CBN blocks LPS-induced inflammatory effects in mice

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INTRODUCTION

Opioids are the gold standard in pain management
- Have negative side effects; high abuse potential
- Need for novel treatments

The endocannabinoid system maintains homeostasis and modulates pain and inflammation
- Cannabinoids are analgesic–limited by psychotropic effects
  - e.g., THC

Cannabinol (CBN) is a minor phytocannabinoid
- Lesser characterized component of cannabis
- Availability and use are on the rise

Lipopolysaccharide (LPS) is a component of gram-negative bacteria
- Used to induce an acute inflammatory response

The current study aimed to assess the analgesic and anti-inflammatory potential of the minor cannabinoid CBN

HYPOTHESES

1. **CBN will block LPS-induced paw edema, mechanical allodynia, and temperature preference.**
2. **CBN will block LPS-induced proinflammatory cytokine production**

METHODS

Subjects: 40 adult male and female C57BL/6J mice were used. Each treatment group was stratified by sex (i.e., 50% mice of each sex), but otherwise mice were randomly assigned. A power analysis of my own preliminary data, using a power of 0.8, an alpha of 0.05, and medium effect sizes, indicated a required minimum sample size of 8 mice per treatment, so we used 10 mice (5F/5M) per group to account for possible attrition during the experiment. All experimental procedures were approved by the IACUC at UConn.

Drugs: the minor cannabinoid cannabinol (CBN) was generously provided by our collaborator, Dr. David Sarlah (University of Illinois), and the steroid dexamethasone (dex) was purchased from Sigma (St. Louis, MO). All drugs were warmed to room temperature and dissolved into a vehicle consisting of 5% ethanol, 5% Kolliphor EL, and 90% normal saline by volume (i.e., 1:1:18 vehicle) prior to injecting (i.p.) at a volume of 10 µL/g body mass.

Procedure: Inflammatory pain was induced by injecting 25µg LPS from Escherichia coli 026:B6 Sigma (St. Louis, MO, USA) in 50µl of physiological saline into the plantar surface of both hind paws of each mouse. In control mice, 50µl of sterile saline will be injected into both hind paws. Paw thickness was measured both before and 24 h after LPS injection, using digital calipers (Mitutoyo, Japan) and expressed to the nearest 0.01 mm. Mechanical allodynia and thermal preference were assessed 24 h after LPS injection using von Frey filaments (North Coast Medical, Morgan Hill, CA) and a thermal gradient ring, respectively.

ELISA: at the study's conclusion, hind paw soft tissue was collected, homogenized in PBS, and measured, in duplicate via enzyme-linked immunosorbent assay (ELISA), for cytokines involved in the inflammatory response.

CBN reduces paw inflammation

CBN reduces mechanical allodynia

CBN does not shift thermal preference in TGR, but does reduce locomotion

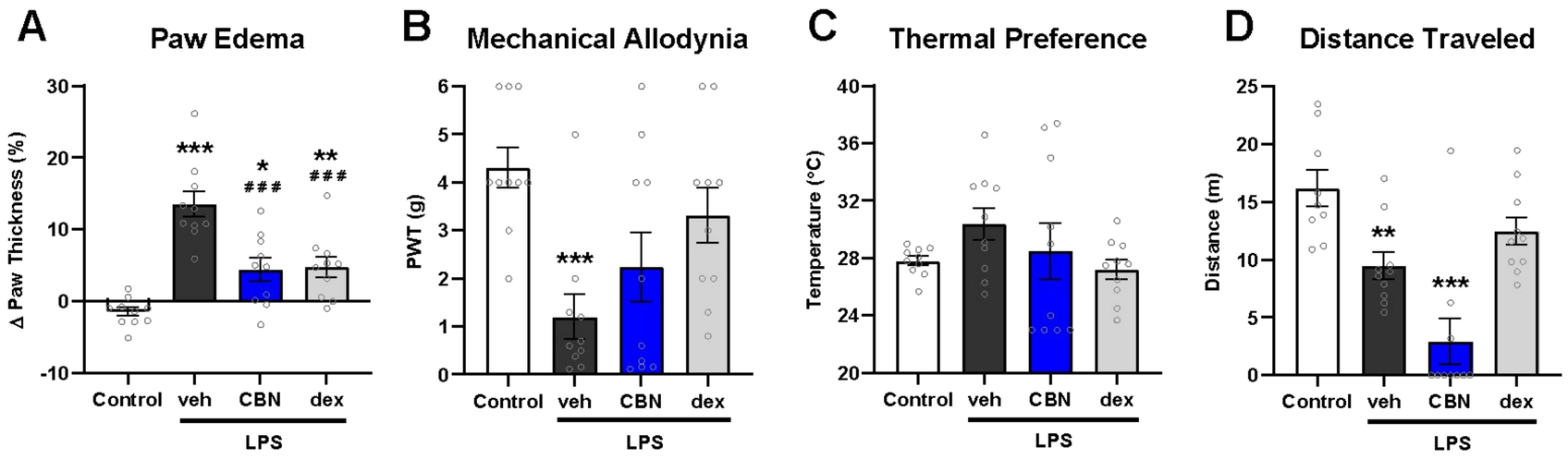

Fig 1. CBN blocks LPS-induced pain and inflammation. All data are expressed as mean ± SEM (n=10 [5M/5F]). *-***p<0.05-0.0005 vs control; ###p<0.0005 vs LPS.

CBN blocks LPS-induced cytokine increase in hind paws

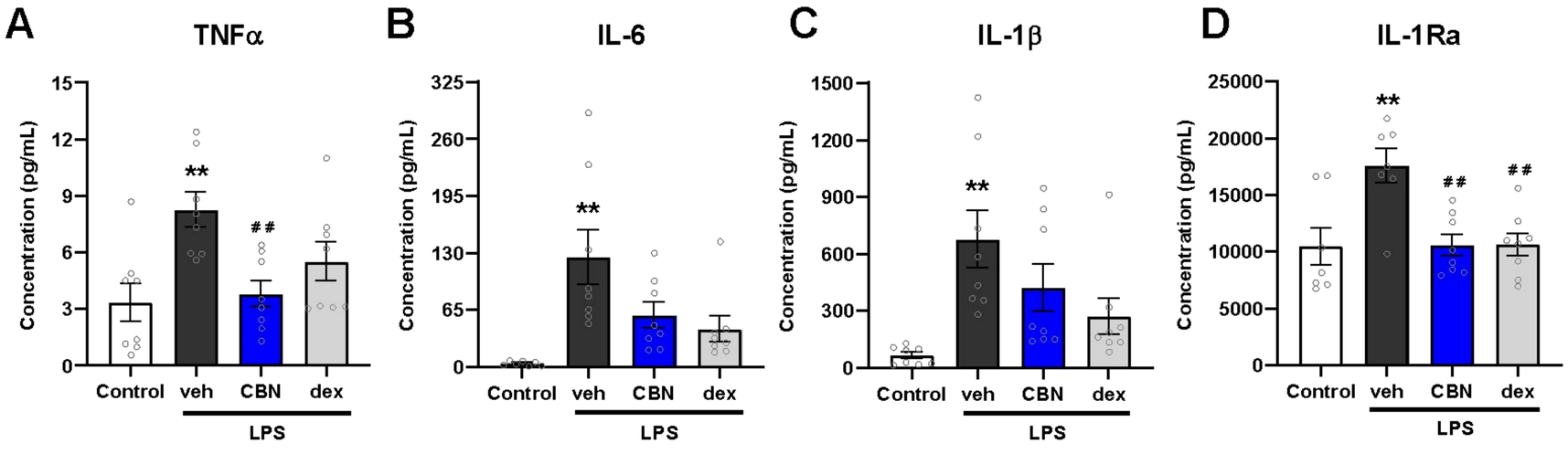

Fig 2. CBN blocks the increase in cytokines caused by LPS. All data are expressed as mean ± SEM (n=10 [5M/5F]). *[*]p<0.005 vs control; [##]p<0.005 vs LPS.

CONCLUSIONS

- **CBN blocked paw edema and mechanical allodynia caused by LPS**
- **CBN blocked LPS-induced proinflammatory cytokine increase**

The minor phytocannabinoid CBN is anti-inflammatory and analgesic

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