrated)

Hypotonic (hydrated)

High Cl⁻_{in}

Low Cl⁻_{in}

1 2 3 4 5 6
-110
-100
-90
-80
-70
-60
V_{rest}, mV

WT

gating pore leak

HypoPP

1 2 3 4 5 6
-110
-100
-90
-80
-70
-60
V_{rest}, mV

WT

HypoPP

1 2 3 4 5 6
-110
-100
-90
-80
-70
-60
V_{rest}, mV

WT

HypoPP
State of hydration (osmolarity) affects susceptibility to HypoPP

“dehydrated”

(Wu et al. Brain 2013)
State of hydration (osmolarity) affects susceptibility to HypoPP

“dehydrated”

“super hydrated”

(Wu et al. Brain 2013)
What’s wrong with my muscles during an attack of periodic paralysis?

Leaky channels in HypoKPP: insights on disease mechanism and a possible opportunity to develop new drugs

Mouse models of periodic paralysis; what are we learning?
  - first accurate measure of potassium sensitivity in periodic paralysis
  - For HypoKPP, chloride is important too
  - acid/base balance may be a factor for exercise-induced attacks and for the beneficial effect of carbonic anhydrase inhibitors
Post-Exercise Reduction of Muscle Excitability in HypoKPP

Controls

R528H calcium

Exploring the Mechanism for Post-Exercise Attacks of Weakness in HypoPP

Clinical Features:

• Strength is preserved during exercise

• Weakness occurs several minutes after exercise

• Strenuous and Prolonged exercise (> 20 min) is necessary to trigger an attack

Hypothesis: Acidosis is an important contributor to exercise-associated weakness in HypoPP
Recovery from acidosis triggers weakness in HypoPP (but not HyperPP)

The graph shows the changes in pH (internal) and CO₂ % over time for different conditions.

- **pH 7.4** and **pH 6.8**

**Graph Details:**
- **Relative Force** vs **Time (min)**
- **CO₂ %** vs **Time (min)**
- **WT**, **NaV1.4-M1592V HyperPP**, **NaV1.4-R669H HypoPP**, **CaV1.1-R528H HypoPP**

(unpublished)
Post-acidosis loss of force is caused by a loss of excitability

Simultaneous measure of force and excitability (CMAP) Na\textsubscript{v}1.4 – R669H

Post-acidosis loss of force is caused by a loss of excitability
A Prolonged Exposure to acidosis is Required to Trigger an Attack

CaV1.1-R528H

$\tau = 33$ min

25% CO$_2$
Proposed Mechanism for Post-Exercise Weakness in HypoPP

**Muscle Fiber**

- **Resting State**: Normal Excitability
  - **Normal [Cl]_{in}**: Normal chloride conductance

**Strenuous Exercise**

- **Muscle Acidosis**: Decreased excitability / weakness
  - **↓ Conductance Chloride Channel**

**Stop Exercise**

- **Recover from Acidosis**
  - **↑ Conductance Chloride Channel**

**Susceptible to Depolarization**

- **↓ Excitability / weakness**

**High [Cl]_{in}**

- **↑ Chloride Muscle**
Will a slow recovery from acidosis reduce the loss of force?

Evidence of a “warm-down” protection

(unpublished)
Mechanistic Insights on Hypokalemic Periodic Paralysis

- Susceptibility to paradoxical depolarization in low K\(^+\) is caused by a “leak” in the voltage sensors of mutant calcium channels or sodium channels.

- Internal [Cl] is the “switch” that biases \(V_{\text{rest}}\) when bi-stability exists.
  - Low internal [Cl] promotes hyperpolarization \(\rightarrow\) normal excitability
  - High internal [Cl] promotes depolarization \(\rightarrow\) reduced excitability

- Post-exercise attacks of weakness may be caused by a transient rise of internal [Cl] resulting from reduced outflow (acidosis \(\rightarrow\) ↓ chloride conductance)
Therapeutic Implications for the Role of Cl in HypoPP

• Minimize activities that increase myoplasmic [Cl].
  • Avoid hyperosmolar states (dehydration, high salt diet, hyperglycemia) that stimulate the NKCC cotransporter.

• Precautions for vigorous exercise.
  • Stay well hydrated
  • Slow “warm down” after exercise. Slow recovery from acidosis → slower recovery of Cl conductance → time for internal Cl washout before full recovery of Cl conductance.

• Pharmacologic intervention for symptom management.
  • NKCC inhibitor for abortive or preventive therapy: bumetanide or furosemide in combination with K$^+$ repletion. (Proof of principle in mouse model. No human controlled trials yet).
  • Carbonic anhydrase inhibitors (acetazolamide, dichlorphenamide): chronic mild metabolic acidosis → maintain excitability from a partial ↓ chloride conductance ???
### Collaborators:

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dennis Burns</td>
<td>UT Southwestern</td>
<td></td>
</tr>
<tr>
<td>Ben Nelson</td>
<td>UT Southwestern</td>
<td></td>
</tr>
<tr>
<td>Eric Olson</td>
<td>UT Southwestern</td>
<td></td>
</tr>
<tr>
<td>Martin Schneider</td>
<td>U Maryland</td>
<td></td>
</tr>
<tr>
<td>Erick Hernandez-Ochoa</td>
<td>U Maryland</td>
<td></td>
</tr>
<tr>
<td>Marino DiFranco</td>
<td>UCLA</td>
<td></td>
</tr>
<tr>
<td>Marbella Quinonez</td>
<td>UCLA</td>
<td></td>
</tr>
<tr>
<td>Nicoletta Savilla</td>
<td>UCLA</td>
<td></td>
</tr>
<tr>
<td>Riccardo Olcese</td>
<td>UCLA</td>
<td></td>
</tr>
<tr>
<td>Perry Shieh</td>
<td>UCLA</td>
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### Postdocs / Faculty

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larry Hayward</td>
<td>MGH / Harvard</td>
<td></td>
</tr>
<tr>
<td>Arie Struyk</td>
<td>MGH / UTSW</td>
<td></td>
</tr>
<tr>
<td>David Francis</td>
<td>UT Southwestern</td>
<td></td>
</tr>
<tr>
<td>Wentao Mi</td>
<td>UT Southwestern</td>
<td></td>
</tr>
<tr>
<td>Volod Rybalchenko</td>
<td>UT Southwestern</td>
<td></td>
</tr>
<tr>
<td>Fenfen Wu</td>
<td>UCLA</td>
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### Graduate Students:

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<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Location</th>
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<tbody>
<tr>
<td>Nathaniel Elia</td>
<td>UCLA</td>
<td></td>
</tr>
<tr>
<td>Vicky Yu</td>
<td>UT Southwestern</td>
<td></td>
</tr>
<tr>
<td>Jadon Webb</td>
<td>UT Southwestern</td>
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