ECRONICON OPEN ACCESS

EC PHARMACOLOGY AND TOXICOLOGY
Research Article

The Involvement of the Endocannabinoid System in the Peripheral Antinociceptive Action of Epoxyeicosatrienoic Acids (EETs)

Flávia CS Fonseca, Walace CP Barra, Daniel PD Machado, Douglas L Almeida, Danielle D Aguiar, Bárbara FG Queiroz, Andrea C Perez, Thiago RL Romero and Igor DG Duarte*

Department of Pharmacology, Federal University of Minas Gerais (UFMG), Brazil

***Corresponding Author:** Igor DG Duarte, Department of Pharmacology, ICB-UFMG, Av. Antônio Carlos, 6627-Pampulha Campus, Belo Horizonte, MG, Brazil.

Received: April 08, 2025; **Published:** April 22, 2025

Abstract

Epoxyeicosatrienoic acids (EETs) are cytochrome P450-epoxygenase-derived metabolites of arachidonic acid that act as endogenous signaling molecules in multiple biological systems, including their controversial effects on nociception. The EETs and the endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) originate from the same precursor, arachidonic acid, and share similar structural characteristics. Different isomers of EETs were described as capable of binding in cannabinoid receptors in rats' brains. To verify whether the peripheral antinociceptive effect of EETs depends on the mechanisms of the cannabinoid system. The mechanical paw pressure test was used to induce hyperalgesia by intraplantar injection of prostaglandin E2, evaluating the effect of exogenous EET administration and its interaction with the antagonism of CB_1/CB_2 receptors and the modulation of endocannabinoids. The EETs (5,6-, 8,9-, 11,12-, and 14,15-EET) and the drugs of the cannabinoid system were administered intraplantarly to male Swiss mice (n = 4). Statistical analysis was performed using ANOVA and the Bonferroni post hoc test. The selective CB_1 cannabinoid receptor antagonist, AM251, reversed the antinociceptive effect of EETs in a dose-dependent manner. Otherwise, the selective CB_2 cannabinoid receptor antagonist, AM630, did not reverse the antinociceptive effect of EETs. Furthermore, an endocannabinoid reuptake inhibitor (VDM11) and an enzyme inhibitor responsible for anandamide degradation (MAFP) potentiated the antinociceptive effect of low doses of EETs. On the other hand, an inhibitor of the enzyme responsible for the diacylglycerol lipase hydrolysis (JZL184) did not potentiate this effect. Our findings showed that the peripheral antinociceptive effect of 5,6-, 8,9-, 11,12-, and 14,15-EET could be due to the release of the endocannabinoid anandamide and subsequent activation of the CB_1 receptor, but not the CB_2 receptor, at the peripheral level, thereby inhibiting PGE_2-induced hyperalgesia.

Keywords: *Epoxyeicosatrienoic Acids; EETs; Cannabinoids; Anandamide; Antinociception*

Abbreviations

PGE2: Prostaglandin E2; EET: Epoxyeicosatrienoic Acid; IPl: Intraplantar

Introduction

The action of select isozymes of cytochrome P450 (CYP) on arachidonic acid (AA) results in the synthesis of metabolites known as epoxyeicosatrienoic acids (EETs) [1]. These molecules are widely distributed in the brain and periphery [2,3]. In the nervous system, EETs are shown to be considerable modulators of cerebral blood flow regulation, axonal growth, and neuronal survival [4], while in the periphery, effects such as vasodilatation and immunomodulation were described in the literature [2,3,5].

Beyond these effects, EETs have demonstrated their potential to modulate nociception [6,7]. This is evidenced by a thermal hyperalgesia test showing that EETs are antinociceptive in LPS-treated rats and pro-nociceptive in LPS-naïve animals. EETs have also shown antinociceptive action when administered directly into the ventrolateral periaqueductal gray area of the mesencephalon, suggesting a central mechanism of analgesia [8].

Endocannabinoids, one of the classical nociceptive modulators, are similar to eicosanoids, which act as lipid signal messengers that affect nociception [9]. The cannabinoid system is capable of endogenous modulation of nociception by decreasing the excitability of nociceptors through the activation of cannabinoid receptors, CB_1 and/or CB_2, which is mediated mainly by the endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG), respectively [10-12].

The EETS and the endocannabinoids AEA and 2-AG come from the same precursor, AA, and share similar structural characteristics [9]. The EETs can directly act on the CB_2 receptor [13]. The 5,6-EET-EA is derived from the oxidation of AEA by the CYP enzymes [14] and can act as an agonist of CB_2 receptors, exhibiting a binding affinity that exceeds that of the parent anandamide 1000-fold and demonstrating greater stability [15]. A different isomer, 14,15-EET-EA, was described as capable of binding to CB1 receptors in rats' brains, although weaker than AEA.

Material and Methods

Animals

Male Swiss mice weighing between 30 and 40g, with free access to food and water, in a light/dark cycle of 12h (6:00-18:00h), kept at a controlled temperature room (24 ± 2°C) were used at the Bioterism Center of the Federal University of Minas Gerais (CEBIO-ICB/UFMG). After the tests, the animals were euthanized by high-dose intraperitoneal injection of an anesthetic mixture (300 mg/kg ketamine hydrochloride and 15 mg/kg of xylazine hydrochloride, both Sigma-Aldrich, USA). The Ethics Committee on Animal Experimentation approved the project under protocol 75/2017.

Drugs

Prostaglandin E_2 (PGE_2; Sigma-Aldrich, USA) was diluted in a 2% ethanol solution in a sterile 0.9% sodium chloride (NaCl) aqueous solution. 5,6-epoxieicosatrienoic acid (5,6-EET) (Cayman Chemical, EUA) was dissolved in methyl acetate in a saline solution. 8,9-Epoxyeicosatrienoic acid (8,9-EET) (Cayman Chemical, USA) and 11,12-Epoxyeicosatrienoic acid (11,12-EET) (Cayman Chemical, USA) were dissolved in a 6.4% ethanol solution in saline. 14,15-Epoxieicosatrienoic acid (14,15-EET) (Cayman Chemical, USA) was dissolved in a 25.6% ethanol solution in saline. The cannabinoid CB_1 and CB_2 receptor antagonists, AM251 (Tocris, USA) and AM630 (Tocris, USA), respectively, were dissolved in 10% dimethyl sulfoxide (DMSO) in saline. JZL 184 (Tocris, EUA), an inhibitor of the enzyme (monoacylglycerol lipase; MAGL) that catalyzes the endocannabinoid 2-arachidonoylglycerol (2-AG), and VDM11 (Tocris, EUA), an inhibitor of the membrane transporter of the endocannabinoid anandamide (AEA), were dissolved in 1% DMSO in saline solution. MAFP (Tocris, EUA), an inhibitor of the FAAH enzyme that degrades AEA, was dissolved in 1% methyl acetate (AC) in a saline solution. All the drugs were injected via intra-plantar (i.pl.) in a volume of 20 µl.

Measurement of nociceptive threshold

Hyperalgesia was induced by an intraplantar injection of prostaglandin E2 (PGE2) into the right hind paw (2 µg). The mechanical nociceptive threshold was expressed in grams (g) and assessed by measuring the response to a paw pressure test (Ugo-Basile; SRL, Varese, Italy), as described in rats [16] and in mice [17]. The test consists of a cone-shaped paw-presser with a rounded tip, which applies a linearly increasing force in the hind paw. The weight in grams required to elicit the nociceptive response of paw withdrawal was determined as the nociceptive threshold. A cutoff value of 150g was used to minimize the risk of paw damage.

The nociceptive threshold was measured in the right paw and determined as the average of three consecutive trials recorded before and 180 minutes after PGE_2 injection. Hyperalgesia was calculated as the difference between these 2 averages (Δ of nociceptive threshold).

Experimental protocol

The basal threshold was measured immediately before the experiments, with no drug effects. Posteriorly, PGE_2 was administered immediately (0 minutes). EETS, or their vehicle, were injected 175 minutes prior. The selective CB_1 and CB_2 cannabinoid receptor antagonists, AM251 and AM630, respectively, were administered over 165 minutes. The non-selective FAAH inhibitor (MAFP), the selective MAGL inhibitor (JZL184), and the anandamide cellular reuptake inhibitor (VDM11) were administered at a time point of 165 minutes. The protocols concerning the dose and the injection time of each drug used in this study were based on previous studies from our research group and literature data [18-21]. The drug injection periods are timed to coincide with the peak of action of each EET, antagonist, or inhibitor, ensuring that all are active when assessing the nociceptive threshold (180 minutes after PGE_2 injection in the hind paw).

Data and statistical analysis

All results are presented in the graphics with the mean ± standard error of the mean (S.E.M.). Statistical analysis was carried out using Graph Prism 8.0.2 software, and the data were submitted to one-way variance analysis (ANOVA) followed by Bonferroni post-test. Only p-values lower than 0.05 ($p < 0.05$) were considered statistically significant.

Results

Study on the participation of CB_1 cannabinoid receptors in the peripheral antinociceptive effect of EETs

The selective cannabinoid receptor antagonist CB_1 (AM 251, 20, 40, and 80 µg/paw) reversed the dose-dependent antinociceptive effect of 5,6-, 8,9-, 11,12-, and 14,15-EET (128, 128, 128, and 512 ng/paw, respectively) (Figure 1).

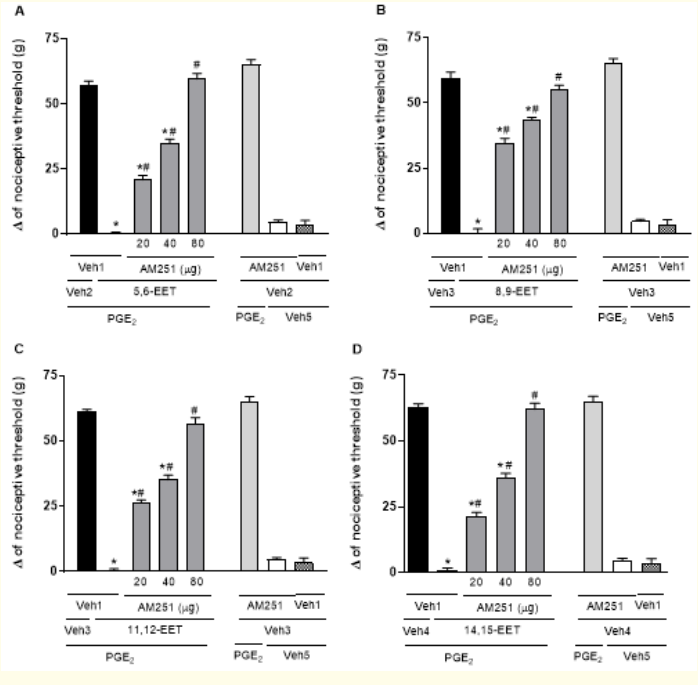

Figure 1: *Administration of a CB_1 receptor antagonist blocks 5,6- (A), 8,9- (B), 11,12- (C) and 14,15-EET (D)-induced peripheral antinociception in hyperalgesic paws. Each column represents the mean ± SEM (n = 4). * and # indicates a significant difference (P < 0.05) compared with PGE2 + Veh and PGE2 + EET, respectively. ANOVA with Bonferroni post test.*

Study on the participation of CB_2 cannabinoid receptors in the peripheral antinociceptive effect of EETs

The selective cannabinoid receptor antagonist CB_2 (AM630, 100 µg/paw) did not reverse the antinociceptive effect of 5,6-, 8,9-, 11,12-, and 14,15-EET (128, 128, 128, and 512 ng/paw) (Figure 2).

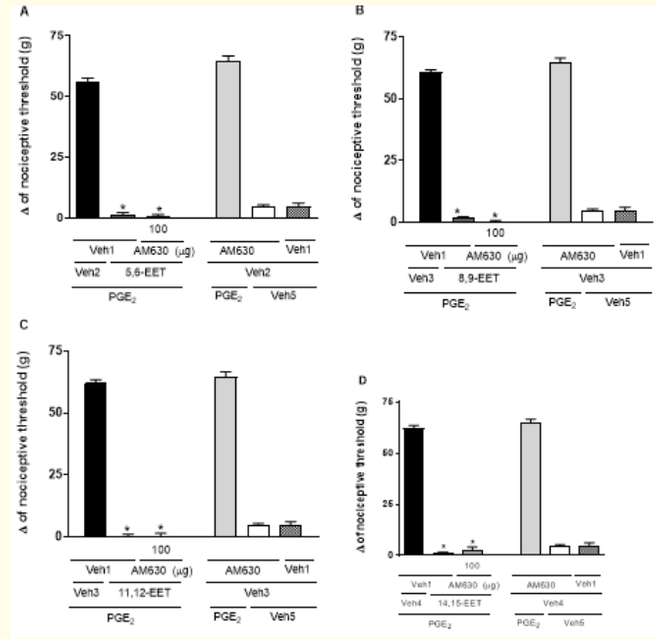

Figure 2: *Administration of a CB_2 receptor antagonist did not block 5,6- (A), 8,9- (B), 11,12- (C), and 14,15-EET (D) -induced peripheral antinociception in hyperalgesic paws. Each column represents the mean ± SEM (n = 4). * and # indicates a significant difference (P < 0.05) compared with PGE2 + Veh and PGE2 + EET, respectively. ANOVA with Bonferroni post test.*

Study on the participation of endocannabinoids in the peripheral antinociceptive effect of EETs

A nonselective inhibitor of the enzyme that catalyzes anandamide hydrolysis, FAAH (MAFP 0.5 µg/paw), potentiated the antinociceptive effect of lower doses of EETS, partially in 8,9- and 11,12-EET, and completely in 5,6- and 14,15-EET (Figure 3). The anandamide cellular reuptake inhibitor (VDM 11, 2.5 µg/paw) potentiated the antinociceptive effect of lower doses of EETs, partially in 8,9- and 11,12-EET, and completely in 5,6- and 14,15-EET (Figure 4).

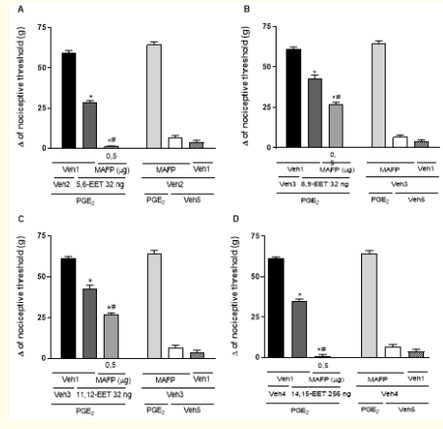

Figure 3: *Potentiation by MAFP of a lower dose of 5,6- (A), 8,9- (B), 11,12- (C), and 14,15-EET (D) -induced peripheral antinociception in hyperalgesic paws. Each column represents the mean ± SEM (n = 4). * and # indicates a significant difference (P < 0.05) compared with PGE2 + Veh and PGE2 + EET, respectively. ANOVA with Bonferroni post test.*

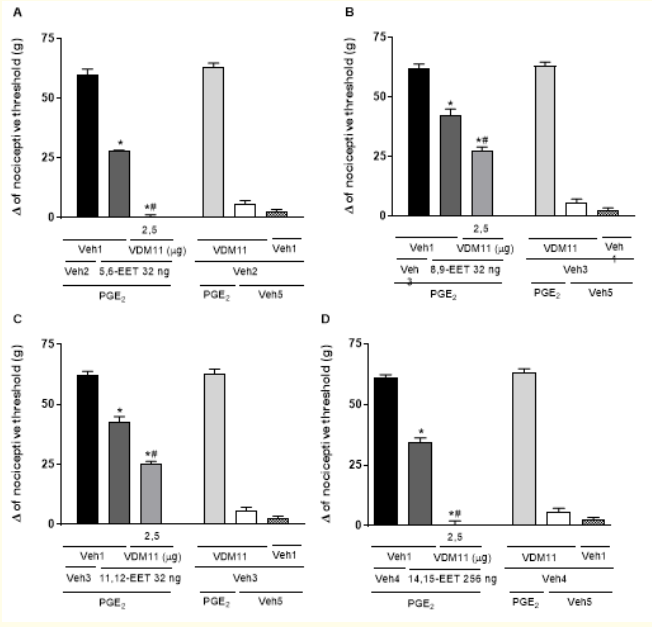

Figure 4: *Potentiation by VDM11 of a lower dose of 5,6- (A), 8,9- (B), 11,12- (C), and 14,15-EET (D) -induced peripheral antinociception in hyperalgesic paws. Each column represents the mean ± SEM (n = 4). * and # indicates a significant difference (P < 0.05) compared with PGE2 + Veh and PGE2 + EET, respectively. ANOVA with Bonferroni post test.*

The inhibitor of the enzyme that catalyzes 2-AG hydrolysis, MAGL (JZL184 4 μg/paw), did not potentiate the antinociceptive effects of lower doses of EETs (Figure 5).

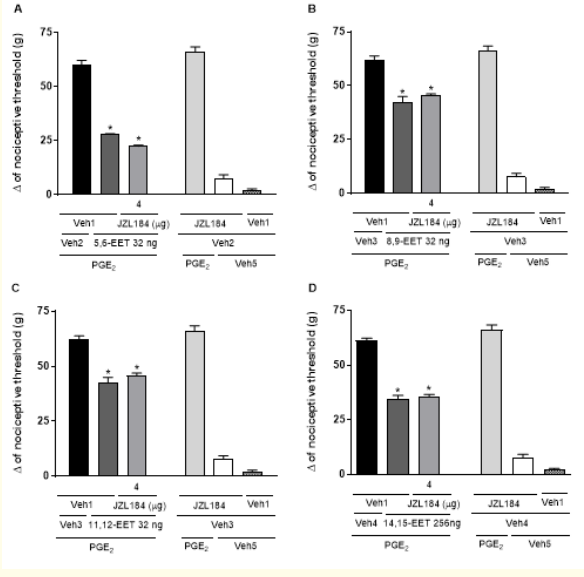

Figure 5: *The treatment with JZL184 did not potentiate a lower doses of 5,6- (A), 8,9- (B), 11,12- (C), and 14,15-EET (D) -induced peripheral antinociception in hyperalgesic paws. Each column represents the mean ± SEM (n = 4). * and # indicates a significant difference (P < 0.05) compared with PGE2 + Veh and PGE2 + EET, respectively. ANOVA with Bonferroni post test.*

Neither the antagonists, inhibitors, nor its vehicles could alter the nociceptive threshold alone.

Discussion

The potential of EETs acting as antinociceptive molecules or modulators of nociceptive transmission has been explored in different ways and applications. Thenceforth, the capacity of topical EETs to cause a significant reversal of LPS-induced thermal hyperalgesia was assessed using a commercial Hargreaves apparatus, which measures thermal hind paw withdrawal latencies. Mechanical hyperalgesia was assessed by measuring mechanical hind paw withdrawal thresholds using von Frey filaments with graded bending forces [6].

Soluble epoxide hydrolase inhibitors administered topically were also tested, and thermal hyperalgesia was significantly attenuated against LPS-treated rats [9]. The central administration of EETs has also shown antinociceptive action. It was demonstrated that administering the drug directly into the ventrolateral periaqueductal gray area of the mesencephalon increased the latency time of the elicited response in the tail-flick test, which was applied by radiant heat to the dorsal surface of the tail, suggesting a central mechanism of analgesia for EETs [8].

Our group first describes the peripheral antinociceptive effects of the four regioisomeric forms of EETs in a PGE2-induced acute pain model, measuring mechanical nociceptive threshold using a paw pressure test, as described in rats [16] and in mice [17]. The maximal analgesic effects were observed at doses of 128 ng for 5,6-, 8-, 9-, and 11,12-EETs, and 512 ng for 14,15-EET. The sub-maximal analgesic effect doses were 32 ng for 5,6-, 8-, 9-, and 11,12-EETs, and 256 ng for 14,15-EET. Both of the described doses were used in the present work to test the participation of the cannabinoid system in the antinociceptive mechanism of EETs [22].

The cannabinoid system is one of the most important mechanisms by which the organisms lead to pain [23]. After the receptor is activated, intracellular signaling pathways propagate the ligand stimulus throughout the cell. Several studies demonstrate that the activation of cannabinoid receptors triggers the NO/cGMP/KATP signaling pathway through Gi protein-coupled receptors, and this activation can be observed with both cannabinoid substances and other exogenous substances [24,25]. The complete idea of activating the NO/cGMP pathway to K_{ATP}, a final effector of the path that causes hyperpolarization of the nociceptive terminal and hinders the afferent of peripheral stimuli, has already been summarized in the literature [26-28].

Besides the endogenous activation of the pathway during pain regulation within one's system, our group has shown that several drugs act through the receptors and mediators of that system. These studies have shown the capacity of the antagonists of cannabinoid receptors CB_1 and CB_2 and inhibitors of reuptake and hydrolysis endocannabinoid molecules AEA and 2-AG to modulate the analgesic activities of exogenous drugs that use that system as a pathway of its analgesia [18-21]. The participation of the cannabinoid system in EETs' antinociception has been poorly explored, despite the importance of that pathway in the context of pain and the proximity between EETs and other activators of the system [9].

It was demonstrated that the P450-mediated epoxidation of anandamide to synthesize 5,6-EET-EA represents a mechanism for endocannabinoid signaling, which may affect microglial activity during neuroinflammation. This study also demonstrates that 5,6-EET-EA can be a potent and selective agonist of cannabinoid receptor type 2 [15]. Unlike this study, in the present study, the antagonism of CB_1 receptors with AM251 (20, 40, and 80 µg/paw) reversed the analgesia of the four isomeric forms of EET. Interestingly, although the authors had described the 5,6-EET-EA as a CB_2 agonist, in our model of peripheral antinociception mediated by EETs, the antagonism of CB_2 receptors with AM630 did not reverse the analgesia of any of the four isomeric forms of EETs.

Similarly, the test was performed to describe if a CB_1 or CB_2 antagonist would block sEHI-mediated antihyperalgesia [9]. This study found that the mixture combined with the four isomeric EETs could displace the CB_2 agonist WIN 55212-2 but not the CB_1 agonist CP

55940 in radioligand displacement assays. It was also noted that this activity is primarily mediated by the 5,6-EET isomer, corroborating the previous results [15]. Thus, the authors considered predictably that CB_2 but not CB_1 antagonists blocked the sEHI anti-hyperalgesia.

Additionally, our study demonstrated that inhibiting the enzyme that catalyzes anandamide hydrolysis (FAAH) and anandamide cellular reuptake potentiated the partial antinociceptive effects of the submaximal dose of EETs. However, the inhibitory of the enzyme that catalyzes 2-AG hydrolysis (MAGL) did not modify the antinociceptive effects of a submaximal dose of any EETs.

Literature data have described the presence of either CB_1 or CB_2 receptors in keratinocytes and immune system cells, which supports the idea that peripheral cells could regulate these analgesic systems and mechanisms after an endogenous or exogenous modulation through the release of endocannabinoids such as AEA and 2-AG [29-33].

Compared to other bioactive lipids, such as endocannabinoids, epoxy fatty acids exhibit unique mechanisms in nociception. Even as endocannabinoids, ETTs can freely diffuse through the cell membranes of neurons that do not fit the classification of neurotransmitters, as they are not stored in vesicles or released from them. In contrast to neurotransmitters, the classification of ETTs and other fatty acids in bioactive metabolites points to a synthesis and/or release from membrane bilayers upon cell stimulation [34].

It is well known that both 2-AG and AEA are substrates for CYP2J2 epoxygenase metabolism. This process utilizes AEA to form four AEA epoxyeicosatrienoic acids, whereas incubations with 2-AG yield detectable levels of two 2-AG epoxides. Nevertheless, the literature has not explored the relationship between the synthesis of EETs and the release of endocannabinoids [33].

Thus, our data on the cannabinoid system first demonstrated that the antinociceptive effect presented by exogenous administration of 5,6-, 8,9-, 11,12- and 14,15-EETs may be due to the release of the endocannabinoid AEA, with subsequent activation of receptors CB_1, but not CB_2 receptors, at the periphery, inhibiting PGE_2-induced hyperalgesia.

Conclusion

In the present work, our findings showed that the peripheral antinociceptive effect of 5,6-, 8,9-, 11,12-, and 14,15-EET could be due to the release of the endocannabinoid anandamide and subsequent activation of the CB1 receptor, but not the CB2 receptor, at the peripheral level, thereby inhibiting PGE_2-induced hyperalgesia.

Acknowledgements

We are grateful for fellowships by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), CNPq (Conselho Nacional de Pesquisa), and FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais).

Conflict of Interest

The authors declare that they have no conflicts of interest.

Bibliography

1. Liu T., *et al*. "EETs/sEHi alleviates nociception by blocking the crosslink between endoplasmic reticulum stress and neuroinflammation in a central poststroke pain model". *Journal of Neuroinflammation* 18.1 (2021): 211.

2. Node K., *et al*. "Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids". *Science* 285.5431 (1999): 1276-1279.

3. Spillman NJ., *et al*. "Exported epoxide hydrolases modulate erythrocyte vasoactive lipids during *Plasmodium falciparum* infection". *mBio* 7.5 (2016): e01538-16.

4. Abdu E., *et al*. "Epoxyeicosatrienoic acids enhance axonal growth in primary sensory and cortical neuronal cell cultures". *Journal of Neurochemistry* 117.4 (2011): 632-642.

5. Sinal CJ., *et al*. "Targeted disruption of soluble epoxide hydrolase reveals a role in blood pressure regulation". *Journal of Biological Chemistry* 275.51 (2000): 40504-40510.

6. Inceoglu B., *et al*. "Inhibition of soluble epoxide hydrolase reduces LPS-induced thermal hyperalgesia and mechanical allodynia in a rat model of inflammatory pain". *Life Science* 79.24 (2006): 2311-2319.

7. Inceoglu B., *et al*. "Soluble epoxide hydrolase and epoxyeicosatrienoic acids modulate two distinct analgesic pathways". *PNAS* 105.48 (2008): 18901-18906.

8. Terashvili M., *et al*. "Antinociception produced by 14,15-epoxyeicosatrienoic acid is mediated by the activation of beta-endorphin and met-enkephalin in the rat ventrolateral periaqueductal gray". *Journal of Pharmacology and Experimental Therapeutics* 326.2 (2008): 614-622.

9. Wagner K., *et al*. "Soluble epoxide hydrolase inhibition, epoxygenated fatty acids, and nociception". *Prostaglandins and Other Lipid Mediators* 96.1-4 (2011): 76-83.

10. Piomelli D. "The molecular logic of endocannabinoid signaling". *Nature Reviews Neuroscience* 4.11 (2003): 873-884.

11. Piomelli D. "Endocannabinoids". Encyclopedia of Biological Chemistry (Second Edition), Academic Press (2013): 194-196.

12. Piomelli D and Sasso O. "Peripheral gating of pain signals by endogenous lipid mediators". *Nature Neuroscience* 17.2 (2014): 164-174.

13. Inceoglu B., *et al*. "Soluble epoxide hydrolase inhibition reveals novel biological functions of epoxyeicosatrienoic acids (EETs)". *Prostaglandins and Other Lipid Mediators* 82.1-4 (2007): 42-49.

14. Snider NT., *et al*. "The endocannabinoid anandamide is a substrate for the human polymorphic cytochrome P450 2D6". *Journal of Pharmacology and Experimental Therapeutics* 327.2 (2008): 538-545.

15. Snider NT., *et al*. "A cytochrome P450-derived epoxygenated metabolite of anandamide is a potent cannabinoid receptor 2-selective agonist". *Molecular Pharmacology* 75.4 (2009): 965-972.

16. Randall LO and Selitto JJ. "A method for measurement of analgesia activity on inflamed tissue". *Archives Internationales de Pharmacodynamie et de Therapie* 111.4 (1957): 409-419.

17. Kawabata A., *et al*. "L-Leucyl-L-arginine, naltrindole and Darginine block antinociception elicited by L-arginine in mice with carrageenin-induced hyperalgesia". *British Journal of Pharmacology* 107.4 (1992): 1096-1101.

18. Guzzo LS., *et al*. "Kahweol, a natural diterpene from coffee, induces peripheral antinociception by endocannabinoid system activation". *Brazilian Journal of Medicine and Biological Research* 54.12 (2021): e11071.

19. Gonçalves WA., *et al*. "Endogenous opioid and cannabinoid systems modulate the muscle pain: A pharmacological study into the peripheral site". *European Journal of Pharmacology* 901 (2021): 174089.

20. Aguiar DD., *et al*. "Curcumin induces peripheral antinociception by opioidergic and cannabinoidergic mechanism: Pharmacological evidence". *Life Sciences* 293 (2022): 120279.

21. Aguiar DD., *et al*. "Participation of the cannabinoid system and the NO/cGMP/KATP pathway in serotonin-induced peripheral antinociception". *Neuroscience Letters* 818 (2024): 137536.

22. Fonseca FCS and Duarte IDG. "5,6-, 8,9-, 11,12-and 14,15-epoxyeicosatrienoic acids (EETs) induce peripheral receptor-dependent antinociception in PGE2-induced hyperalgesia in mice". *Pharmacology and Clinical Pharmacy Research* 9 (2024): 154-167.

23. Howlett AC. "The cannabinoid receptors". *Prostaglandins and Other Lipid Mediators* 68-69 (2002): 619-631.

24. Reis GML., *et al*. "Opioid receptor and NO/cGMP pathway as a mechanism of peripheral antinociceptive action of the cannabinoid receptor agonist anandamide". *Life Science* 85.9-10 (2009): 351-356.

25. Romero TRL., *et al*. "Involvement of the L-arginine/nitric oxide/cyclic guanosine monophosphate pathway in peripheral antinociception induced by N-palmitoyl-ethanolamine in rats". *Journal of Neuroscience Research* 90.7 (2012): 1474-1479.

26. Duarte IDG and Ferreira SH. "The molecular mechanism of central analgesia induced by morphine or carbachol and the L-arginine-nitric oxide-cGMP pathway". *European Journal of Pharmacology* 221.1 (1992): 171-174.

27. Duarte IDG., *et al*. "Analgesia by direct antagonism of nociceptor sensitization involves the arginine-nitric oxide-cGMP pathway". *European Journal of Pharmacology* 217.2-3 (1992): 225-227.

28. Rodrigues ARA and Duarte IDG. "The peripheral antinociceptive effect induced by morphine is associated with ATP-sensitive K(+) channels". *British Journal of Pharmacology* 129.1 (2000): 110-114.

29. Kauser S., *et al*. "Regulation of human epidermal melanocyte biology by β-endorphin". *Journal of Investigative Dermatology* 120.6 (2003): 1073-1080.

30. Cabot PJ., *et al*. "Immune cell-derived beta-endorphin. Production, release, and control of inflammatory pain in rats". *Journal of Clinical Investigation* 100.1 (1997): 142-148.

31. Casanova ML., *et al*. "Inhibition of skin tumor growth and angiogenesis *in vivo* by activation of cannabinoid receptors". *Journal of Clinical Investigation* 111.1 (2003): 43-50.

32. Anthony R., *et al*. "Cannabinoid receptors CB1 and CB2: A characterization of expression and adenylate cyclase modulation within the immune system". *Toxicology and Applied Pharmacology* 142.2 (1997): 278-287.

33. McDougle DR., *et al*. "Endocannabinoids anandamide and 2-arachidonoylglycerol are substrates for human CYP2J2 epoxygenase". *Journal of Pharmacology and Experimental Therapeutics* 351.3 (2014): 616-627.

34. Wang J and Ueda N. "Biology of endocannabinoid synthesis system". *Prostaglandins and Other Lipid Mediators* 89.3-4 (2009): 112-119.

Volume 13 Issue 5 May 2025