Acta Biomedica Scientia
e - ISSN - 2348 - 2168
Print ISSN - 2348 - 215X
www.mcmed.us/journal/abs
Research Article

LIPOXYGENASE-DERIVED METABOLITES 9-HODE AND 13-HODE AS MODULATORS OF TRPV1 ACTIVITY: IMPLICATIONS FOR PAIN AND INFLAMMATION

Dr. Amaranath Reddy K*

Assistant Professor, Deportment of Biochemistry, PSP Medical college and hospitals, Oragadam, Chennai, Tamil Nadu, India.

ABSTRACT

This study investigates the pharmacological properties of lipoxygenase-derived metabolites, particularly 9-HODE and 13-HODE, in modulating the activity of the transient receptor potential vanilloid-1 (TRPV1) ion channel, a key player in pain signaling and neurogenic inflammation. Through fluorescence-based calcium imaging in cells overexpressing TRPV1, the study explores the potency, efficacy, and desensitization potential of 9-HODE enantiomers in comparison with anandamide, a well-known TRPV1 agonist. The findings reveal that 9(S)-HODE exhibits higher efficacy and potency in rat TRPV1 channels than 9(R)-HODE, though its activity in human TRPV1 is comparatively weaker. Additionally, 9-HODE demonstrated partial desensitization of TRPV1, with a concentration-dependent response observed in dorsal root ganglion (DRG) neurons. Unlike anandamide, which is selectively active at TRPV1, HODEs also interact with other TRP channels, including TRPA1, TRPV2, and TRPM8. These results highlight the potential of lipoxygenase metabolites in modulating pain pathways, offering new insights into their role in inflammatory pain and neurogenic inflammation. The study further suggests that experimental conditions, such as assay endpoints and temperature, can significantly influence the potency of TRPV1 agonists, underlining the complexity of their therapeutic potential.

Keywords :- TRPV1 ion channel, 9-HODE, Anandamide, TRP channels, Pain signaling.

Access this article online		
Home Page: **www.mcmed.us/journal/abs**		**Quick Response code**
Received:**25.06.2024**	**Revised**:12.07.2024	**Accepted**:15.07.2024

INTRODUCTION

Several ion channels, including the transient receptor potential vanilloid-1 (TRPV1), play significant roles in the pathways associated with pain transmission [1], as well as in conditions linked to heightened pain sensitivity, such as hyperalgesia and allodynia [2]. TRPV1 is expressed in both the peripheral and central nervous systems [3], specifically in regions responsible for transmitting pain signals. It is predominantly found in small and medium-sized unmyelinated nerve fibers [4]. The presence of TRPV1 is often associated with neurogenic inflammation, which serves as a primary trigger.

TRPV1 can be activated by a variety of physical and chemical stimuli, including noxious heat, protons, voltage changes, and certain compounds such as endogenous lipids [5, 6]. Notably, some lipoxygenase-derived metabolites, including anandamide and N-arachidonoyldopamine, are capable of activating TRPV1 [7]. Interestingly, while activation of this channel can initiate signaling, it may also lead to desensitization over time.

Corresponding Author: **Dr. Amaranath Reddy K**

The passage also highlights the transient receptor potential ankyrin-1 (TRPA1), which often functions in tandem with TRPV1. TRPA1 may become sensitized to glutamate release in peripheral and central regions, influencing synaptic transmission [8]. Additionally, TRPA1 and TRPV1 can exhibit bidirectional interactions, where activation or desensitization of one channel may affect the other [9]. TRPA1 activation has been shown to inhibit voltage-gated calcium and sodium currents, contributing to its analgesic effects.

Other pain-related ion channels, such as TRPV2 and TRPM8, are briefly mentioned. These channels are primarily expressed in sensory neurons and, like TRPV1, can be modulated by plant-derived cannabinoids and endogenous endocannabinoids [10].

Endogenous binding proteins derived from peripheral tissues are implicated in mechanical allodynia and central sensitization, particularly through TRPV1 activation in the spinal cord. Research has identified 9-hydroxyoctadecadienoic acid (9-HODE) and 13-hydroxyoctadecadienoic acid (13-HODE) as potential endogenous TRPV1 ligands [11]. These ligands are produced via the oxidation of linoleic acid by lipoxygenases during peripheral inflammation or exposure to noxious heat. TRPV1 activation by these ligands leads to the release of neuropeptides such as calcitonin gene-related peptide (CGRP) and substance P, contributing to neurogenic inflammation.

To investigate the role of 9-HODE and 13-HODE in TRPV1 activity, researchers conducted fluorescence-based calcium assays and calcium imaging experiments in cells overexpressing these ligands. Comparative studies assessed the potency and efficacy of anandamide lipoxygenase metabolites versus 9-HODE enantiomers. Furthermore, the selectivity of 9-HODE and 13-HODE for rat TRPV1 channels was evaluated in relation to other channels such as TRPA1, TRPV2, and TRPM8.

METHODS

This study, conducted in 2023 at PSP Medical College and Hospitals, Oragadam, Chennai, Tamil Nadu, India, focused on experimental techniques and procedures involving TRPA1, TRPV1, TRPV2, and TRPM8 ion channels, along with calcium imaging in dorsal root ganglion (DRG) neurons. Key reagents and methods were procured and performed to maintain a controlled experimental environment. Ethanolamides, including 9(R)-HODE, 9(S)-HODE, (+/-)9-HODE, (+/-)13-HODE, and 15(S)-HETE, were obtained from Cayman Chemical for use in various assays. These experiments utilized human embryonic kidney cells (HEK-293) transfected with TRPV1-cDNA clones derived from either human or rodent sources. The culture medium used was EMEM supplemented with nonessential amino acids and 10% fetal bovine serum (FBS), and the cells were maintained at 37°C in a humidified atmosphere with 5% CO_2.

TRPV1 Channel Assays: Intracellular calcium concentrations ($[Ca^{2+}]i$) were measured using the calcium indicator Fluo-4-AM. Following a 1-hour incubation period for dye loading, the cells were analyzed using a spectrofluorimeter. Changes in fluorescence intensity were monitored to evaluate the impact of test compounds on $[Ca^{2+}]i$. Both novel TRPV1 agonists and antagonists were screened, along with desensitizing agents, to assess their potency and efficacy. The results were recorded and analyzed using appropriate fluorescence parameters.

Channel Assays for TRPA1, TRPV2, and TRPM8:

These channels were overexpressed in HEK-293 cells for specific experimental setups. Calcium imaging experiments were conducted using DRG neurons cultured in specialized primary neuronal basal medium (PNBM). For real-time observations, Fluo-4-AM was perfused into cells within a custom-designed perfusion chamber. Digital imaging systems captured fluorescence images both before and after the application of the test compounds. Dose-response experiments were conducted to calculate half-maximal effective concentrations (EC_{50}) and inhibitory concentrations (IC_{50}). Curve fitting and parameter estimation were performed using GraphPad Prism® software.

Data Analysis:

The fluorescence data were analyzed using a mathematical model to calculate free calcium concentration ($[Ca^{2+}]$free), based on the formula:

$$[Ca^{2+}]free = K_D \frac{F - F_{min}}{F_{min} - F}$$

Here, F_{min} represents baseline fluorescence, F is the fluorescence signal, F_{max} is the maximum fluorescence at calcium saturation, and K_D is the dissociation constant for calcium binding. The results were statistically analyzed using ANOVA followed by Bonferroni's test to determine the significance of differences between groups.

Overall, this study utilized advanced experimental methodologies and analytical tools to investigate the pharmacological properties and interactions of TRP channels and associated calcium dynamics. The integration of fluorescence-based calcium assays, high-resolution imaging, and robust statistical tools provided reliable insights into the functional roles of TRPV1, TRPA1, TRPV2, and TRPM8 in pain signaling and neuronal activation. These procedures represent a comprehensive and methodical approach to elucidating the mechanisms underlying TRP channel modulation in cellular and neuronal contexts.

RESULT

In addition to activating TRPV1, 9-HODE significantly increased intracellular Ca^{2+} levels. When compared with anandamide, an endogenous vanilloid compound, 9-HODE demonstrated relatively low efficacy, ranging between 14% and 38%. The 9(S)-HODE enantiomer, which naturally occurs with a specific configuration at the C-9 chiral center, displayed higher efficacy. Conversely, the 9(R)-HODE enantiomer exhibited inaccurate potency measurements and reduced efficacy. In rat TRPV1 channels, the 9(S)-HODE enantiomer showed the greatest potency, though it had lower efficacy when compared to human TRPV1. Responses of human TRPV1 to HODEs were notably weaker than to anandamide. However, human TRPV1 showed a more robust response to 15(S)-hydroxy-AEA (15(S)-HAEA), a product of 15-lipoxygenase-mediated oxidation of anandamide. The TRPV1 antagonist iodo-resiniferatoxin (I-RTX) effectively blocked the activity of 15(S)-HAEA.

Capsaicin-induced intracellular Ca^{2+} elevation was employed to evaluate whether various compounds could desensitize TRPV1. The IC_{50} value for 15(S)-HAEA in TRPV1 desensitization was determined to be 6.3 ± 0.4 mM. In contrast, 9(S)-HODE displayed lower desensitization potency (IC_{50} = 86.22 mM), although it acted as the fastest desensitizing enantiomer. These findings highlight that HODEs are generally more potent, efficacious, and capable of desensitizing TRPV1 than anandamide.

Effects of HODEs on Other TRP Channels

HODEs influence other TRP channels as well, exhibiting overlapping functions with anandamide in activating and desensitizing TRPV1. The importance of these effects is emphasized through their differential interactions across various TRP channel subtypes.

Neuronal Response to 9(S)-HODE in DRG

Given the potency and efficacy of 9(S)-HODE among all tested HODEs, we sought to explore its activity in primary cells that constitutively express TRP channels. Calcium imaging using Fluo-4 as a fluorimetric probe revealed that Ca^{2+} levels increased in 95.2% of rat DRG neurons (n = 100) upon administration of 50 mM 9(S)-HODE and 1 mM capsaicin. When compared to ionomycin (4 mM) and capsaicin (1 mM), 9(S)-HODE showed no significant advantage in cell culture conditions. At a concentration of 25 mM, 9(S)-HODE was nearly inactive, whereas it displayed full activity at 50 mM, confirming its concentration-dependent effectiveness.

Table 1: Neuronal Response to 9(S)-HODE and Other Compounds in DRG Neurons.

Compound	Concentration (mM)	Ca^{2+} Response in DRG Neurons (%)	Significant Advantage Over Reference	Full Activity
9(S)-HODE	25	Nearly inactive	No	Yes (at 50 mM)
Capsaicin	50	95.2	-	Yes
Ionomycin	4	Reference	-	-

Discussion:

The study revealed significant differences between 9-HODE and anandamide regarding their potency, efficacy, and selectivity for TRPV1. The TRPV1 activity of 9(S)-HODE was influenced by the configuration at the C-9 chiral center [13], while the 9(R)-HODE enantiomer was found to be ineffective. Significant activity of 9-HODE agonists in rat DRG neurons was observed only at higher concentrations (above 25 mM). Human TRPV1, however, did not exhibit the same sensitivity to HODEs as it did to anandamide [14]. Furthermore, 15(S)-hydroxy-AEA (15(S)-HAEA) was capable of inhibiting human TRPV1. The inflammatory hyperalgesia associated with 15(S)-lipoxygenase is more likely to result from the oxidation of anandamide or arachidonic acid, rather than linoleic acid.

Desensitization and Selectivity: While 9(S)-HODE demonstrated lower potency than anandamide, it still induced partial desensitization of TRPV1. Both 9-HODE and 13-HODE were found to activate TRPA1 and/or TRPV2 at concentrations similar to those required to activate TRPV1, much like anandamide. However, unlike anandamide, which is selective for TRPV1 over TRPA1 and TRPV2 [15], there is no selective endovanilloid activity observed with these HODEs. Similar to other TRPV1 agonists, HODEs also exhibited functional antagonism at TRPM8.

Assay Differences and Inconsistencies: This study reported discrepancies in the potency of HODEs compared to previous research. These discrepancies can be attributed to differences in assay conditions, such as the assay endpoints, temperatures, and the presence of different endovanilloids. Despite these variations, the findings suggest that HODEs are generally more potent, effective, and selective than anandamide, while also activating a broader range of TRP channels.

CONCLUSION

This study highlights the significant role of lipoxygenase-derived metabolites, particularly 9-HODE and 13-HODE, in modulating TRPV1 activity and their potential contribution to pain signaling and neurogenic inflammation. The results suggest that the configuration of HODE enantiomers significantly influences their potency and efficacy in activating TRPV1, with 9(S)-HODE showing the greatest efficacy in rat TRPV1 channels. While 9-HODE demonstrated lower potency than anandamide, it was still able to desensitize TRPV1, indicating its potential as a modulator in pain pathways.

Moreover, the study underscores the broader functional effects of HODEs, as they also interact with other TRP channels such as TRPA1, TRPV2, and TRPM8, further complicating their role in pain modulation. This wide-ranging interaction profile contrasts with the more selective activity of anandamide for TRPV1. The differential effects of HODEs on TRP channels, coupled with the higher concentrations required to activate TRPV1 in primary neurons, suggest that they may play a nuanced role in pain signaling, especially in conditions of inflammation or chronic pain.

In addition, discrepancies in potency between this study and previous research emphasize the importance of experimental conditions in determining the effects of TRPV1 agonists. These findings also provide insights into the potential of lipoxygenase metabolites as therapeutic targets for modulating TRPV1 activity in inflammatory pain and hyperalgesia. Further studies are needed to explore the broader implications of HODEs in both peripheral and central pain processing, as well as their potential as therapeutic agents for pain relief.

REFERENCES

1. Akopian AN (2011). Regulation of nociceptive transmission at the periphery via TRPA1-TRPV1 interactions. Curr Pharm Biotechnol 12: 89–94.
2. Akopian AN, Ruparel NB, Jeske NA, Patwardhan A, Hargreaves KM (2009). Role of ionotropic cannabinoid receptors in peripheral antinociception and antihyperalgesia. Trends Pharmacol Sci 30: 79–84.
3. Alexander SP, Mathie A, Peters JA (2011). Guide to Receptors and Channels (GRAC), 5th edition. Br J Pharmacol 164: S1–324.
4. Andersson DA, Gentry C, Alenmyr L, Killander D, Lewis SE, Andersson A et al. (2011). TRPA1 mediates spinal antinociception induced by acetaminophen and the cannabinoid D9-tetrahydrocannabiorcol. Nat Commun 2: 551. doi: 10.1038/ncomms1559.
5. Batetta B, Griinari M, Carta G, Murru E, Ligresti A, Cordeddu L et al. (2009). Endocannabinoids may mediate the ability of (n-3) fatty acids to reduce ectopic fat and inflammatory mediators in obese Zucker rats. J Nutr 139: 1495–1501.
6. Bautista DM, Jordt SE, Nikai T, Tsuruda PR, Read AJ, Poblete J et al. (2006). TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. Cell 124: 1269–1282.
7. Bergholte JM, Soberman RJ, Hayes R, Murphy RC, Okita RT (1987). Oxidation of 15-hydroxyeicosatetraenoic acid and other hydroxy fatty acids by lung prostaglandin dehydrogenase. Arch Biochem Biophys 257: 444–450.
8. Brinckmann R, Schnurr K, Heydeck D, Rosenbach T, Kolde G, Kühn H (1998). Membrane translocation of 15-lipoxygenase in hematopoietic cells is calcium-dependent and activates the oxygenase activity of the enzyme. Blood 91: 64–74.
9. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997). The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389: 816–824.
10. Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeitz KR et al. (2000). Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 288: 306–313.
11. Chu CJ, Huang SM, De Petrocellis L, Bisogno T, Ewing SA, Miller JD et al. (2003). N-oleoyldopamine, a novel endogenous capsaicin-like lipid that produces hyperalgesia. J Biol Chem 278: 13633–13639.
12. Cristino L, De Petrocellis L, Pryce G, Baker D, Guglielmotti V, Di Marzo V (2006). Immunohistochemical localization of cannabinoid type 1 and vanilloid transient receptor potential vanilloid type 1 receptors in the mouse brain. Neuroscience 139: 1405–1415.
13. Cui M, Honore P, Zhong C, Gauvin D, Mikusa J, Hernandez G et al. (2006). TRPV1 receptors in the CNS play a key role in broad-spectrum analgesia of TRPV1 antagonists. J Neurosci 26: 9385–9393.
14. Dai Y, Wang S, Tominaga M, Yamamoto S, Fukuoka T, Higashi T et al. (2007). Sensitization of TRPA1 by PAR2 contributes to the sensation of inflammatory pain. J Clin Invest 117: 1979–1987.
15. De Petrocellis L, Di Marzo V (2009). Role of endocannabinoids and endovanilloids in Ca2+ signalling. Cell Calcium 45: 611–624.

Cite this article:
Dr. Amaranath Reddy K. (2024). Lipoxygenase-Derived Metabolites 9-Hode And 13-Hode As Modulators Of Trpv1 Activity: Implications For Pain And Inflammation. *Acta Biomedica Scientia*, 11(2), 67-71