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Research Article

Electrophysiological evidence of neuronal action potential alterations by a hydroxycinnamic acid derivative at the peripheral level

Feyza Alyu Altinok[1], Abderaouf Boubekka[2], Ahmed Hasan[2], Ilhem Dallali[3], Yusuf Burak[4], Nilgun Ozturk[5], Yusuf Ozturk[6]✉

[1]Anadolu University, Faculty of Pharmacy, Department of Pharmacology, Eskisehir, Türkiye

[2]Anadolu University, Graduate School, Department of Pharmacology, Eskisehir, Türkiye.

[3]KU Leuven, VIB-KU Leuven Center for Brain and Disease Research and Department of Cellular and Molecular Medicine, Leuven, Belgium

[4]Anadolu University, Faculty of Pharmacy, Eskisehir, Türkiye

[5]Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, Eskisehir, Türkiye

[6]Istanbul Aydın University, Faculty of Pharmacy, Department of Pharmacology, Istanbul, Türkiye

✉ Yusuf Ozturk
yusufozturk10@aydin.edu.tr

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ABSTRACT

Hydroxycinnamic acid derivatives have emerged as promising agents in pain management due to their multifaceted actions on various processes, including neuronal excitability. In this study, we investigated the electrophysiological effects of ferulic acid, a hydroxycinnamic acid derivative, on primary cultured dorsal root ganglion neurons using the whole-cell patch-clamp technique. Acute application of ferulic acid at a dose of 100 µM to the external solution resulted in a significant hyperpolarization of the resting membrane potential, indicating a stabilization of the neuronal membrane and a reduction in baseline excitability. Furthermore, ferulic acid induced a more negative afterhyperpolarization phase, suggesting enhanced potassium conductance and prolonged refractory periods. Notably, the threshold for action potential initiation became less negative following treatment, demonstrating that a stronger depolarizing stimulus was required to elicit neuronal firing. These changes collectively reflect a decrease in neuronal excitability that extends beyond anti-inflammatory or antioxidant effects, highlighting direct modulation of ion channel dynamics as a key mechanism. The findings provide mechanistic insight into the analgesic potential of ferulic acid by demonstrating its ability to alter action potential parameters and suppress excitability in peripheral sensory neurons. This study supports the therapeutic relevance of hydroxycinnamic acid derivatives in the development of novel analgesics targeting peripheral nociceptive pathways.

Keywords: Action potential, dorsal root ganglion neurons, ferulic acid, patch clamp technique

1. INTRODUCTION

Dorsal root ganglion (DRG) neurons are commonly used as a model to investigate the role of peripheral mechanisms in the processing of nociceptive information and are considered a therapeutic target in pain research [1, 2]. The hydroxycinnamic acid derivative bioactive phytochemical ferulic acid has been shown to exert anti-hyperalgesic and anti-allodynic effects in various pain models, as reported in recent literature [3-7]. The sodium salt of ferulic acid has been shown to inhibit capsaicin-induced currents and the associated increase in intracellular calcium concentration in DRG neurons [8]. In another study, the sodium salt of ferulic acid was found to inhibit currents activated by a P2X3 agonist in DRG neurons [9]. These studies suggest a decrease in neuronal excitability and thus potential analgesic effects, highlighting the need for further research on ferulic acid. There are also studies investigating the effects of various compounds on action potentials (APs) in DRG neurons using electrophysiological methods. Changes in AP parameters in DRG neurons have been associated with analgesic effects [10, 11].

The present study aimed to investigate the electrophysiological effects of ferulic acid on AP parameters in primary DRG neurons using the patch-clamp technique. Given the previously reported antihyperalgesic and antiallodynic properties of ferulic acid, we hypothesized that ferulic acid would modulate neuronal excitability in DRG neurons by altering AP characteristics. By exploring these effects, this study seeks to provide mechanistic insight into the potential analgesic actions of ferulic acid and support its relevance as a therapeutic candidate in pain management.

Derivatives of hydroxycinnamic acid alleviate pain through interactions with multiple molecular components of pain pathways, such as ion channels, neurotransmitter systems, and various receptors. Their ability to influence these targets suggests potential in creating new pain-relief drugs that function through mechanisms beyond just anti-inflammatory or antioxidant effects [12, 13]. Based on this, this study aimed to contribute data focusing on their electrophysiological effects on primary cell culture.

2. MATERIALS AND METHODS

2.1. Animals

This study was conducted using Sprague-Dawley rats, aged between 8 and 12 weeks, with body weights ranging from 250 to 300 grams. The animals were kept in groups of no more than five per cage under standard laboratory conditions, with free access to both food and water. All procedures involving animals were carried out in full compliance with the European Directive 2010/63/EU on the protection of animals used for scientific purposes. The experimental protocol received approval from the Local Animal Ethics Committee at Anadolu University in Eskisehir, Turkey (Approval No: 2022-11).

2.2. Chemicals

Ferulic acid and phosphate-buffered saline (PBS) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All salts used in the preparation of both internal and external solutions, along with trypsin, were obtained from Multicell (Wisent Inc., St-Bruno, Quebec, Canada). Collagenase type IV and Dulbecco's Modified Eagle Medium (DMEM) were sourced from Gibco (Life Technologies, Carlsbad, CA, USA). EGTA was provided by Biomatik (Cambridge, Ontario, Canada), and ATP was acquired from Genaxxon Bioscience (Biberach an der Riß, Germany).

2.3. Primary DRG Cell Culture Preparation

DRG neurons were freshly isolated and cultured in accordance with a previously described protocol developed in our laboratory [14]. The dissection included DRGs from the general thoracic and lumbar regions. Briefly, animals were anesthetized via intraperitoneal injection of a ketamine/xylazine combination (90 mg/kg and 10 mg/kg, respectively; 1 mL/kg), followed by rapid decapitation. The spinal column was carefully removed and immediately submerged in ice-cold PBS. DRGs were promptly extracted and placed into chilled DMEM. Using a surgical lancet and fine iris scissors, connective tissue and surrounding debris were meticulously cleared from the ganglia. The cleaned DRGs were

incubated at 37°C with 5% CO_2 for 45 minutes in DMEM containing 1x Penicillin/Streptomycin and collagenase type IV (2 mg/mL). After enzymatic digestion, tissues were rinsed three times with PBS to stop the reaction. Next, 1 mL of DMEM supplemented with 1x Penicillin/Streptomycin and 100 µL of 0.25% trypsin (1:250) was added, and samples were incubated for an additional 7 minutes. Trypsin activity was terminated by multiple washes with fresh DMEM. Finally, 4 mL of complete culture medium DMEM containing 1x Penicillin/Streptomycin and fetal bovine serum) was introduced. Mechanical dissociation was achieved by gentle trituration using a 1000 µL pipette tip to yield a single-cell suspension. Cells were plated into glass petri dishes and maintained at 37°C with 5% CO_2. Electrophysiological recordings were performed 1–2 hours after dissociation, allowing sufficient time for neuronal recovery from enzymatic and mechanical processing.

2.5. Patch Clamp Recordings

The small to medium-sized (25-35 µm) neurons exhibiting a spherical shape with well-defined borders, with no apparent neurites, were specifically selected for recordings. Ferulic acid (100 µM) was applied extracellularly to primary cultured DRG neurons using a micropipette positioned 50–100 µm from the recorded cell, with the concentration calculated based on the volume of the recording chamber. Electrophysiological measurements were performed at ambient room temperature (18–20ºC) using the whole-cell patch-clamp technique. Borosilicate glass pipettes with internal filaments (Sutter Instrument BF150-110–10) were pulled on a P-97 Micropipette Puller (Sutter Instrument) to achieve resistances between 2 and 5 MΩ. High-resistance gigaseals were formed before breaking into the membrane by applying gentle negative pressure, either manually via mouth suction or using a 1 mL syringe. Successful establishment of the whole-cell configuration was confirmed by observing capacitive transients, a significant decrease in access resistance, and a drop in membrane resistance from the gigaohm range to approximately 500 MΩ. After the control recording, ferulic acid was applied for a duration of approximately 2 minutes prior to the start of the experimental protocol.

Data acquisition was performed using the Sutter Integrated Patch Amplifier (IPA) equipped with a single headstage, with current-clamp control managed through SutterPatch® software (version 2.0.4) running on a Windows® 10 platform. Currents were digitized at 25 kHz and filtered at 5 kHz by the amplifier's internal low-pass filter to maintain signal fidelity. Automatic compensation for series resistance and pipette capacitance was applied throughout the recordings. All data collection and subsequent analysis were carried out using SutterPatch and Graphpad software.

The cells were bathed in external solution (in mM): NaCl 140, KCl 5, $CaCl_2$ 2.5, $MgCl_2$ 1.2, HEPES ACID 10, D-glucose 10 (pH = 7.4 with NaOH, 321 mOsm). The internal (pipette) solution contained (in mM): KCl 130, NaCl 10, HEPES ACID 10, Mg-ATP 4, EGTA 5, D-glucose 10 (pH = 7.3- 7.4 with KOH, 313 mOsm).

Spontaneous firing activity was recorded during several minutes of whole-cell patch-clamp recording. The AP threshold was identified by applying depolarizing current steps of 10 pA with a duration of 10 ms, incrementally increased from 0 pA up to 1200 pA. The smallest current amplitude that successfully evoked an AP was selected as the threshold.

Using this threshold current, multiple APs were triggered to establish a stable baseline. Once stability was confirmed, the test compound was applied, and subsequent changes in AP parameters—including threshold and afterhyperpolarization (AHP)—were continuously monitored. The AP threshold was determined using SutterPatch® software (Sutter Instrument). The AP was defined as the voltage at which the first derivative of the voltage trace (dV/dt) rapidly rose, indicating the onset of AP upstroke. The software detects this key point in the analysis by identifying the voltage at which dV/dt intersects a predetermined value, representing the AP inflection. For each recorded event, the threshold value is included in the results summary.

2.5. Statistical Analysis

Origin Pro 2021 (64-bit) 9.8.0.200 (Learning Edition), and GraphPad Prism (ver. 10.0.2) programs have been used for the electrophysiological data

analysis. Paired Student's *t*-test has been used to compare the effect of the drug treatment within the same cell. A value of P<0.05 was considered significant. Results are given as mean ± standard error of mean (S.E.M.) (n=7).

3. RESULTS AND DISCUSSION

Application of ferulic acid (100 µM) to primary cultured DRG neurons resulted in a significant hyperpolarization of the resting membrane potential (RMP), as indicated by a downward shift of the membrane potential in the voltage-time trace (Figure 1A) via the implementation of the protocol (Figure 1B). A more negative RMP places the neuron further from the threshold required to trigger an AP, thereby reducing excitability. This shift suggests that the neurons are in a more stable, hyperpolarized resting state, decreasing the likelihood of spontaneous or stimulus-evoked firing—an effect that may contribute to pain suppression.

This hyperpolarization could result from increased K^+ conductance or activation of K^+ channels, which drive the membrane potential closer to the K^+ equilibrium potential. Such a mechanism is observed with opioid peptides and other analgesic agents that enhance K^+ efflux in DRG neurons without altering AP amplitude or duration. Alternatively, ferulic acid may inhibit inward currents that normally maintain a more depolarized RMP, such as the hyperpolarization-activated cation current (I_h). For instance, clonidine has been shown to inhibit I_h, leading to mild hyperpolarization in DRG neurons [15]. Additionally, suppression of persistent Na^+ currents—especially TTX-resistant Na^+ currents—can reduce depolarizing drive at rest, further contributing to membrane hyperpolarization [16].

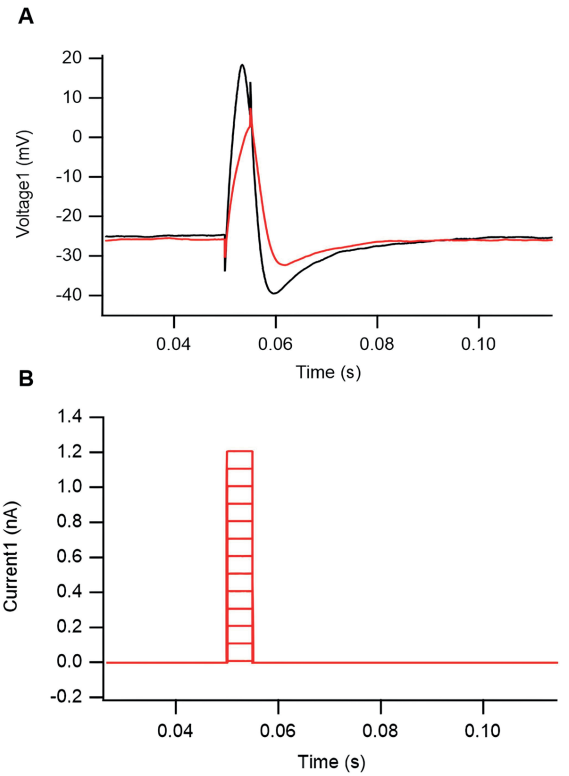

Figure 1. Representative APs recorded from DRG neurons (A) and the protocol applied (B). APs were elicited by brief depolarizing current injections (1.2 nA for 0.4 ms), and key AP parameters were subsequently measured. To determine the AP threshold, current pulses of 10 ms duration were applied in incremental steps of 0.1 nA until an AP was triggered.

3.1. Effect of Ferulic Acid on The AP Threshold

The AP threshold was found to be less negative following ferulic acid treatment (P<0.05, n = 7, Figure 2A), indicating that a stronger depolarizing stimulus was required to elicit an AP after drug administration, an observation supporting the results regarding the data in Figure 1. The neuron's membrane potential must reach a higher (less

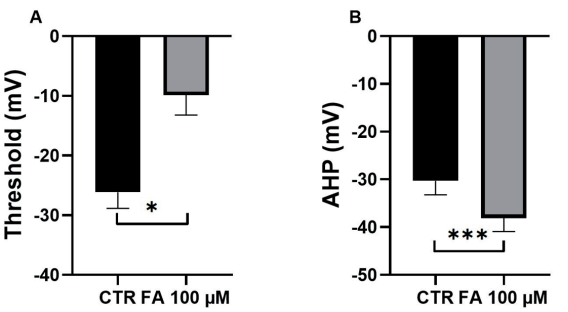

Figure 2. Ferulic acid (100 µM) alters AP threshold and afterhyperpolarization in DRG neurons. (A) Bar graph showing a significant depolarizing shift in the action potential threshold following ferulic acid (FA) administration (P < 0.05, n = 7). (B) Bar graph demonstrating a significantly more negative afterhyperpolarization (AHP) post-FA treatment (P < 0.05, n = 7). Data are presented as mean ± SEM. Statistical analysis was performed using paired Student's t-test; P < 0.05 was considered significant.

negative) voltage to open enough voltage-gated Na^+ channels to initiate an AP. This effectively makes the neuron less excitable because it is harder to reach the threshold for firing.

Ferulic acid may reduce the availability or function of voltage-gated Na^+ channels responsible for the rapid depolarization phase of the AP, raising the threshold. It could modulate the gating properties of Na^+ channels, requiring a larger depolarization to activate them. Alternatively, ferulic acid might enhance K^+ currents or other hyperpolarizing influences that counteract depolarization, indirectly increasing the threshold.

A less negative threshold reduces the likelihood of spontaneous or evoked firing, contributing to decreased neuronal excitability. This effect is consistent with analgesic or inhibitory drug actions that dampen sensory neuron firing, such as riluzole, blocking persistent Na^+ currents in DRG neurons, which raises the threshold and inhibits spontaneous activity [17]. In pathological states like neuropathic or bone cancer pain, a lowered (more negative) threshold is often associated with hyperexcitability; thus, a shift to a less negative threshold can counteract such hyperexcitability [18].

3.2. Ferulic Acid Administration Enhances AHP

When the AHP phase of the AP in a DRG neuron shifts to more negative values following drug administration (Figure 2B), it indicates a stronger or prolonged hyperpolarization. The AHP occurs after the AP and is characterized by the membrane potential falling below the RMP, primarily due to increased K^+ conductance. A more negative AHP suggests enhanced activation of K^+ channels—particularly voltage-gated and Ca^{2+}-activated K^+ channels—which remain open after the spike, allowing greater K^+ efflux and driving the membrane potential closer to the K^+ equilibrium potential.

This enhanced AHP reduces neuronal excitability by increasing the refractory period, making it more difficult for the neuron to fire subsequent APs in rapid succession. As a result, firing frequency decreases, serving as a negative feedback mechanism to prevent excessive excitability.

4. CONCLUSION

Hydroxycinnamic acid derivatives exhibit analgesic properties by targeting multiple molecular components involved in pain signalling, including neurotransmitter systems, ion channels, and receptor pathways. This multifaceted mode of action highlights their potential as promising candidates for the development of novel analgesics with mechanisms extending beyond traditional anti-inflammatory and antioxidant effects. As limitations, DRG neuron cultures do not fully capture *in vivo* complexity, and experiments were limited to 8–12-week-old Sprague-Dawley rats, a single compound, and one dose, which may restrict generalizability. Exposure durations may not reflect long-term effects, and behavioral correlations were not assessed, though prior studies report analgesic effects [3-7]. While molecular mechanisms remain unclear and single-cell recordings limit network-level insights, these findings provide valuable electrophysiological data and shed light on key directions for future studies to explore mechanisms, dose-responses, and functional outcomes.

Our electrophysiological findings demonstrate that ferulic acid induces significant hyperpolarization of the RMP and enhancement of the AHP amplitude in primary cultured DRG neurons, effects that suggest modulation of potassium conductance or activation of potassium channels. K^+ channels are the main ionic mechanisms responsible for neuronal hyperpolarization and AHP because potassium ion efflux drives these processes. Additionally, the observed depolarizing shift in threshold potential suggests reduced neuronal excitability, possibly via modulation of Na^+ channel function or the enhancement of opposing hyperpolarizing currents [19]. Further studies will elucidate the precise mechanisms underlying these observations. These combined effects point to a stabilizing influence on sensory neuron excitability, which may underlie the compound's analgesic action. Theoretically, this study contributes to the understanding of ion channel modulation by natural compounds in nociceptive pathways. Practically, it positions ferulic acid as a potential lead compound for the development of non-opioid, ion channel-targeting therapies for pain conditions.

Ethical approval

This study was conducted using Sprague-Dawley rats. All procedures involving animals were carried out in full compliance with the European Directive 2010/63/EU on the protection of animals used for scientific purposes. The experimental protocol received approval from the Local Animal Ethics Committee at Anadolu University in Eskisehir, Turkey (Approval No: 2022-11).

Author contribution

Conceptualization, N.O., F.A.A. and Y.O.; Validation, R.B., A.H., F.A.A, I.D. and Y.O.; Formal analysis, A.H. and I.D.; Investigation, A.B., Y.B., F.A.A.; Resources, F.A.A., A.H., N.O., Y.O.; Data curation, A.B. and A.H.; Writing—original draft preparation, F.A.A., Y.B.; Visualization, A.H.; Supervision, Y.O.; Project administration and funding acquisition, N.O., Y.O., F.A.A. All authors have been contributed to the writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declared that there is no conflict of interest.

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