Afferent sensory input by orally-administered TRPV1 and TRPA1 activators inhibits electrically-induced muscle cramps in normal healthy volunteers

Glenn F. Short III, Brooke W. Hegarty, Bruce P. Bean, Roderick MacKinnon, Christoph H. Westphal and Jennifer M. Cermak

Flex Pharma, Boston, MA

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Drs. G.F. Short, B.W. Hegarty, C.H. Westphal, and J.M. Cermak are employees of Flex Pharma, Inc.
Dr. B.P. Bean and R. MacKinnon hold stock and receive compensation for service on the Scientific Advisory Board of Flex Pharma.

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The causes of muscle cramping are poorly understood

- Muscle cramping is a common problem with no approved clinical treatment.
- Recent research has shown that most cramping is not caused by dehydration or electrolyte imbalance.
- The best current hypothesis is that cramping reflects persistent firing of alpha motor-neurons, not a direct property of the muscle itself.
- There is no laboratory animal model of cramping.
The key element in muscle cramping may be the same as in muscle spasms: persistent motor neuron firing

Muscle spasms in a rat model of spinal cord injury reflect long-lasting firing of motoneurons.

K.C. Murray, M.J. Stephens, E.M. Ballou, C.J. Heckman, D.J. Bennett  
*Neurophysiol.* 105: 731-748, 2010

Self-sustained long-lasting firing produced by Ia synaptic input in the motoneuron of a decerebrate cat.

R.H. Lee and C.J. Heckman  
Muscle cramp can be reproducibly induced by brief repetitive stimulation of motor end-plates at 5-20 Hz.

Stimulated with 180 microsecond biphasic square pulses at 8, 10 or 12 Hz for 5 seconds.
Drinking pickle juice decreases cramp duration and acts within 90 seconds (Miller et al., 2010)

<table>
<thead>
<tr>
<th>TABLE 1. Cramp duration, intensity, and MF_{trt} before (Pre) and after (Post) ingestion of pickle juice and deionized water.</th>
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<tbody>
<tr>
<td><strong>Pickle Juice</strong></td>
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<tr>
<td>Duration (s)</td>
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<tr>
<td>Intensity (% of MVIC EMG activity)</td>
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<tr>
<td>MF_{trt} (Hz)</td>
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- We hypothesized that the acetic acid in pickle juice acts by stimulating sensory nerve endings in the mouth, esophagus or stomach via activation of TRPV1 and/or TRPA1 channels.

- More potent and efficacious TRPV1 and TRPA1 activators are available in natural products, including capsicum (TRPV1), gingerols (TRPV1 and TRPA1), and cinnamaldehyde (TRPA1).
Drinking Flex beverage containing natural extracts produces long-lasting inhibition of electrically-induced muscle cramping

Subjects drank 50 mL of a beverage containing aqueous extracts of capsicum, cinnamon and ginger.
Collected data in 37 subjects showed highly significant effect of Flex treatment vs. vehicle control

- Flex treatment and vehicle data were compiled across three independent, randomized, blinded crossover studies (n=37, normal healthy volunteers)
- Flex treatment decreased the mean cramp intensity within 15 min and effect lasted 6-8h
- Flex treatment significantly reduced cramp intensity by 3-fold (ANOVA, p<0.0001)
- Flex treatment was well-tolerated with no serious adverse events
Primary sensory neurons can be activated by TRPV1 and TRPA1 agonists in Flex natural extracts

Rat trigeminal ganglion neurons exposed to highly-diluted capsicum, cinnamon, or ginger extracts. Stimulation monitored by intracellular calcium indicator.

Highly synergistic action of combined effects of individual extracts at low dilutions.

Data by Yong Ho Kim and Seog Bae Oh
Identification of beverage components

Ginger Extract
- 6-Gingerol

Capsicum Extract
- Capsaicin

Cinnamon Extract
- Cinnamaldehyde
Tests of purified components of Flex treatment to activate human sensory neurons

- Naturally-occurring compound components of the Flex treatment activate human DRGs
- Co-activation of TRPV1 and TRPA1 potentiates and prolongs stimulation

![Graphs showing potentiation of 
$[\text{Ca}^{2+}]$ by combination and single agent activators of TRPV1 and TRPA1](image-url)
Molecular structures of TRPV1 and TRPA1 channels are known from Cryo-EM.


Muscle cramping occurs in a spectrum of neuromuscular disorders

**Nocturnal Leg Cramps**
Sudden painful contraction reducing sleep quality
No drug approved in the U.S.

**Severe Neuromuscular Conditions**
Spinal Cord Spasticity, Dystonias, Multiple Sclerosis

**Exercise Associated Muscle Cramps (EAMCs)**

U.S. Patient Population
- 37% prevalence for 50+ yo
- ~4M over 65 yo suffer daily
- No evidence that common ‘remedies’ such as electrolyte replacement, bananas and hydration afford relief
- No approved drug in US to treat

U.S. Patient Population
- Cervical dystonia: 90K patients
- Spinal spasticity: 150K patients
- MS: 250K – 350K patients, 84% of whom experience spasticity

- Impacts both weekend warriors and elite athletes

2 Estimates based on third party survey results
3 National Spasmodic Torticollis Association
4 Own estimates
5 National Institute of Neurological Disorders and Stroke
Summary and Clinical Implications

- TRPV1 and TRPA1 activation demonstrates a significant reduction in muscle cramp intensity compared to vehicle control.

- Results from three independent human studies:
  - Treatment effect observed at 15 minutes lasting up to 6-8 hours.
  - Cramp muscle contractions reduced by 3-fold.
  - Well-tolerated with no serious adverse events.

- Identification of active components may allow improved formulations.
Acknowledgements

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Christoph H. Westphal, MD, PhD

Rat Trigeminal Ganglion Recording
Yong Ho Kim, PhD
Seog Bae Oh, DDS, PhD
**Flex beverage inhibits natural cramping**

Subject G
Spontaneous cramping of flexor hallucis brevis

- **Control #1**
  - 8 sec cramp

- **Control #2**
  - 5 sec cramp

- **10 min after TRP-Stim**
  - No cramp

- **15 min after TRP-Stim**
  - No cramp
Ease of electrically-induced cramping correlates with susceptibility to natural cramping

ELECTRICAL STIMULATION CRAMP THRESHOLD FREQUENCY CORRELATES WELL WITH THE OCCURRENCE OF SKELETAL MUSCLE CRAMPS

KEVIN C. MILLER, MS, and KENNETH L. KNIGHT, PhD

Human Performance Research Center, Brigham Young University, 106 Smith Fieldhouse, Provo, Utah 84602, USA


RESULTS

Subjects with a history of cramping had a significantly lower cramp TF (14.9 ± 1.3 Hz) than subjects without a history of cramping (25.5 ± 1.6 Hz; F_{1,29} = 26.0, P < 0.001). Threshold frequency was also slightly lower on day 2 (18.3 ± 0.26 Hz) than day 1 (19.7 ± 0.25 Hz; F_{1,29} = 5.5, P = 0.03).

Subjects with a history of muscle cramping reported that prior cramping episodes occurred primarily in the lower extremity, with the foot and toes being the most frequently afflicted (13/19, 68%) followed by the calves (12/19, 63%) and hamstrings (6/19, 32%). Moreover, 89% (16/18) of subjects in the history of cramping group had a family history of cramping compared to 27% (3/11) in the no history of cramping group (χ² = 11.5; P < 0.001). Reliability over days was high (r = 0.92).
Subjects drank 50 mL of a beverage containing aqueous extracts of capsicum (0.075%), cinnamon (1%) and ginger (1.5%) diluted in 1:1 mixture of water and corn syrup.
Inhibition of calf cramps

Subject E
Calf muscle

Stimulation
2 Hz
28 Hz
20 sec

Control
59 sec cramp

3 min after TRP-Stim
3 sec cramp

ELICITABILITY OF MUSCLE CRAMPS IN DIFFERENT LEG AND FOOT MUSCLES
MARCO ALESSANDRO MINETTO, MD, PhD1,2 and ALBERTO BOTTER, MS2
MUSCLE & NERVE October 2009
The causes of muscle cramping are poorly understood

- There is no laboratory animal model of cramping.
- Recent research has shown that most cramping is not caused by dehydration or electrolyte imbalance.

Significant and serious dehydration does not affect skeletal muscle cramp threshold frequency


<table>
<thead>
<tr>
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<th>Euhydrated</th>
<th>Dehydrated (4.7±0.5% of body mass) by exercise</th>
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</thead>
<tbody>
<tr>
<td>Cramp threshold frequency</td>
<td>15±5 Hz</td>
<td>13±6 Hz (=0.12)</td>
</tr>
<tr>
<td>Cramp Intensity</td>
<td>94.2±41%</td>
<td>115.9±73% (p=0.2)</td>
</tr>
<tr>
<td>Cramp Amplitude</td>
<td>0.18±0.06 mV</td>
<td>0.18±0.09 mV</td>
</tr>
<tr>
<td>[Na⁺] plasma</td>
<td>141.9±3.1 mM</td>
<td>149.5±1.8 mM</td>
</tr>
<tr>
<td>[K⁺] plasma</td>
<td>4.9±0.4</td>
<td>5.0±0.4</td>
</tr>
</tbody>
</table>
The causes of muscle cramping are poorly understood

- Recent research shows that muscle cramping originates in the motor neurons innervating muscle, not in the muscle itself.

Mechanisms of cramp contractions: peripheral or central generation?
M.A. Minetto, A. Holobar, A. Botter, R. Ravenni, D. Farina