Preemptive Analgesic Effect of Intrathecal Applications of Neuroactive Steroids in a Rodent Model of Post-Surgical Pain

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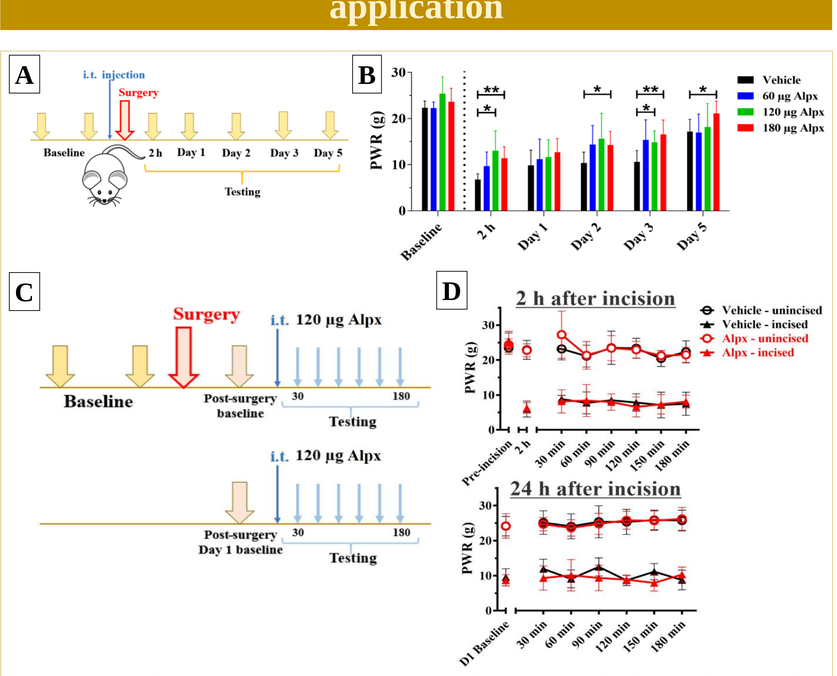
Introduction

Tissue injury is a common occurrence after surgery and is highly predictable. Recent surveys show that more than 80 % of patients suffer with acute post-operative pain. Opioids have been traditionally used to treat acute pain; however, it carries risks of side effects and habit-forming. Our project targets in finding new therapeutic modalities with a focus on preemptive application in blocking pain development.

Previous studies have been focusing on GABA-A receptors. However, calcium channels play important role in controlling calcium-modulated release of synaptic vesicles from presynaptic terminals in spinal dorsal horn. In this project, we utilize different neuroactive steroids (ECN, CNC24, and Alphaxalone) in comparison to opioid to examine preemptive antinociceptive potential of each steroid.

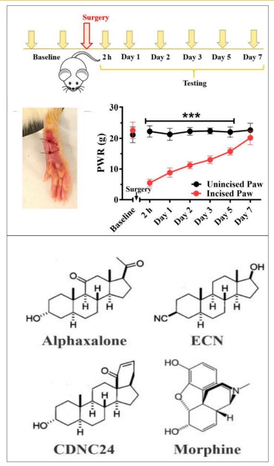
Alphaxalone increases the post-incisional mechanical hypersensitivity threshold in preemptive application



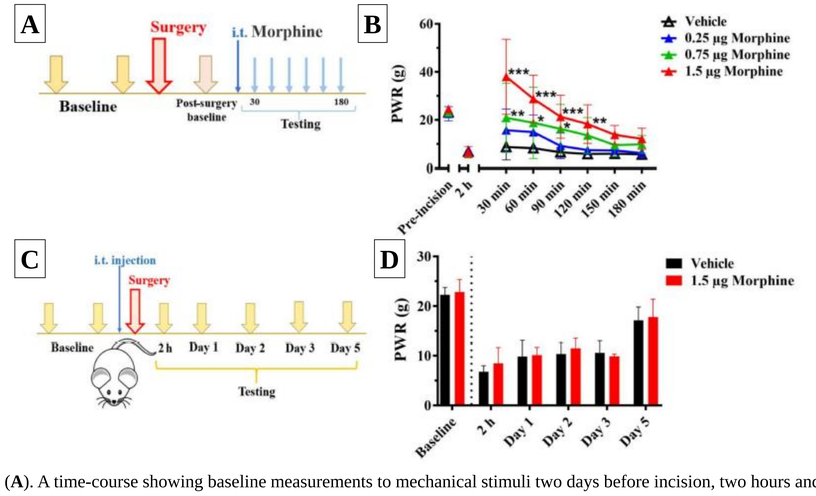
(A) A time course showing presurgical baseline measurements, the timing of intrathecal (i.t.) Alphaxalone (Alpx) injection and postsurgical testing time points. **(B)** The preemptive i.t. application of Alpx alleviated postincisional mechanical hypersensitivity in rats in a dose-dependent manner (n=9 for Vehicle, 60 µg and 120 µg Alpx groups; n=8 for the 180 µg Alpx group). Each data point is represented as mean + SD. *p<0.05 and **p<0.01 vs. Vehicle group, Two-way RM ANOVA followed by Tukey's *post hoc* test. **(C)** A time course showing baseline measurements to mechanical stimuli two days before incision, two hours and 24 hours after incision. The paw withdrawal responses were measured every thirty minutes after i.t. injection of 120 µg Alpx. **(D)** Repeated administration of 120 µg Alpx at 2 h (top; n=5 per group) or 24 h after incision (bottom; n=6 per group) does not alleviate mechanical hypersensitivity in rats. Each data point is represented as mean ± SD. Data was analyzed using Two-way RM ANOVA.

Method

- Incisional pain model (upper panel)
  - Female Sprague Dawley rats by performing a 1-cm longitudinal incision on the plantar muscle of rat hind paw. Animals were allowed to recovery 2 hours in their cages.
- Drugs (lower panel)
  - Alphaxalone, ECN, and CDNC24 were dissolved in 15% Cyclodextrin solution and pH-balanced to 7.4
    - Alphaxalone: combined GABAergic agent and T-channel inhibitor (low-voltage activated-type Ca^{2+} channels)
    - ECN: T-channel inhibitor
    - CDCN24: GABAergic agent
  - Morphine was dissolved in saline
- Intrathecal Injection:
  - Injection site at L4-L6 lumbar region either experimental compound or vehicle with total volume of 50 µL.
- Mechanical sensitivity:
  - Using the electronic Von Frey apparatus with single rigid filament that exert pressure to plantar surface of the paw up to 50 g.
  - Maximum force was exerted until animal withdraw response and the paw withdrawal response (PWR) in unit of force in grams was recorded.

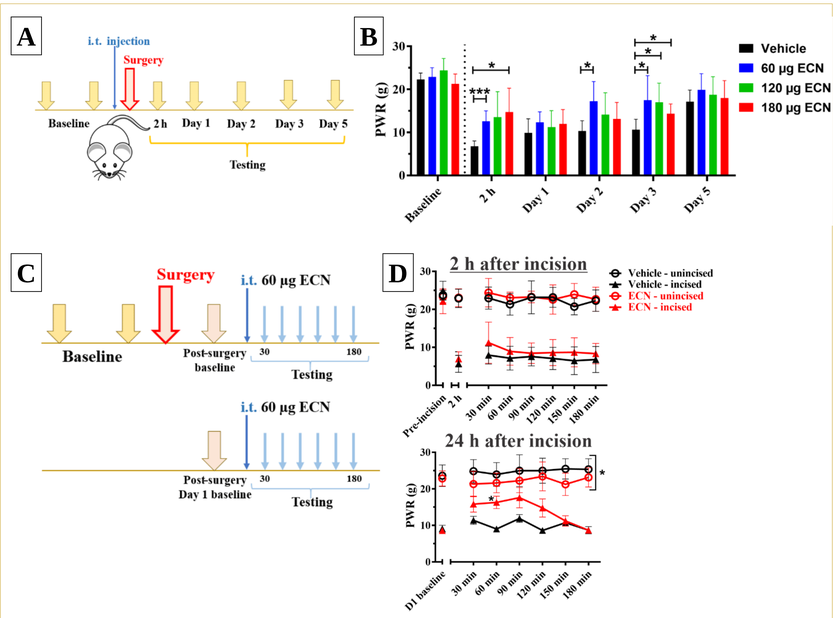


The preemptive administration of morphine does not alleviate post-procedure mechanical hypersensitivity



(**A**). A time-course showing baseline measurements to mechanical stimuli two days before incision, two hours and 24 h after incision. The paw withdrawal responses were measured every thirty minutes after intrathecal (i.t.) injection of morphine (0.25 g, 0.75 g or 1.5 g). (**B**). Morphine significantly increases the mechanical sensitivity threshold in the incised paw in a dose-dependent manner (n = 8 for Vehicle; n = 7 for 0.25 g, n = 9 for 0.75 g and n = 8 for 1.5 g morphine groups). Each data point is represented as mean SD. * p < 0.05, ** p < 0.01 and *** p < 0.001 vs. Vehicle group, Two-way ANOVA followed by Tukey's post hoc test. (**C**). A time-course showing pre-surgical baseline measurements, the timing of i.t. morphine injection and post-surgical testing time points. (**D**). Preemptive treatment of 1.5 g morphine does not reduce mechanical hypersensitivity after surgery (n = 9 for Vehicle and n = 5 for 1.5 g morphine group). Each data point is represented as mean + SD. Data were analyzed using Two-way ANOVA.

ECN increases the post-incisional mechanical hypersensitivity threshold in preemptive application and also in post-procedure application



(A) A time course showing presurgical baseline measurements, the timing of intrathecal (i.t.) ECN injection and post-surgical testing time points. **(B)** The preemptive i.t. application of ECN alleviated post-incisional mechanical hypersensitivity in rats in a dose-dependent manner (n=9 for Vehicle; n=8 for 60 µg, n=7 for 120 µg and n=8 for 180 µg ECN groups). Each data point is represented as mean + SD. *p<0.05 and ***p<0.001 vs. Vehicle group, Two-way RM ANOVA followed by Tukey's *post hoc* test. **(C)** A time course showing baseline measurements to mechanical stimuli two days before incision, two hours and 24 hours after incision. The paw withdrawal responses were measured every thirty minutes after i.t. injection of 60 µg ECN. (**D)** A single i.t. injection of 60 µg ECN two hours after incision does not affect mechanical hypersensitivity (top; n=6 for Vehicle and n=9 for ECN groups). Second dose of ECN 24 hours after incision significantly increases the mechanical sensitivity threshold in the incised paw (bottom; n=7 for Vehicle and n=8 for ECN groups). Each data point is represented as mean ± SD. *p<0.05 vs. Vehicle group, Two-way RM ANOVA followed by Sidak's *post hoc* test.

Discussion

- In contrast to GABAergic potentiator with no analgesic effect on incisional pain, preemptive blockade of low-voltage activated-type Ca^{2+} leads to lasting relief of post-incisional pain.
- Previous studies suggested that several subtypes of voltage-gated calcium channels play a crucial role in shaping action potentials and controlling cellular excitability and synaptic neurotransmission in pain pathway.
- Our study shows that neuroactive steroids with a core pregnane ring system carry this role and have analgesic properties with specific cellular-targeted signaling (T-channels), suggesting the importance of T-channels in a multimodal approach in future pain management..
- Complete paper can be found on:

Tat QL, Joksimovic SM, Krishnan K, Covey DF, Todorovic SM, Jevtovic-Todorovic V. Preemptive Analgesic Effect of Intrathecal Applications of Neuroactive Steroids in a Rodent Model of Post-Surgical Pain: Evidence for the Role of T-Type Calcium Channels. *Cells*. 2020;9(12):2674. Published 2020 Dec 12. doi:10.3390/cells9122674