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Analgesia by intrathecal delta-9-tetrahydrocannabinol is dependent on Cav3.2 calcium channels

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Abstract

Delta-9-tetrahydrocannabinol (Δ^9-THC) is known to produce systemic analgesia that involves CB_1 and CB_2 cannabinoid receptors. However, there is compelling evidence that Δ^9-THC can potently inhibit Cav3.2T-type calcium channels which are highly expressed in dorsal root ganglion neurons and in the dorsal horn of the spinal cord. Here, we investigated whether spinal analgesia produced by Δ^9-THC involves Cav3.2 channels vis a vis cannabinoid receptors. We show that spinally delivered Δ^9-THC produced dose-dependent and long-lasting mechanical anti-hyperalgesia in neuropathic mice, and showed potent analgesic effects in models of inflammatory pain induced by formalin or Complete Freund's Adjuvant (CFA) injection into the hind paw, with the latter showing no overt sex differences. The Δ^9-THC mediated reversal of thermal hyperalgesia in the CFA model was abolished in Cav3.2 null mice, but was unaltered in CB_1 and CB_2 null animals. Hence, the analgesic effects of spinally delivered Δ^9-THC are due to an action on T-type calcium channels, rather than activation of spinal cannabinoid receptors.

Keywords Δ^9-THC, Cannabinoid receptors, Cav3.2 channel, Analgesia, Pain

T-type Ca^{2+} channels are known to be important regulators of pain transmission in primary afferent sensory neurons and the spinal cord [1]. Among the three isoforms of T-type Ca^{2+} channels that are expressed in the mammalian genome, the Cav3.2 channel isoform appears to be the predominant T-type channel subtype involved in this process [2]. It is expressed in a subpopulation of primary afferent fibers and the spinal dorsal horn [3], and its expression is enhanced in these tissues in a wide range of chronic pain conditions in rodents [1]. Consequently, systemic or intrathecal delivery of T-type channel inhibitors mediates analgesia (for review see [1, 4]. T-type channels can be inhibited by different types of endocannabinoids [5], terpenes [6] and phytocannabinoids such as cannabidiol and Δ^9-THC [7, 8]. In particular, Δ^9-THC mediates strongly state dependent inhibition of Cav3.2 channels with a preference for binding to inactivated channels [7, 8]. It is known that spinally delivered Δ^9-THC inhibits mechanical and cold allodynia in models of neuropathic pain [9], and analgesia exerted by Δ^9-THC delivered to the brain involves modulation of both CB_1 and CB_2 receptors [10]. However, it is unclear whether the spinal actions of Δ^9-THC involve T-type channels, cannabinoid receptors, or a combination thereof. Thus, the present study was designed to investigate contributions of spinal CB receptor subtypes and Cav3.2 channels on the antihyperalgesic effect of spinally delivered Δ^9-THC. All experiments were carried out with approval of an animal protocol by the Institutional Animal Care and

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Use Committee, and all efforts were made to minimize animal suffering according to the policies and recommendations of the International Association for the Study of Pain. Δ^9-THC was delivered by intrathecal injection as described before [6, 8] into male and female C57BL/6J (wild-type), or male CB1 null, CB2 null, or Cav3.2 null mice (20 − 25 g, 8–10 weeks; Jackson Laboratories). We first assessed the analgesic action of spinally delivered Δ^9-THC in the acute nociceptive (phase 1) and inflammatory pain (phase 2) phases of a standard formalin test [11]. Intrathecally delivered Δ^9-THC, 20 min before testing, significantly and dose-dependently reduced the duration of nocifensive responses in the first (Fig. 1a) and second (Fig. 1b) phases of the formalin test. Next, we verified whether spinal Δ^9-THC was also able to inhibit mechanical hyperalgesia caused by chronic neuropathy 21 days after partial sciatic nerve injury which was performed as described by us before [11]. Mechanical hyperalgesia was measured using a Dynamic Plantar Aesthesiometer (Ugo Basile, Varese, Italy). When compared to the neuropathic control group, treatment of mice with Δ^9-THC (10.0 µg/i.t.), but not vehicle (PBS, 10 µl/i.t.) produced marked anti-hyperalgesia when evaluated 45 min after treatment (Fig. 1c). These data show that Δ^9-THC mediates robust analgesia in wild type mice. Next, we investigated the effect of Δ^9-THC in a model of persistent inflammatory pain. 20 µl of Complete Freund's Adjuvant (CFA) were given intraplantarly (i.pl.) in the ventral surface of the right hindpaw, whereas sham groups received 20 µl of PBS. Thermal hyperalgesia was examined by measuring the latency to withdrawal of ipsilateral hind paws in response to a focused beam of radiant heat (IR = 30) using a plantar test apparatus (UgoBasile, Varese, Italy). Two days after CFA injection, intrathecal treatment with Δ^9-THC (10 µg/i.t) but not with vehicle (10 µl/i.t.) resulted in anti-hyperalgesia that remained significant up to 3 h (Fig. 1d). Δ^9-THC was also effective in increasing paw withdrawal latencies when delivered to female mice (tested 45 min after its spinal delivery, Fig. 1e). To determine whether the analgesic effects observed for spinally delivered Δ^9-THC were mediated by cannabinoid CB_1 or CB_2 receptors, we repeated the CFA model using male CB_1 (Fig. 1f) and male CB_2 (Fig. 1g) null mice in comparison with male wild-type mice that were simultaneously tested. For this purpose, mice were injected intrathecally with either vehicle (control) or Δ^9-THC (10.0 µg/i.t.) and tested 45 min later. Similar to wild type animals, Δ^9-THC produced significant analgesic effects, indicating that neither of these two receptors are essential for the observed analgesia even though this compound is an agonist of both receptor types [12].

We then tested the analgesic effect of spinal Δ^9-THC in Cav3.2 null mice. These mice develop CFA-induced hypersensitivity despite the absence of Cav3.2 channels [11], most likely due to compensatory mechanisms that are not fully understood. As shown in Fig. 1h, Δ^9-THC lost its analgesic effects when delivered to Cav3.2 null mice, indicating that the key biological target for spinally delivered Δ^9-THC are T-type channels.

There is considerable evidence that CB_1 receptor activation mediates analgesia [13], however there are also reports that the analgesic activity of Δ^9-THC is lost in CB receptor null mice [10, 14]. We do not challenge a possible involvement of these receptors when Δ^9-THC is delivered systemically. Our focus was to specifically isolate a spinal effect, and this can be cleanly accomplished by the intrathecal route of delivery used in our study (Additional file 1: Fig S1). What we do not know is the overall contribution of the spinal action to the overall analgesic properties of Δ^9-THC. We attempted testing the effect of systemically delivered Δ^9-THC in Cav3.2 null mice, however, we found that these mice became lethargic, thus confounding the types of pain behavioral measurements that we typically perform. Finally, our laboratory has previously reported that the analgesic effect of intrathecally delivered mixed CB receptor/Cav3.2 ligands are abolished in Cav3.2 null mice, but they retain activity upon blocking CB_1 receptors with AM-281 [15]. Interestingly, inhibition of CB_2 receptors with AM-630 did attenuate the analgesic effects of these compounds

(See figure on next page.)

Fig. 1 Δ^9-THC produces spinal analgesia in mice that is Cav3.2 channel-dependent. Dose response action of Δ^9-THC (delivered 20 min before formalin) in the **a** first and **b** second phases of the formalin test. Each bar represents the mean of 5 animals, error bars denote S.E.M. Data are representative of 2 independent sets of experiments. Statistical analyses were performed by two-way ANOVA followed by Tukey's test. Asterisks denote a significant difference, $^{**}P < 0.01$ and $^{***}P < 0.001$ when compared with the control groups. **c** Mechanical threshold of PSNI mice 45 min after treatment with Δ^9-THC (10 ug/i.t.). Bars represent the mean of 7 animals, error bars denote S.E.M. Data are representative of 2 independent sets of experiments. Two-way ANOVA followed by Tukey's test revealed significance, $^{###}P < 0.001$ and $^{****}P < 0.0001$ when compared with the control groups. **d** Time-course of the effect of Δ^9-THC (10 µg/i.t.) on thermal withdrawal latencies of CFA-injected male mice. **e** Effect on CFA-treated female mice when evaluated 45 min following treatment. In **d** and **e**, error bars are S.E.M. Data are representative of 2 independent sets of experiments Two-way ANOVA followed by Tukey's test revealed statistical differences, $^{*}P < 0.05$ $^{**}P < 0.01$ or $^{***}P < 0.001$ when the CFA + treated group is compared with the CFA + vehicle control group, and $^{###}P < 0.001$ when the PBS group is compared with the control groups. **f**, **g** Comparison of the effect of 10 µg/i.t. Δ^9-THC on CFA-injected wild type and **f** CB_1, **g** CB_2, and **h** Cav3.2 knockout mice. Each bar represents the mean of 6–10 mice, error bars are S.E.M. Data are representative of 2 independent sets of experiments. Two-way ANOVA followed by Tukey's test revealed statistical differences, $^{*}P < 0.05$ or $^{****}P < 0.0001$ when compared with the control group

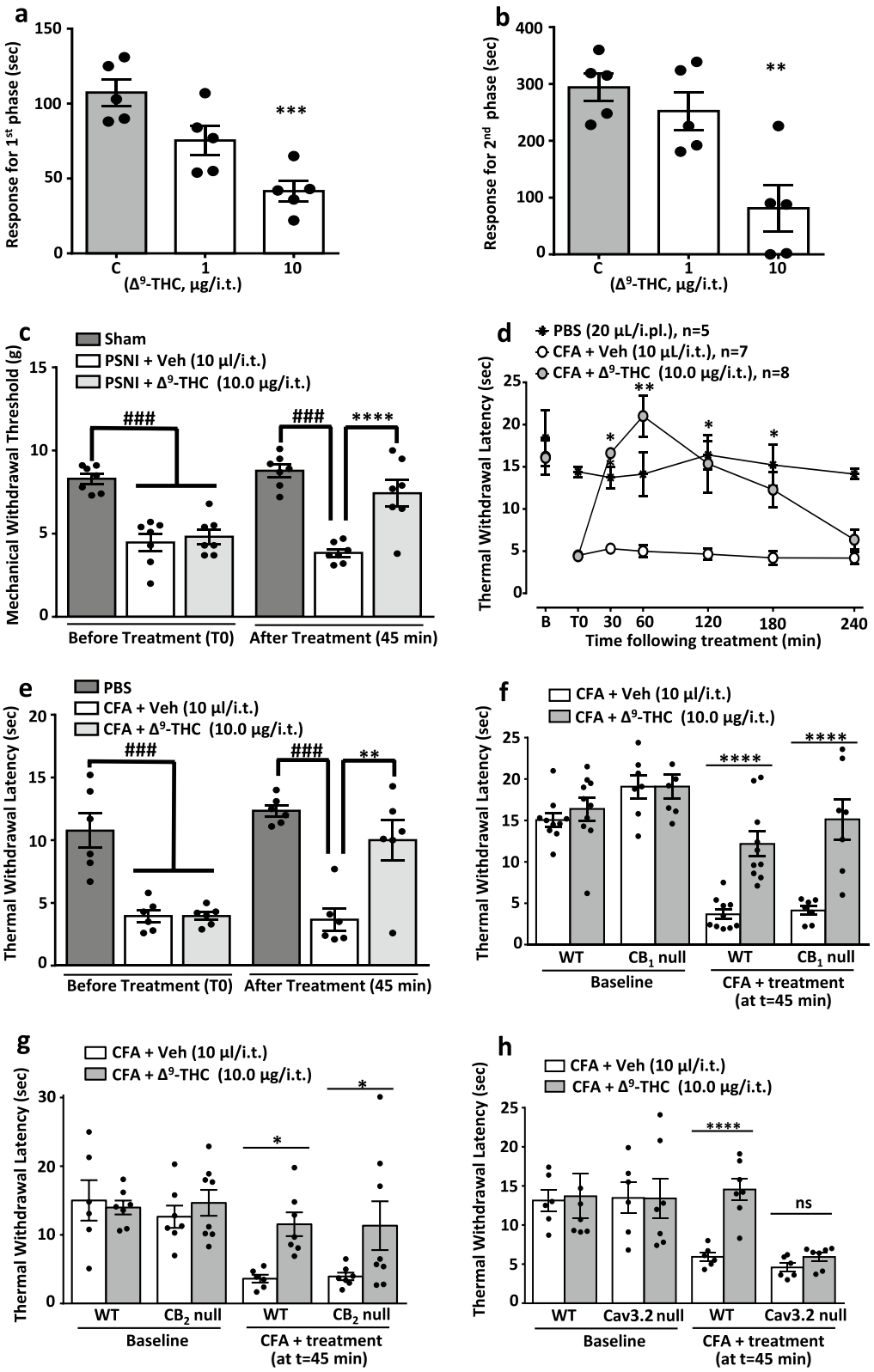

Fig. 1 (See legend on previous page.)

and we concluded that although CB_2 receptors may be involved in their actions, this may be due to CB_2 receptor modulation of Cav3.2 channel activity. Hence, we cannot rule out the possibility that Δ^9-THC might activate spinal CB_2 receptors which may in turn inhibit Cav3.2 in addition to the direct inhibitory actions of Δ^9-THC on these channels. We note that CB_1 receptors do not functionally inhibit Cav3.2 in heterologous systems [8] but we could at that time not explore such coupling for CB_2 receptors for technical reasons. Nonetheless, even if CB_2 receptors augment direct inhibition of Cav3.2 channels, our results clearly implicate Cav3.2 channels as an essential target of Δ^9-THC in the actions of spinal Δ^9-THC as an analgesic, whereas CB receptors are not required.

Abbreviations

Δ^9-THC	Delta-9-tetrahydrocannabinol
CB	Cannabinoid
CFA	Complete Freund's adjuvant
PSNI	Partial sciatic nerve injury
i.t.	Intrathecal

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13041-023-01036-8.

Additional file 1: Figure S1: Graphical representation of the primary afferent pain pathway. Intrathecal injection of Δ9-THC induces analgesia in mice lacking either the CB1 or the CB2 receptor, but not in Cav3.2 null mice.

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Author contributions
VMG and FTTA performed experiments. VMG and GWZ designed the experiments. VMG performed data analysis. VMG, FTTA and GWZ wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
This study was approved by the University of Calgary's ethics committees.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interest.

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References

1. Harding EK, Zamponi GW. Central and peripheral contributions of T-type calcium channels in pain. Mol Brain. 2022;15:39.
2. Bourinet E, Alloui A, Monteil A, Barrère C, Couette B, Poirot O, et al. Silencing of the Cav3.2 T-type calcium channel gene in sensory neurons demonstrates its major role in nociception. EMBO J. 2005;24:315–24.
3. François A, Schüetter N, Laffray S, Sanguesa J, Pizzoccaro A, Dubel S, et al. The low-threshold calcium channel Cav3.2 determines low-threshold mechanoreceptor function. Cell Rep. 2015;10:370–82.
4. Snutch T, Zamponi GW. Recent advances in the development of T-type calcium channel blockers for pain intervention. Br J Pharmacol. 2018;175:2375–83.
5. Chemin J, Monteil A, Perez-Reyes E, Nargeot J, Lory P. Direct inhibition of T-type calcium channels by the endogenous cannabinoid anandamide. EMBO J. 2001;20:7033–40.
6. Gadotti VM, Huang S, Zamponi GW. The terpenes camphene and alpha-bisabolol inhibit inflammatory and neuropathic pain via Cav3.2 T-type calcium channels. Mol Brain. 2021;14:166.
7. Ross HR, Napier I, Connor M. Inhibition of recombinant human T-type calcium channels by Delta9-tetrahydrocannabinol and cannabidiol. J Biol Chem. 2008;283:16124–34.
8. Harding EK, Souza IA, Gandini MA, Gadotti VM, Ali MY, Huang S, et al. Differential regulation of Cav 3.2 and Cav 2.2 calcium channels by CB1 receptors and cannabidiol. Br J Pharmacol. 2023. https://doi.org/10.1111/bph.16035.
9. Casey SL, Mitchell VA, Sokolaj EE, Winters BL, Vaughan CW. Intrathecal actions of the cannabis constituents Δ(9)-Tetrahydrocannabinol and cannabidiol in a mouse neuropathic pain model. Int J Mol Sci. 2022;23(15):8649.
10. Wang XF, Galaj E, Bi GH, Zhang C, He Y, Zhan J, et al. Different receptor mechanisms underlying phytocannabinoid- versus synthetic cannabinoid-induced tetrad effects: Opposite roles of CB1 /CB2 versus GPR55 receptors. Br J Pharmacol. 2020;177:1865–80.
11. García-Caballero A, Gadotti VM, Stemkowski P, Weiss N, Souza IA, Hodgkinson V, et al. The deubiquitinating enzyme USP5 modulates neuropathic and inflammatory pain by enhancing Cav3.2 channel activity. Neuron. 2014;83(5):1144–58.
12. Pertwee RG. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: Δ9-tetrahydrocannabinol, cannabidiol and Δ9-tetrahydrocannabivarin. Brit J Pharmacol. 2008;153:199–215.
13. Elikottil J, Guopota P, Gupta K. The analgesic potential of cannabinoids. J Opioid Manag. 2009;5:341–57.
14. Zimmer A, Zimmer AM, Hohmann AG, Herkenham M, Bonner TI. Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. Proc Natl Acad Sci U S A. 1999;96:5780–5.
15. Gadotti VM, You H, Petrov RR, Berger ND, Diaz P, Zamponi GW. Analgesic effect of a mixed T-type channel inhibitor/CB2 receptor agonist. Mol Pain. 2013;9:32.

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