Different roles of T-type calcium channel isoforms in hypnosis induced by an endogenous neurosteroid epipregnanolone
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Background: Common general anesthetics that target GABA A and NMDA receptors are associated with developmental neurotoxicity in rodents and non-human primates. Hence, it is important to investigate new hypnotic agents with different mechanisms of action. Epipregnanolone ([3β,5β]-3-hydroxyepipregnan-20-one) is an endogenous neuroactive steroid that blocks T-type calcium channels but lacks any GABA-mimetic and NMDA receptor-blocking properties. Here, we utilized mouse genetics, behavioral experiments, and EEG analysis to investigate potential sedative/hypnotic and immobilizing properties of epipregnanolone (EpiP).

Methods:
- **Loss of Righting Reflex**
  - Flip mouse
  - If mouse doesn’t re-right in 30s = LORR
- **Loss of Withdrawal Reflex**
  - Pinch tail with alligator clip
  - If no response for 30s = LOWR

**Figure 1:** Epipregnanolone is a dose dependent hypnotic agent
A. Dose-dependent decrease in time to LORR with increasing concentration of epipregnanolone. B. Dose-dependent increase in LORR duration with epipregnanolone. C. The dose at which half of animals underwent LORR (ED 50) with epipregnanolone is 72.53 ± 4.00 mg/kg

**Figure 2:** Epipregnanolone significantly lowers isoflurane concentration necessary to immobilize WT mice.
A. A low dose of epipregnanolone (EpiP) yielded a significant decrease in the isoflurane concentration necessary to induce LORR. B. Epipregnanolone lowered the concentration of isoflurane necessary to immobilize WT mice and inhibit LOWR

**Figure 3 - Total EEG power is increased after EpiP injections**
Analysis of recordings from 11 animals. A. Representative heat maps during baseline recordings and 30 minutes after EpiP injection. B. Total (left) and relative (right) power 15 minutes after EpiP i.p. injection. Analysis of total power revealed increase in δ, β, α and γ frequency. Analysis of relative power revealed increase in δ and drop in a frequency range after EpiP. C. Total (left) and relative (right) power 30 minutes after i.p. injection of the neurosteroid. Analysis of total power revealed increase in δ, β, α and γ frequency. Analysis of relative power revealed increase in β and drop in δ frequency.

**Figure 4 - Total and relative EEG power during baseline recordings, 15 and 30 min after neurosteroid injections.**
Analysis of recordings from 11 animals. A. Representative heat maps during baseline recordings and 30 minutes after EpiP injection. B. Total (left) and relative (right) power 15 min after EpiP i.p. injection. Analysis of total power revealed increase in δ, β, α and γ frequency. Analysis of relative power revealed increase in δ and drop in a frequency range after EpiP. C. Total (left) and relative (right) power 30 minutes after i.p. injection of the neurosteroid. Analysis of total power revealed increase in δ, β, α and γ frequency. Analysis of relative power revealed increase in β and drop in δ frequency.

**Figure 5 - Knockout of the CaV3.1 channel confers resistance to the hypnotic effects of epipregnanolone.**
A. No dose-dependent changes in LORR onset in CaV3.1 KO mice (p = 0.3661). B. Dose-dependent hypnosis in CaV3.1 KO mice (p < 0.0001). C. No difference in LORR onset between CaV3.1 KO and WT mice (p = 0.4379). D. Male CaV3.1 KO mice demonstrate an overall shorter LORR duration than WT males (p = 0.0032).

**Figure 6 - EpiP exerts dose-dependent hypnosis in CaV3.2 KO mice with delayed induction but same duration when compared to WT mice.**
A. CaV3.2 KO mice exhibited dose-dependent LORR onset in response to EpiP (p < 0.0001). B. EpiP generated a dose-dependent hypnosis in CaV3.2 KO mice (p = 0.0001). C. No difference in LORR onset between CaV3.2 KO and WT mice (p = 0.2939). D. LORR Duration in CaV3.2 KO male mice was not significantly different from WT male mice (p = 0.6134) and LORR duration was dose-dependent (p < 0.0001).

**Figure 7 - Epipregnanolone induces dose-dependent hypnosis over CaV3.3 KO mice that is significantly longer from WT mice at a high dose.**
A. Dose-dependent increase in LORR onset (p < 0.0001). B. Dose-dependent hypnosis duration in response to EpiP (p = 0.0006). C. No difference in LORR onset between CaV3.3 KO and WT mice (p = 0.4367). D. No significant difference in LORR duration between CaV3.3 KO and WT mice (p = 0.4567). Despite the insignificant finding, there appears to be a trend indicating that C3.3 KO mice show longer LORR duration than WT mice. Post-hoc analysis demonstrates that CaV3.3 KO mice exhibited longer LORR duration than WT at 100 mg/kg (p = 0.0173).

**Conclusions:**
- Epipregnanolone is an efficacious dose-dependent hypnotic in rodents.
- Epipregnanolone significantly lowers the required concentration of isoflurane needed to induce immobilization and loss of withdrawal to a painful stimulus.
- EEG changes are consistent with other sedative/hypnotic drugs.
- We noted differential response to epipregnanolone based on T-channel expression. WT mice ED 50: 54.1 mg/kg; CaV3.1 KO mice ED 50: 67.1 mg/kg; CaV3.2 KO mice ED 50: 56.1 mg/kg; CaV3.3 KO mice ED 50: 51.1 mg/kg

**Future Directions:**
- Investigate male to female differences in hypnotic response
- Consider other receptor targets of epipregnanolone in the brain

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