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Effect of dexpramipexole in Neuropathic pain in prenatal alcohol exposed female subjects

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Introduction

- Neuropathic pain can be described as the damage caused to the somatosensory system manifested as an increase in sensitivity to light choice, clinically referred to as allodynia.
- Prenatal alcohol exposure is a risk factor for developing allodynia via enhanced neuroimmune, proinflammatory actions in the central nervous system (CNS) such as the spinal cord.
- Neuropathic pain is mediated by cytokines secreted from infiltrating peripheral immune cells to CNS and glial cells that reside within the CNS.
- Interelukin-10 (IL-10) is a potent anti-inflammatory cytokine that inhibits the production of a variety proinflammatory cytokines and chemokines released by different types of immune cells such as T helper (Th1) and natural killer (NK) cells, and macrophages, which prevent further tissue damage.
- Interferon-γ (IFNγ) is a soluble cytokine that serves as a T cell and macrophage activator in response to inflammation and can further enhance inflammatory processes.
- While pramipexole serves as a dopamine receptor agonist (D2/D3 receptors) used to reduce progressive movement disorders observed in Parkinson's disease, dexpramipexole [(+)-pramipexole] is an enantiomer of (-)-pramipexole and has very weak actions at the D2/D3 receptors. However, dexpramipexole reduces caspase-3 activation, and decreases cell death, suggesting that this compound may play a role in anti-inflammatory processes.
- Prior unpublished data from our lab show that an i.v. injection of dexpramipexole reverses ongoing allodynia in non-PAE mice.
- Further unpublished work from our lab revealed intrathecal (i.t.; subarachnoid, surrounding the spinal cord) dexpramapixole reverses chronic allodynia in PAE male rats that were susceptible to developing allodynia from minor peripheral nerve injury.
- In the current experiments, we administered an i.t. injection to female rats to test whether dexpramipexole can act directly on spinal glia and spinal immune cells to reverse allodynia.
- In a parallel experiment, to test the possible anti-inflammatory effect of dexpramipexole on peripheral immune cells, cells were collected from the spleen because these cells include a large population of T cells.
- In vitro manipulation of splenocytes with an immune stimulator was performed to determine if co-treatment with dexpramipexole could elevate mRNA levels of IL-10 while simultaneously decreasing IFNγ mRNA levels.

Hypothesis
Direct i.t. injection of dexpramapixole will reduce allodynia in female rats and increase IL-10 mRNA while decreasing IFN mRNA levels from splenocytes.

Methods

Development of Sac and PAE rats:
Rat dams were provided Sac-sweetened alcohol (5%) or Sac-sweetened water for 4 hours/day during gestation (BECs: ~60 mg/dl).

Habituation and Chronic Constriction Injury (CCI).
Long-Evans 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 (CCI) model is a rat model of neuropathic pain based on a unilateral loose ligation of the sciatic nerve. Chronic constriction injury consisted of a single loose ligation of the left sciatic nerve using (4-0).

Intrathecal (i.t.) injection
Lightly anesthetized rats received a single i.t. injection via acute subarachnoid puncture with drug delivered via a sterile PE-10 tubing threaded 3 cm rostrally to the spinal cord L5/L6 level. Injections occurred on Day 10/11 post-surgery. Immediately following drug administration, tubing is removed, and rats are allowed to recover before being placed in their home cage. All rats recovered within 5 min of drug administration and resumed normal locomotor function prior to being placed back into their home cages.

Dexpramipexole effect on Allodynia in Female Rats

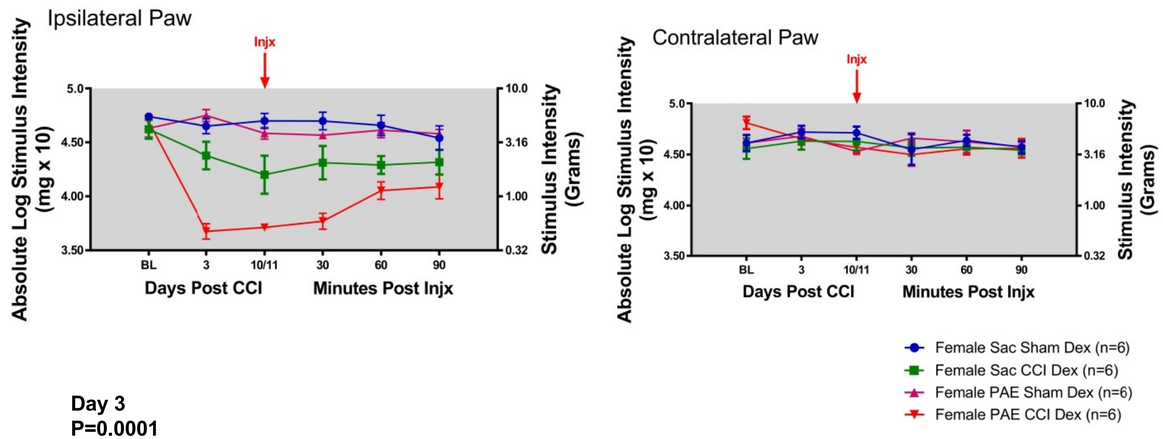

Figure 1: Intrathecal dexpramipexole does not significantly reverse allodynia. Following CCI, female saccharin (SAC) rats develop a weak ipsilateral allodynia that persists through day 10 or 11. Rats with prenatal alcohol exposure (PAE) develop a much greater ipsilateral allodynia compared to SAC rats, with allodynia persisting through day 10 or 11. In both cases allodynia is not significantly reversed by i.t. dexpramipexole. Both PAE and SAC fail to develop contralateral allodynia, as predicted.

Dexpramipexole effect on inflammatory cytokines in cells from female rats

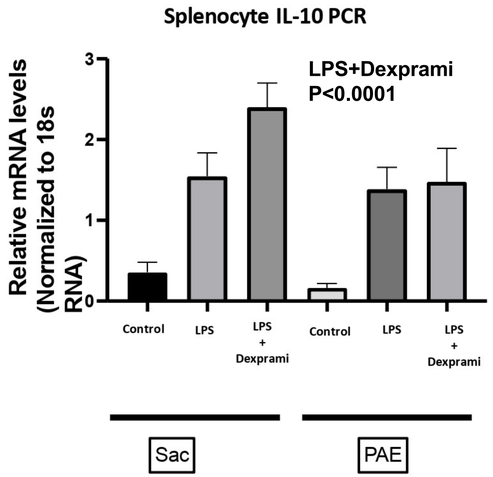

Figure 2: Dexpramipexol induces greater production of IL-10 when stimulated with LPS in splenocytes from SAC treated rats but not in cells from PAE rats. Prenatal alcohol treated offspring reveal blunted IL-10 production from dexpramipexole with LPS stimulated compared SAC controls. These data suggest that spinal application of dexpramipexole fails to elevate IL-10 levels to achieve reversal from allodynia in females.

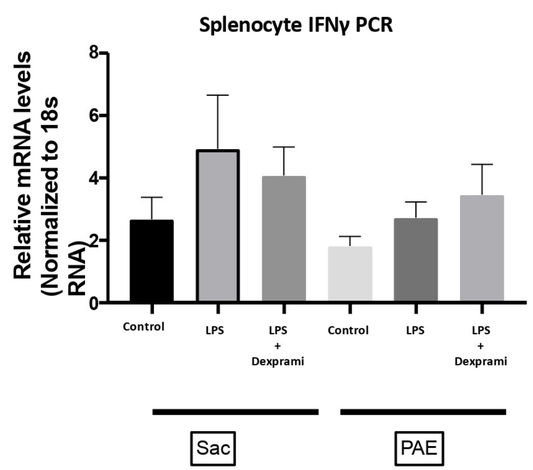

Figure 3: Dexpramipexol does not significantly reduce IFNγ mRNA in splenocytes from Sac rats nor from splenocytes from PAE when stimulated with LPS. Prenatal alcohol treated offspring did not reveal statistically significant difference in the production of IFNγ when treated with dexprampipexole.

Methods

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 surgery 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 computer program, PsychoFit (L. Harvey, University of Colorado), to acquire a 50% paw withdrawal threshold response rate, and finally, the data were plotted using GraphPad Prism.

Spleen cell collection
Cells were plated 0.4 million/per well in a 24 well tissue culture plate. Cells were stimulated for 24 hr with lipopolysaccharide (LPS; 1ug/ml) and dexpramipexole at 2ug/ml

RNA Extraction and Real-Time PCR
RNeasy Micro Kit (Qiagen) was used to process tissues for total RNA extraction from spleenocyte cell culture. The concentration and purity of RNA were then assessed using Quibit (Invitrogen) technology. The initial concentration of each sample varied to reach a minimum concentration of 2ng/ul. Reverse transcription to obtain cDNA was performed, and for the endogenous controls, was diluted 1:50, while the target genes Il-10 and IFNγ were undiluted. The target genes were measured in real time with a polymerase chain reaction (qRT-PCR) with Taqman Gene Expression Assays (ThermoFisher Scientific)

Two-way Anova Test
Result where analyzed for statistical significance with a two-way ANOVA test. In the case of the behavioral results, the independent variables where Sac vs. PAE and Sham vs. CCI. We preformed one test per time period (BL, day 3, 10 or 11 after surgery, and 30, 60, and 90 minutes after injection. In the case of the polymerase chain reaction our variables were alcohol exposure. (saccrine or prenatal alcohol exposure)

Conclusions

Our female rat model of PAE and neuropathic pain shows that damage to the sciatic nerve results in clear susceptibility to developing neuropathic pain (allodynia). Furthermore, peripheral immune cells such as splenocytes (consisting predominantly of T cells) from PAE rat offspring reveal a blunted anti-inflammatory response. In terms of the effectiveness of dexpramipexole, we can see that PAE offspring do not show significant reversal of allodynia after an i.t. injection. This could be because the dose used was too low, and larger doses may be required to reverse allodynia in these female rats. On the other hand, data from cell culture qRT-PCR suggest that immune cells from PAE rats reveal a blunted anti-inflammatory response to dexpramipexole treatment, while immune cells from Sac controls robustly produce IL-10 in response to dexpramiexole. In splenocytes from either Sac or PAE rats, dexpramipexole did not significantly alter the proinflammatory T cell cytokine, IFNγ.

Reference

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Acknowledgment

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