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Effect of dexpramipexole on neuropathic pain in prenatal alcohol exposed male and female subjects

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Introduction

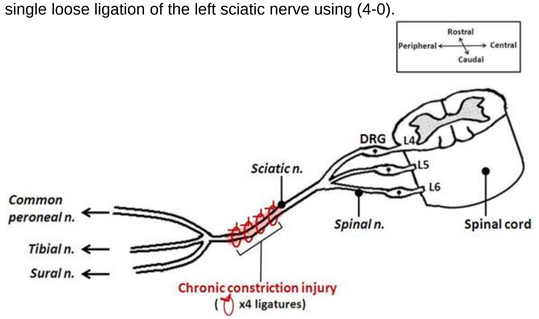
- Neuropathic pain can be described as the result of damage caused to the somatosensory system manifested as a pathological sensitivity to light touch, clinically referred to as allodynia
- Neuropathic pain is mediated, in part, by cytokines released from activated resident glial cells and peripheral immune cells that invade the central nervous system (CNS).
- Our lab has previously demonstrated that prenatal alcohol exposure (PAE) is a risk factor for developing chronic neuropathic pain from very minor injuries to peripheral nerves of the somatosensory system that project to and communicate to spinal pain projection neurons.
- IL-1β is a potent pro-inflammatory cytokine that is elevated in spinal cords and peripheral immune cells from PAE offspring with either nerve damage or immune challenge, respectively.
- IL-1β is released by macrophages and to a lesser extent, T cells.
- Dexpramipexole [(+)-pramipexole] is an enantiomer of (-)-pramipexole and has virtually none of the dopamine receptor binding activity that (-)-pramipexole exerts. However, given that dexpramipexole reduces caspase-3 activity and cell death, we speculated that dexpramipexole may exert anti-inflammatory properties on immune cells and glia.
- Therefore, we tested the **hypothesis** that **administration of dexpramipexole will result in a decreased inflammatory response and reduced allodynia in PAE rats**
- To test this hypothesis, we administered an intrathecal (i.t., subarachnoid, peri-spinal) injection of dexpramipexole to determine if spinal glia and local immune cells could respond to dexpramipxole and lead to reversal of allodynia.
- In addition, we collected immune cells from the spleen (majority T-cells) and peritoneal cavity (PEC; majority monocytes/macrophages), and stimulated them with lipopolysaccharide (LPS) alone, or in combination with dexpramipexole to test whether dexpramipexole can decrease IL-1β protein levels

Methods

Prenatal Alcohol Exposure model. Rat dams were provided Sac-sweetened alcohol (5%) or Sac-sweetened water for 4 hours/day during gestation (BECs: ~60 mg/dl). Offspring were acclimated to a 12:12 light cycle and remained in the colony room for ~4 months until the time of experimentation.

Habituation and Chronic Constriction Injury (CCI).

Rats were housed in the colony room for 4 months prior to the initiation of experiments. They were then habituated for a week for an hour a day. Chronic constriction injury model is a rat model of neuropathic pain based on a unilateral loose ligation of the sciatic nerve. Chronic constriction injury (CCI) consisted of a single loose ligation of the left sciatic nerve using (4-0).



Intrathecal (i.t.) injection

On Day 10/11 post-surgery, lightly anesthetized rats received a single i.t. injection via acute subarachnoid puncture with drug delivered at the spinal level of L5/L6 via a sterile PE-10 catheter threaded 3 cm rostrally.

Behavioral Analysis

Hindpaw withdrawal responses to light touch were assessed using the von Frey fiber test. Behavior was assessed at baseline (BL) and days 3 and 10 or 11 following CCI for all groups. Rats were reassessed for light touch hindpaw response at 30, 60, and 90 minutes after the injection. Raw behavioral responses were then entered into the software program, PsychoFit (L. Harvey, University of Colorado), to acquire a 50% absolute threshold paw withdrawal response rate and the data were plotted using GraphPad Prism.

i.t. Dexpramipexole in Allodynic Male PAE and Sac Rats

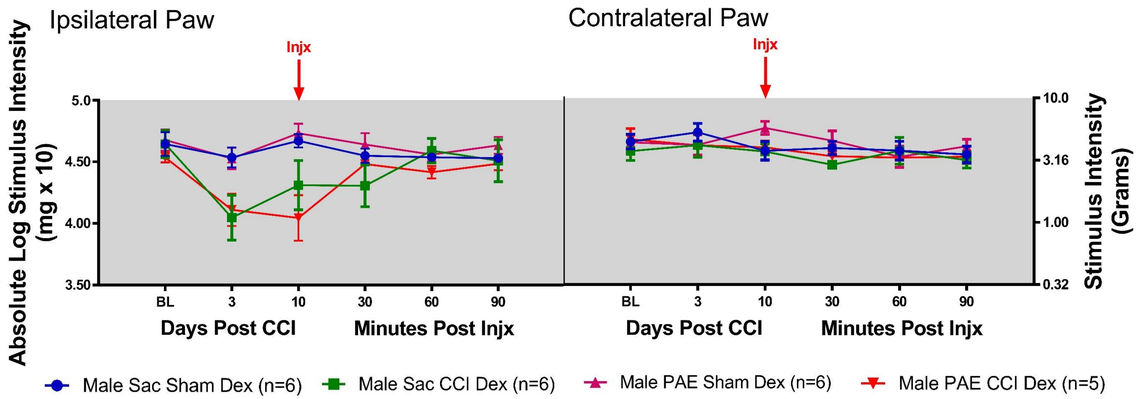


Figure 1: Intrathecal injection of dexpramipexole reverses allodynia in male Sac and PAE rats. Male Sac and PAE rats subjected to a single suture CCI develop ipsilateral allodynia 3 days after the CCI, and this allodynia persists through day 10 or 11. Following i.t. dexpramipexole, allodynia reversal was observed 30 minutes later, with hindpaw threshold responses similar to those of Sham-operated rats. Maximal reversal occurs at 60 and 90 min in both Sac and PAE rats. Both PAE and Sac male rats fail to develop contralateral allodynia, as predicted. Day 3 interaction p = 0.0215; Day 10 surgery p = 0.0003

IL-1β After Dexpramipexole Treatment in Female PAE and Sac Rats

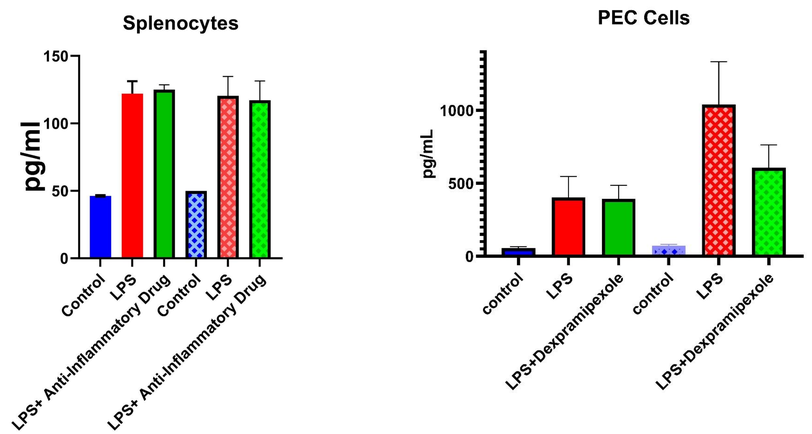


Figure 2: IL-1β levels in splenocytes from female PAE rats were not significantly reduced following stimulation with LPS in combination with dexpramipexole. However, in PEC cells collected from female rats, a reduction in IL-1β levels was observed following LPS stimulation in combination with dexpramipexole treatment. Main interaction of LPS and LPS / Dex; p = 0.0048.

Methods (Continued)

Tissue Collection

Splenocytes were prepared and plated at 0.4 million cells/well. PEC cells were enriched for macrophages described previously[2,3] and plated at 0.3 million cells/well. Both sets of cells were cultured in 24 well tissue culture plates, and both sets of cells were stimulated for 24 hours with 1ug/ml LPS and 2ug/ml dexpramipexole. Cells were collected and analyzed for IL-1β protein content via ELISA assay (R&D Systems**).**

Results

- In male PAE and SAC rats, robust ipsilateral allodynia occurred on days 3 and 10 following CCI surgery. In PAE rats, this allodynia was reversed 30 minutes after the i.t. injection of dexpramipexole, with hindpaw withdrawal thresholds returning to normal sensitivity for at least one hour. The allodynia in SAC rats was reversed 60 minutes after the i.t. injection of dexpramipexole. Both PAE and SAC rats did not develop contralateral allodynia.
- A mild CCI surgery causes greater sciatic nerve injury and induces robust allodynia in both SAC and PAE male rats on Days 3 and 10 after CCI.
- Both splenocytes and PEC cells showed an increase in IL-1β levels following stimulation with LPS. IL-1β levels were significantly reduced in PEC cells from PAE offspring following treatment of the cells with LPS in combination with dexpramipexole. In splenocytes, neither SAC nor PAE cells showed a decrease in IL-1β levels following treatment with LPS in combination with dexpramipexole.

Conclusions

- Both PAE and non-PAE male rats develop allodynia from mild left sciatic nerve injury.
- I.t. injection of dexpramipexole reverses allodynia induced by CCI surgery in PAE and non-PAE male rats.
- Treatment of PAE and SAC splenocytes and PEC cells with LPS effectively raises IL-1β expression in splenocytes and PEC cells.
- Treatment of PEC cells with dexpramipexole is effective in reducing IL-1β following stimulation with LPS

References

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Acknowledgements

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