Thromboxane A2 Receptors Contribute to the Exaggerated Exercise Pressor Reflex in Rats with Heart Failure


Departments of Kinesiology - Kansas State University

Background

- Exercise requires complicated cardiovascular control
- Cardiovascular control is altered in heart failure patients with reduced ejection fraction (HF-rEF)
- The exercise pressor reflex (EPR) contributes to altered cardiovascular control in HF-rEF

What we know: Thromboxane A2 (TxA2) receptors on sensory neurons sensitize mechanically activated channels (Butenas et al., 2020; Autonomic Neuroscience)

What we do not know: If inhibiting TxA2-Rs will reduce the exaggerated EPR in HF-rEF

Methods

- Sprague Dawley rats
  - HF-rEF induced coronary artery ligation
  - Sham ligations were performed on rats
- Measurements:
  - MAP: carotid artery catheter
  - Heart rate: ECG
  - RSNA: stainless steel electrodes.
- Rats were decerebrated and unanesthetized
- Autonomic functions intact

Protocol 1

First, we compared the sympathetic, pressor, and cardioaccelerator response to 30s of 1 Hz dynamic contraction between rats with HF-rEF and SHAM rats

Protocol 1 Results

- Effects of TxA2 receptor blockade on exercise pressor reflex:
  - SHAM rats—no effect
  - HF-rEF rats—reduced exercise pressor reflex

Protocol 2: SHAM rats

Protocol 2: HF-rEF rats

Hypothesis

Inhibiting TxA2 receptors on sensory neurons would reduce the EPR in HF-rEF rats, but not healthy rats

Activation of EPR in SHAM and HF-rEF rats

Confirmed exercise pressor reflex is exaggerated in heart failure

Protocol 2

Second, we determined the effect of TxA2 receptor blockade on these responses HF-rEF and SHAM rats

Dynamic Contraction

Daltroban injected i.a.

Dynamic Contraction

Tighten snare

Release snare after 5 mins. Repurfuse 10 mins

Conclusions

- The exercise pressor reflex is exaggerated in HF-rEF
- TxA2 receptors on sensory neurons contributes to exaggerated exercise pressor reflex in HF-rEF
- Altered intracellular signaling within sensory neuron is likely responsible
Enrichment of the Microbial Community in a Spacecraft Assembly Facility in Extreme Brines Relevant to Mars

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Abstract
Spacecraft are assembled in clean rooms to reduce microbial contamination that could confound life detection missions. Mars' near-surface is hyperarid and rich in dry hygroscopic sulfate and (per)chlorate salts that can absorb humidity and deliquesce at certain times to produce dense brines with extremely low water activity that preclude microbial growth. Here we challenge the microbial community in samples from the Jet Propulsion Laboratory Spacecraft Assembly Facility (JPL SAF) with the extreme chemical conditions of brines relevant to Mars. Wipe samples from JPL SAF floors were enriched in water medium with 50% MgSO4 or 20% NaClO (w/v). Samples were taken weekly for a month and then after six months to follow changes in the SAF microbial community. Metagenomic DNA extracts of community enrichments were characterized using Illumina sequencing of 16S rRNA gene sequences using bacterial and fungal primers. Culturable microbial diversity in six-month enrichment cultures was characterized and appeared limited, dominated by Oceanobacillus and Staphylococcus. A succession of bacterial and fungal species was observed over the six-month incubation; Firmicutes dominated the cultures early, with increases in Actinobacteria observed over time, along with increased overall diversity. Ascomycota dominated the fungal communities. Basidiomycota were common early in the incubation, but nearly disappeared by four weeks. The microbes persisting from the JPL SAF microbial community in aged cultures, enriched at extreme salinities, are arguably the most capable organisms to subsequently survive and proliferate at the near-surface of Mars.

Succession of a Microbial Community
Fluids squeezed from polyester wipes were introduced into flasks of SP medium supplemented with 50% MgSO4 (0.9 A2) or 20% NaClO (0.84 A2). Four weekly and six-month samples were taken for DNA extraction. Aliquots of culture at one month were fully dried in desiccator before extracting DNA. Illumina sequencing of 16S and 18S rRNA genes identified members of the bacterial and fungal communities present and created a narrative of what species were present week to week and eventually at six months. Flasks exhibited a decrease in diversity over time as the microbial communities developed and changed during the six-month period.

JPL SAF Bacterial Isolate Collection
Bacteria living in flasks at six months were isolated and characterized. Colonies were selected based on morphology and isolated through five sequential streak plates. A total of 40 isolates were characterized to describe their morphology and enzyme activity.

Salt Tolerances of JPL SAF Bacterial Isolates
Limits of salt tolerance in bacterial isolates were tested by measuring growth in increasing salt concentrations (NaCl, NaClO3, and MgSO4). Isolates were measured five days after inoculation. Growth was indicated by a 0.1 OD increase. Note that growth of several isolates was inhibited as salt concentrations increased.

Bacterial and Fungal Community
Microorganisms from JPL SAF can survive in high-salt brines representative of Mars' surface. A succession of bacterial and fungal species was observed over a six-month period showing a decrease in community diversity over time. Bacteria present at six months were mainly found in the Firmicutes phylum, specifically species of the Staphylococcus genus. Most fungal species found in the flask cultures at six months belong to the Mucoromycota phylum.

The Mars Environment
Near surface of Mars is harsh due to high levels of salt and desicating conditions. Presence of sulfates, chlorates, and perchlorates create a highly oxidizing environment. Areas on Mars’ surface regarded as Special Regions are of particular interest and include gullies and recurring slope lineae.

Supported by awards from National Aeronautics and Space Administration (NASA), Research Opportunities in Space and Earth Science (ROSES), Planetary Protection Research (09-PPR09-0004 and 14-PPR14-2-0002) and Kansas INBRE, National Institute of General Medical Sciences (NIGMS), National Institutes of Health (NIH) (P20 GM103418); University of Kansas Genome Sequencing Center.
**Wheat streak mosaic virus** prevalence in Kansas winter wheat fields

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**Abstract**

*Wheat streak mosaic virus* (WSMV) is a common wheat virus that causes significant yield loss in wheat production. Intermittent epidemics of this viral disease cause losses of millions of dollars. The key management strategy against WSMV is genetic resistance, along with the use of good cultural practices. One of the important threats to the durability of genetic resistance is the presence of potential new variants of the virus in the field population. The frequent monitoring and identification of field isolates of WSMV is essential to determine the population structure of the virus. A statewide survey was conducted to collect the WSMV isolates in the Kansas winter wheat fields. A total of 101 and 81 symptomatic wheat leaves were collected from 60 and 31 different counties of Kansas in 2019 and 2020 respectively. Enzyme-Linked Immunosorbent Assay (ELISA) results showed that there was a high prevalence of WSMV. Out of 101 samples, 72 samples (71.3%) and out of 81, 73 samples (90.12%) were positive for WSMV. Our data from the WSMV survey and its prevalence provide the information to estimate future incidences and potential for an epidemic year of the virus. The positive WSMV samples will be subjected to whole-genome sequencing technology to characterize Kansas WSMV isolates. The knowledge of WSMV prevalence and unique isolates present in the Kansas wheat fields will enable plant breeders to use relevant isolates in their future breeding programs.

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**Introduction**

- *Wheat streak mosaic virus* (WSMV) is a common wheat viruses causing significant economic loss in wheat production all over the wheat-growing regions of the world.
- In 2017, Kansas wheat producers lost 19.2 million bushels of wheat worth $76.8 million due to wheat streak mosaic.
- A key management strategy against WSMV is genetic resistance. There are limited commercially available wheat cultivars with resistance genes and resistance breaks down at higher temperatures.
- Due to several evolutionary forces such as mutation, selection, and bottlenecks, variation in WSMV isolates in the field population is common.
- Therefore, frequent monitoring, identification, and characterization of WSMV field isolates is essential. These activities facilitate WSMV research and use in breeding programs to screen new cultivars for resistance to WSMV with relevant isolates.

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**Materials and Methods**

- A state-wide survey of Kansas wheat field was carried out in 2019 and 2020.
- A total of 101 and 81 symptomatic wheat leaves were collected from 60 and 31 different counties of Kansas in 2019 and 2020 respectively.
- Double antibody sandwich Enzyme-linked immunosorbent assay (DAS-ELISA) was performed to identify the WSMV positive samples according to the company’s protocol (Agdia, Inc.)
- The ELISA plate was measured by a plate reader at Abs 405 nm (Biotek, Gen 5 Model EL800, Winooski, VT). If the absorbance value of each well was 3X the average of the negative samples, then the sample was considered positive for WSMV infection.

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**Results**

- There was high incidence of WSMV in the fields as 71.3% to 90.12% of the samples were positive for WSMV.
- Total loss of crop in localized fields was observed.
- Whole genome sequencing of WSMV on positive samples will be carried out by using Nanopore sequencing and characterization of WSMV isolates will be done after sequencing.

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**Conclusion and Future work**

- There was high incidence of WSMV in the fields as 71.3% to 90.12% of the samples were positive for WSMV.
- Total loss of crop in localized fields was observed.
- Whole genome sequencing of WSMV on positive samples will be carried out by using Nanopore sequencing and characterization of WSMV isolates will be done after sequencing.
Studies of Salinotolerant Bacteria at the Great Hydrostatic Pressure within Europa and the Icy Ocean Worlds

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Abstract

Coral bodies throughout the solar system have the potential to harbor life in the saltwater oceans under frozen crusts. Large icy worlds (Callisto, Europa, Ganymede, Pluto, and Titan) have deep global oceans in contact with rocky cores rich in minerals. Even modest coral bodies (Ariel, Ceres, Enceladus, Mimas, Miranda, Oberon, and Titania) harbor at least regional oceans. The oceans of icy worlds appear to be salty, with current disagreement on their compositions and salinities. Native life or terrestrial microbes delivered as spacecraft contaminants may have to withstand hypersalinity, temperatures below 0°C, and high physical pressure in ocean worlds. Here we measured the piezotolerances of bacteria from Hot Lake, WA and the Great Salt Plains, OK. Halomonas sp. STR. HL12 cells held in sealed straws were exposed to hydrostatic pressures of ~8000 psi (55 MPa; 550 atm) for 48 h and culture density was determined by standard plate count. Survival relative to unpressurized controls was high, although certain experiments showed substantial (>1 log) decreases in cell numbers with pressure treatment. Not that ~30 MPa is typical at Earth’s ocean floor, while 55 MPa represents a depth of 40 to 60 km in large icy moons (halfway between the ice shell and rocky core of Europa) and far greater than the ~5 MPa of the smaller icy worlds. We show here that salinotolerant, psychrotolerant, terrestrial microbes can survive the great physical pressure expected in icy ocean worlds.

Planetary Protection

Planetary protection is focused on preventing the forward and backward contamination of both the bodies targeted for exploration within our solar system and the Earth. Given the right conditions, however improbable, it may be possible that a microbial organism from a hypersaline environment could survive near the surface of Mars or in the ocean of an icy world

Hot Lake, Washington

Hot Lake is in Washington state, located about 5 km from Oroville. It is a meromictic athalassohaline lake dominated by sulfate salts of magnesium to saturation. Crystalline epsomite (MgSO₄ • 7H₂O) precipitates. This lake is characteristically shallow, does not freeze, and has a thick layer of solid epsomite under its sediments.

The Icy Ocean Worlds

<table>
<thead>
<tr>
<th>Europa</th>
<th>Mere</th>
<th>Titan</th>
<th>Oberon</th>
<th>Triton</th>
<th>Pluto</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ice Thickness, km</td>
<td>Ocean Thickness, km</td>
<td>Core Radius, km</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>89.5</td>
<td>140.5</td>
<td>347.2</td>
<td>519.8</td>
<td>481.0</td>
<td>1037.0</td>
</tr>
</tbody>
</table>

Life at High Physical Pressure

Salinotolerant bacterium Halomonas sp. STR. HL12 was subjected to 8000 psi while held in sealed straws in SP medium with low and high NaCl or MgSO₄. After 48 h, culture density was measured by plate counts and compared. Losses due to enclosure in straws were small. Initial results indicate that pressure treatment led to a reduction in cell numbers, but this was variable and the effect of salts uncertain at this point.

<table>
<thead>
<tr>
<th>Pressure Effect</th>
<th>Halite</th>
<th>NaCl (%w/v)</th>
<th>MgSO₄ (%w/v)</th>
<th>Culture Density (CFU/ml ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 (Atm)</td>
<td>1</td>
<td>0.10</td>
<td>0.76×10⁶ ± 0.05×10⁶</td>
<td>-</td>
</tr>
<tr>
<td>8000</td>
<td>550</td>
<td>0.10</td>
<td>2.6×10⁵ ± 0.35×10⁵</td>
<td>3-fold reduction</td>
</tr>
<tr>
<td>14 (Atm)</td>
<td>1</td>
<td>10.0</td>
<td>3.5×10⁵ ± 0.56×10⁵</td>
<td>-</td>
</tr>
<tr>
<td>8000</td>
<td>550</td>
<td>10.0</td>
<td>0.47×10⁶ ± 0.35×10⁶</td>
<td>7-fold reduction</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Epsomite</th>
<th>Pressure Effect</th>
<th>MgSO₄ (%w/v)</th>
<th>Culture Density (CFU/ml ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 (Atm)</td>
<td>1</td>
<td>0.10</td>
<td>3.7×10⁶ ± 1.6×10⁶</td>
</tr>
<tr>
<td>8000</td>
<td>550</td>
<td>0.10</td>
<td>6.2×10⁵ ± 7.4×10⁵</td>
</tr>
<tr>
<td>14 (Atm)</td>
<td>1</td>
<td>50.0</td>
<td>2.6×10⁵ ± 0.56×10⁵</td>
</tr>
<tr>
<td>8000</td>
<td>550</td>
<td>50.0</td>
<td>1.7×10⁶ ± 0.56×10⁶</td>
</tr>
</tbody>
</table>

Funding

Supported by awards from National Aeronautics and Space Administration (NASA), Research Opportunities in Space and Earth Science (ROSES), Planetary Protection Research (09-PPR09-0004 and 14-PPR14-2-0002) and Kansas INBRE, National Institute of General Medical Sciences (NIGMS), National Institutes of Health (NIH) (P20 GM103418).

Extremely Salty European Ocean

Two conflicting models of the composition of the European ocean can be made from Cassini data. In one, the ocean composition is saturated with respect to Na, Mg, and SO₄ with a relatively neutral pH overall. The other has salts comparable to Earth’s ocean, which easily supports life. Halomonas sp. STR. HL12 survives in medium with European ocean salts and can be transferred to fresh medium several times. However, it is not clear yet whether there is growth and whether that would persist at temperatures below 0°C.

<table>
<thead>
<tr>
<th>Europe Ocean Salts</th>
<th>NaCl</th>
<th>Na₂SO₄</th>
<th>NaCl</th>
<th>Na</th>
<th>Cl⁻</th>
<th>Mg</th>
<th>SO₄⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressures</td>
<td>2.6 M</td>
<td>0.6 M</td>
<td>0.3 M</td>
<td>-</td>
<td>-</td>
<td>2.6 M</td>
<td>3.5 M</td>
</tr>
</tbody>
</table>

Icy Ammonia-Water Ocean Worlds

Ocean worlds beyond Europa, at lower temperatures, have liquid oceans that must rely on ammonia-water mixtures to remain liquid; all salty brines would freeze. Pluto, Titan, and Ceres seem to harbor ammonia-water oceans. The ammonia-water eutectic is ~100 °C (at 34 wt% ammonia) and the coldest liquid water common in the solar system.

While ammonia is used commercially as a disinfectant, we have observed substantial resistance in initial studies. For instance, strong growth was observed for Halomonas sp. STR. HL12 at 1 M ammonium sulfate or chloride. At higher concentrations, up to saturation at ~35% salt, we observed survival of isolates for days to weeks and continued presence of culturable microbes from soil for weeks.

Cryovolcanism, Global Melting, and Pansperma

The thick ice layers burying the deep oceans of icy worlds are unstable and regularly crack or at times are entirely melted by impacts, causing ocean waters to be in direct contact with the frigid hard vacuum of space, boiling furiously. Brines spewed from cryovolcanoes at will instantly freeze-dry in space and mainly deposit on the surface. Spacecraft at Europa and Enceladus have directly sampled brine plumes, finding evidence of geothermal vents at the bottom of buried oceans, a source of energetic reduced mineral nutrients. At the surface are oxidants that need to mix with ocean waters. Extant native life ejected from the oceans may persist in stasis until the ice shell melts or perhaps escape to seed other worlds, even exoplanets, through pansperma.
Status of Alfalfa Weevils in Kansas

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Abstract

The alfalfa weevil (Hypera postica) is one of the most damaging insect pests of alfalfa crops in Kansas and the United States causing yield losses of 10-15% annually. H. postica was introduced three separate times to North America and leading to the development of three strains, or subspecies: the Eastern, Western, and Egyptian that differ in life history traits that can play a role in management strategies. IPM strategies exploit knowledge of the pest’s biology and life history to survey its abundance and determine optimal timing of control efforts to reduce damage. The alfalfa weevil is found throughout the United States; however, it is still unknown which strains are present in Kansas. This project seeks to conduct a population genetic study and identify the strains of H. postica present in Kansas. Identifying the strain of weevil is important for interpreting field research results. In order to do this, we visited six alfalfa fields across the state of Kansas in the mid-spring to collect alfalfa weevil larvae. Then, we visited these six fields again in late spring to collect the adults once they were more prevalent. Using molecular genetic methods we can identify the western strain from the eastern and Egyptian strains based on a 5% sequence divergence of the mitochondrial DNA sequence, however, the closely related eastern and Egyptian strains can only be distinguished by sequencing the mitochondrial DNA. We are currently processing 100 samples for strain identification from across Kansas.

Background

• The alfalfa weevil (Hypera postica) is the number one pest of alfalfa in the U.S. causing more than $100 million in loss every year
• There are 3 strains of weevils: Western, Eastern, and Egyptian, it is unknown what the distribution of these 3 strains in in Kansas and how their different life history traits are affecting Integrated Pest Management strategies
• Pyrethroid resistance is of particular concern to many farmers
• Eastern and Egyptian strains are closely related and must be sequenced to identify the difference; Western strain can be identified using restriction enzymes
• This research aims to identify which strains of alfalfa weevils are found in Kansas and their distribution to identify new IPM strategies

Methods

• Collected samples using sweep net in six counties across Kansas: Lyon, Neosho, Reno, Brown, Ford, and Saline counties
• Sampled each county twice, once for larvae and later in the season for adults
• Captured 50 alfalfa weevils, per sample
• Extracted and amplify DNA from 10 larvae and 10 adults from each location
• Used restriction enzyme AluI to identify Western from Eastern and Egyptian strains
• Sent off unidentified Eastern/Egyptian strains for sequencing

Results

• 117 Alfalfa weevils identified using restriction enzymes
• Western: 19
• Eastern/Egyptian: 98
• Currently Eastern/Egyptian strain samples have been sent off to the lab and waiting to be sequenced

Discussion

While a mix of strains was expected, we expected that strains would have geographic patterns. Western and Eastern/Egyptian strains were found at each sampling location. Western strain was found more with the spring sampling and less frequently in the summer sampling. Current Pest Management Strategies in Kansas should focus on Eastern/Egyptian strain, although Western strain could be reduced due to current Pest Management Strategies. Many of the fields sampled were using pest control and were still infested with alfalfa weevils. Current research is investigating the pesticide resistance of different strains and developing a degree day model for alfalfa weevils in the Midwest region.

Figure 1: Lifecycle of the Alfalfa Weevil. Photo credits: Kevin Wanner and Ruth O’Neil, Montana State University

Figure 3: Molecular identification of weevil strains. PCR with C1-J-2797 and C2-N-3686 primers produces a 927 bp product (lane 2 & 4). When the western strain is the source of the band, its digestion with AluI restriction enzyme produces three smaller bands sized 486, 357 and 84 (lane 7).

Table 1: Alfalfa weevils identified by strain and the county sampled.

<table>
<thead>
<tr>
<th></th>
<th>Lyon</th>
<th>Neosho</th>
<th>Reno</th>
<th>Brown</th>
<th>Saline</th>
<th>Ford</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western strain</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>7</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Eastern/Egyptian</td>
<td>17</td>
<td>18</td>
<td>19</td>
<td>13</td>
<td>16</td>
<td>16</td>
</tr>
</tbody>
</table>

Figure 4: Map of Kansas showing sampled counties. Brown counties indicate presence of both Western and Eastern/Egyptian strains present. Tan counties were not sampled. Map created by Tania Wiest.

Acknowledgments: I would like to thank Emporia State FRCC for funding; the farmers who allowed us to sample their fields; Nick Rutherford and Tania Wiest for assisting in sampling; and my wife for assisting in the lab.