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Repository Citation

Kozak, J. A. (2022). Suppression of Store-operated Calcium Entry Channels and Cytokine Release by Cannabinoids. *Function*, 3 (5), zqac044.

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PERSPECTIVES

Suppression of Store-operated Calcium Entry Channels and Cytokine Release by Cannabinoids

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Key words: calcium signaling; calcium measurement; calcium release activated calcium channels; minor cannabinoids; cytokine; lymphocyte; immunity

A Perspective on “Acidic Cannabinoids Suppress Proinflammatory Cytokine Release by Blocking Store-operated Calcium Entry”

A single *Cannabis sativa* plant can be the source of more than 120 cannabinoids.^{1,2} These include the two major neutral phytocannabinoids trans- Δ^9 tetrahydrocannabinol (Δ^9 THC) and cannabidiol (CBD), which are used as therapeutics for emesis, nausea, cancer-induced cachexia, metabolic syndrome, high intraocular pressure, and a variety of inflammatory conditions, including dry-skin syndrome, chronic pain, and edema. In addition, carboxylic acid derivative phytocannabinoids are synthesized by the plant and can be decarboxylated upon exposure to oxygen and heat, resulting in neutral cannabinoids.¹⁻³ The main acidic cannabinoids are Δ^9 tetrahydrocannabinolic acid (Δ^9 THCA), cannabichromenic acid (CBCA), cannabidiolic acid (CBDA), and cannabigerolic acid (CBGA). Albeit less studied than their neutral counterparts, they hold the advantage of being nonpsychoactive.¹ Cannabidiolic acid reduced inflammation, hyperalgesia, and edema in an inflammatory pain animal model,⁴ and Δ^9 THCA, CBCA, and CBGA have antiemetic properties. Additionally, CBGA had a cytotoxic effect on colon cancer cells.

Cannabinoids were previously shown to inhibit transient receptor potential (TRP) cation channels, some of which are expressed in nociceptors and transduce painful stimuli.⁵ In particular, acidic cannabinoids CBDA and CBGA can activate TRPV1

channels gated by capsaicin, heat, and acid pH. It has been suggested that the cannabinoid analgesic action is due to the subsequent TRPV1 channel desensitization. TRPM8 is expressed in nociceptive neurons where it is activated by cold temperatures and menthol, and Δ^9 THCA, CBDA, and CBGA potentially inhibit these channels.⁵ To what extent the observed modulation of TRP channels by cannabinoids in vitro accounts for their known analgesic effects is not yet fully understood.^{2,5}

The recent report by Faouzi *et al.*⁶ describes for the first time the inhibitory action of several acidic cannabinoids on store-operated calcium entry (SOCE) in immune cells. Store-operated calcium entry through the plasma membrane occurs through the calcium-release activated calcium (CRAC) channels, which open in response to ER calcium store depletion.⁷ While two major cannabinoids CBD and THC were largely ineffective, several acidic cannabinoids such as CBGA, potentially inhibited SOCE and CRAC channel currents at low micromolar concentrations. Channel blockade was slow in onset and largely irreversible. Store-operated calcium entry in Jurkat T cells treated with thapsigargin was sensitive to these purified cannabinoids with IC₅₀ values ranging from 530 nM (CBGA) to 24 μ M (CBCV, cannabichromevarin). Moreover, the secretion of interleukin 2 (IL2) cytokine was also suppressed. As is well known, IL2 synthesis and secretion are calcium-dependent, and inhibition of the main calcium entry channel in lymphocytes, CRAC, would be expected to suppress both processes. In resting T cells, nuclear factor of activated T cells (NFAT), a transcription factor

Submitted: 28 August 2022; Accepted: 29 August 2022

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belonging to a 4-member family, is located in the cytoplasm in a highly phosphorylated state.⁸ Upon activation of the T cell with antigen or mitogen, cytoplasmic calcium is elevated and NFAT is dephosphorylated by the calcium/calmodulin-dependent serine/threonine phosphatase calcineurin (protein phosphatase 2B). The dephosphorylated NFAT then translocates to the nucleus, where it binds to DNA and initiates various transcription programs necessary for the adaptive immune response, including IL2 and IL2 receptor (CD25) transcription. Conversely, reducing SOCE results in reduced activity of calcineurin keeping NFAT phosphorylated and therefore in the cytoplasm, where it is inactive.⁸ The importance of calcineurin/NFAT pathway in immunity is underscored by decrease in IL2 production by immunosuppressants tacrolimus (FK506) and cyclosporine, which inhibit calcineurin, preventing NFAT dephosphorylation and translocation into the nucleus.⁹ Using a luciferase-based plate reader assay, the authors find that CBGA inhibits NFAT activity at 3.3 μM in the absence of serum. Interestingly, the potency of the acidic cannabinoids in cell-based assays strongly depended on the serum concentration: in the presence of 10% serum, commonly used in cell culture, IC_{50} for CBGA jumped to 101 μM in the NFAT activity assay. Size exclusion chromatography showed that CBGA binds to serum albumins, explaining its reduced potency in the presence of serum. This finding will likely be important for determining the dosage in any future studies of acidic cannabinoids in animal models of inflammatory disease and pain. Another important factor in this context will be CBGA cytotoxicity exhibited above 20 μM .⁶

There is some evidence that blockade of SOCE may alleviate pain in animal models. Store-operated calcium entry inhibition with BTP2 (YM-58483) relieved complete Freund's adjuvant-induced inflammation and hyperalgesia as well as formalin-induced and acute pain.¹⁰ Since the dorsal root ganglion neurons, including nociceptive cells, express the CRAC channel components Orai and STIM,^{8,10} more experimentation will be required to determine the exact target(s) of SOCE inhibitors for pain: immune cells, neurons, or both.

The initial characterization of acidic cannabinoid inhibition of CRAC channels raises several interesting questions. What mechanism underlies the observed slow onset of inhibition and its irreversibility? Because IP_3 was present, ER calcium store refilling is unlikely, but the disruption of interaction between ER calcium sensor STIM and Orai channel is a possibility. Since CBGA did not inhibit from the cytoplasmic side, the slow time course is probably not due to drug diffusion through the plasma membrane. Would prolonged CBGA exposure be expected to prevent the refilling of calcium stores after T-cell receptors are engaged?

The authors examined the effects of individual cannabinoids not only in isolation but also in the presence of other cannabinoids. The goal here was to test whether simple additivity, synergy, or inhibition results from mixing of the compounds. Cannabigerolic acid potency was modulated by other cannabinoids such as tetrahydrocannabinol (THCVA) and $\Delta^8\text{THC}$ in both directions, but overall, the effects were rather modest, below 10%.

Finally, the authors discovered significant variability between potencies of the same compound in various cell lines, majority

of them of human origin. The explanations here might include different complements of Orai1-3 or STIM1,2 depending on the cell type or the presence of other proteins interacting with Orai/STIM.⁸ Since some cannabinoids were potent in T cells and mast cells but not in other cell types, an exciting possibility is proposed for targeted therapeutic uses for diseases in which a particular cell type plays a major role.

Funding

This study received funding from the National Institute of Allergy and Infectious Diseases (R21AI144337).

Conflict of Interest Statement

None declared.

Data Availability

No new data were generated or analyzed in support of this research.

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