Behavioral and pharmacological characterization of the isosorbide dinitrate model of headache in male and female rats

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Background

Headache disorders including tension-type headache and migraine affect 40-50% of the global population and represent a leading cause of years lived with disability. The National Institutes of Health Helping to End Addiction Long-term Initiative, or NIH HEAL Initiative, Preclinical Screening Platform for Pain (PSPP) program aims to accelerate the discovery of novel pain therapeutics for pain disorders including headache and migraine.

The identification of translatable preclinical models is an important part of the drug discovery process to establish initial target validation. Translatable preclinical models of headache and migraine include administration of nitric oxide (NO) donors, since these compounds produce facial allodynia in rodents and headache and migraine in human subjects. Isosorbide dinitrate (ISDN) is a NO donor that is unique in that it is largely peripherally restricted and soluble in saline vehicle. Here, we characterize the behavioral pain phenotype and pharmacology of the ISDN model in rats to determine the utility of this model to evaluate novel mechanisms for the treatment of headache and migraine disorders.

Methods

Animals: Adult male and female Sprague Dawley rats (200 – 300 g, Envigo) were used in all studies. All housing and testing of animals was in accordance with the Principles of Laboratory Animal Care and the approval of the PsychoGenics Inc., Institutional Animal Care and Use Committee in AAALAC-accredited facilities.

ISDN model: Rats were initially acclimated to Bowman restrainers for three consecutive days (Day 1 to Day 3) for approximately 1- hour each day. On Day 4, baseline (BSL) facial sensitivity thresholds were determined, and rats that had a BSL facial sensitivity threshold < 8 g were excluded from the study.

Single administration phenotype: Rats were dosed with ISDN (10 mg/kg; 10 mL/kg; IP) or vehicle (saline; 10 mL/kg, IP) on Day 5 and effects on facial sensitivity thresholds were determined at 1-, 2-, and 4- hours post-dosing.

Repeated administration phenotype: Rats were dosed with ISDN (10 mg/kg; 10 mL/kg; IP) or vehicle (saline; 10 mL/kg; IP) once per day for 5 consecutive days and effects on facial sensitivity thresholds were determined following discontinuation of dosing on Days 6, 7.

Pharmacology: To examine effects of reference compounds on the development of transient facial mechanical allodynia, rats were dosed with a reference analgesic or vehicle on Day 5 and then received a single injection of ISDN (10 mg/kg; 10 mL/kg; IP) approximately 5 minutes following drug administration.

Measurement of facial mechanical sensitivity: Rats were placed in Bowman restrainers for facial sensitivity testing. Von Frey filaments (1 – 8 g) were applied in ascending order to the forehead above the eyes to determine the facial sensitivity threshold. A positive response was defined as either a recoil of the head, stroking of the face, vocalization, or aggressive behavior towards the filament, and a positive response in 2/3 trials was defined as the facial sensitivity threshold. If a positive response in 2/3 trials was not found for any filament, a maximum facial sensitivity threshold of 10 g was assigned. Rats displaying baseline facial sensitivity thresholds <8.0 g were excluded from the study.

Reference compounds: Morphine sulfate (0.3-6 mg/kg, SC; mu opioid receptor agonist), SNC80 (3-30 mg/kg, SC; delta opioid receptor agonist), sumatriptan (0.1-1 mg/kg, IP; 5-HT1B/1D receptor agonist), olcegepant (0.3-3 mg/kg, IP; CGRP receptor antagonist), duloxetine (3-60 mg/kg, PO; SNRI), celecoxib (3-30 mg/kg, PO; COX-2 inhibitor) .

Data and statistics: Results were graphed and analyzed using GraphPad Prism version 10.4.1. Data are represented as individual animal responses and mean ± SEM and analyzed using two-way repeated measures ANOVA with appropriate post-hoc test. An effect was considered significant at $p < 0.05$ level.

Rigor

- Experimenters were blinded to treatments
- Inclusion/exclusion criteria were applied according to baseline responses
- Groups were balanced according to baseline responses

This project has been funded in whole or in part with Federal funds from the National Institute of Neurological Disorders and Stroke, National Institutes of Health, Department of Health and Human Services, under Contract No. 75N95024D00038.

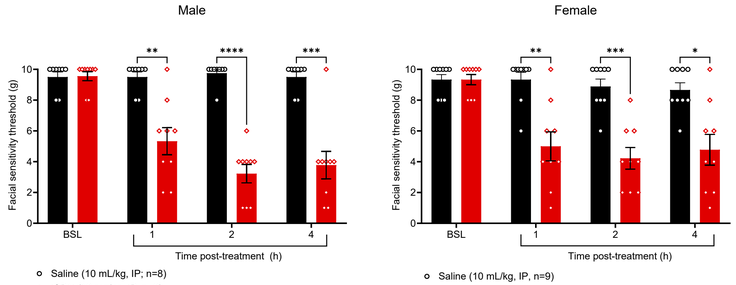
NIH National Institute of Neurological Disorders and Stroke

NIH HEAL INITIATIVE

PsychoGenics

Behavioral Pain Phenotype

Facial Mechanical Allodynia Following Single Administration of ISDN (10 mg/kg, IP)

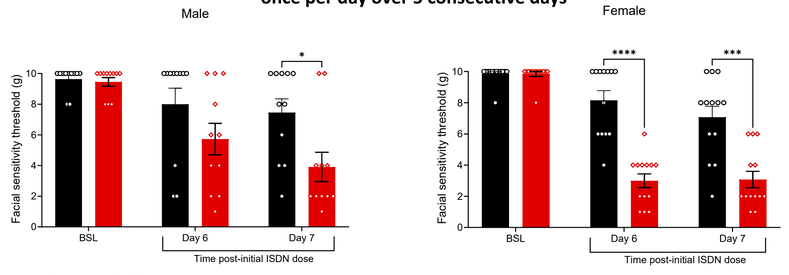


○ Saline (10 mL/kg, IP; n=8)
◇ ISDN (10 mg/kg, IP, n=9)

○ Saline (10 mL/kg, IP, n=9)
◇ ISDN (10 mg/kg, IP, n=9)

A) Single administration of ISDN (10 mg/kg, IP) produced facial mechanical allodynia which persisted for 4 hours in male and female rats.

Facial Mechanical allodynia Following Repeated Administration of ISDN (10 mg/kg, IP) once per day over 5 consecutive days



○ Saline (10 mL/kg, IP); n=11
◇ ISDN (10 mg/kg, IP); n=11

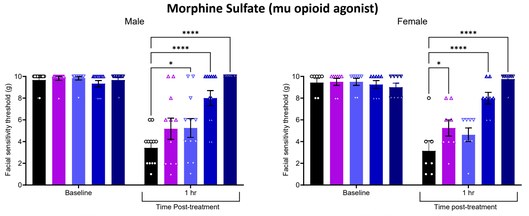
○ Saline (10 mL/kg, IP); n=13
◇ ISDN (10 mg/kg, IP); n=13

B) Repeated administration of ISDN (10 mg/kg, IP) once per day for 5 consecutive days (Day 1 – 5) produced persistent facial mechanical following discontinuation of dosing on Day 6 (females) and Day 7 (males and females)

**** p<0.0001, *** p<0.001, ** p<0.01, * p<0.05 Bonferroni's test

Pharmacology

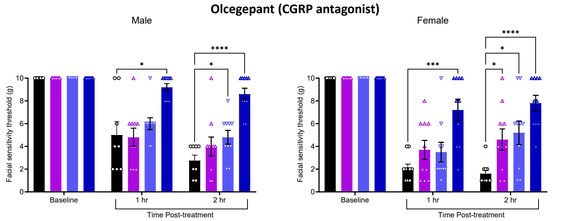
Morphine Sulfate (mu opioid agonist)



○ Vehicle (saline, SC); n=12
△ Morphine (0.3 mg/kg, SC); n=11
▽ Morphine (1 mg/kg, SC); n=12
▲ Morphine (3 mg/kg, SC); n=12
▼ Morphine (6 mg/kg, SC); n=12

○ Vehicle (saline, SC); n=7
△ Morphine (0.3 mg/kg, SC); n=8
▽ Morphine (1 mg/kg, SC); n=8
▲ Morphine (3 mg/kg, SC); n=8
▼ Morphine (6 mg/kg, SC); n=8

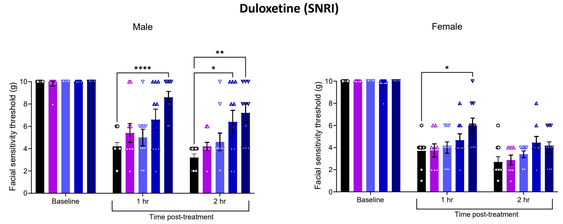
Olcegepant (CGRP antagonist)



○ Vehicle (saline, IP); n=8
△ Olcegepant (0.3 mg/kg, IP); n=10
▽ Olcegepant (1 mg/kg, IP); n=10
▲ Olcegepant (3 mg/kg, IP); n=10

○ Vehicle (saline, IP); n=10
△ Olcegepant (0.3 mg/kg, IP); n=10
▽ Olcegepant (1 mg/kg, IP); n=10
▲ Olcegepant (3 mg/kg, IP); n=10

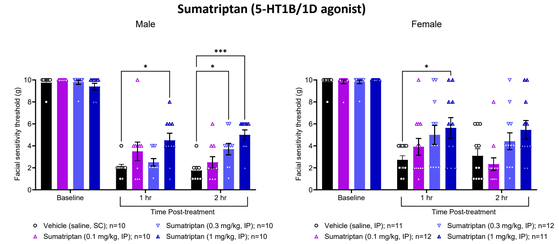
Duloxetine (SNRI)



○ Vehicle (Water, PO, n=10)
△ Duloxetine (3 mg/kg, PO, n=10)
▽ Duloxetine (10 mg/kg, PO, n=10)
▲ Duloxetine (30 mg/kg, PO, n=10)
▼ Duloxetine (60 mg/kg, PO, n=10)

○ Vehicle (Water, PO, n=10)
△ Duloxetine (3 mg/kg, PO, n=8)
▽ Duloxetine (10 mg/kg, PO, n=10)
▲ Duloxetine (30 mg/kg, PO, n=9)
▼ Duloxetine (100 mg/kg, PO, n=10)

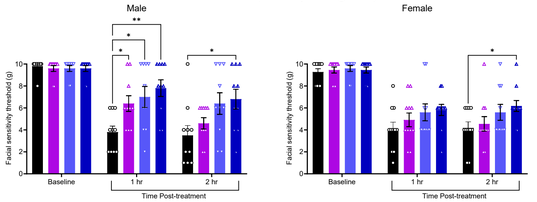
Sumatriptan (5-HT1B/1D agonist)



○ Vehicle (saline, SC); n=10
△ Sumatriptan (0.1 mg/kg, IP); n=10
▽ Sumatriptan (0.3 mg/kg, IP); n=10
▲ Sumatriptan (1 mg/kg, IP); n=10

○ Vehicle (saline, IP); n=11
△ Sumatriptan (0.1 mg/kg, IP); n=12
▽ Sumatriptan (0.3 mg/kg, IP); n=12
▲ Sumatriptan (1 mg/kg, IP); n=11

SNC80 (delta opioid agonist)

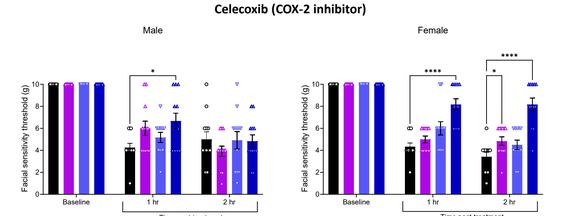


○ Vehicle (SC); n=10
△ SNC80 (3 mg/kg, SC); n=10
▽ SNC80 (10 mg/kg, SC); n=10
▲ SNC80 (30 mg/kg, SC); n=10

○ Vehicle (SC); n=11
△ SNC80 (3 mg/kg, SC); n=11
▽ SNC80 (10 mg/kg, SC); n=10
▲ SNC80 (30 mg/kg, SC); n=11

Vehicle: 10% DMSO, 40% PEG300, 5% Tween-80, 45% Saline

Celecoxib (COX-2 inhibitor)



○ Vehicle (0.5% MC, PO, n=12)
△ Celecoxib (3 mg/kg, PO, n=12)
▽ Celecoxib (10 mg/kg, PO, n=12)
▲ Celecoxib (30 mg/kg, PO, n=12)

**** p<0.0001, *** p<0.001, ** p<0.01, * p<0.05 Dunnett's test

Conclusions

- The isosorbide dinitrate (ISDN) headache model produces reliable facial mechanical allodynia in male and female Sprague Dawley rats
- Reference analgesics that are clinically effective for the treatment of migraine (i.e. triptans, CGRP blockers) are efficacious in the ISDN model, demonstrating that the pharmacology associated with this model is consistent with migraine
- This model may be used to evaluate the efficacy of novel mechanisms for the treatment of headache and migraine
- Additional characterization of this model is warranted to further understand the behavioral phenotype in terms of migraine pain (i.e. photophobia/phonophobia, biomarkers)