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Research Paper

Hepatic Acyl CoA Oxidase1 Inhibition Modifies Brain Lipids and Electrical Properties of Dentate Gyrus

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ABSTRACT

Introduction: Peroxisomes are essential organelles in lipid metabolism. They contain enzymes for β-oxidation of very long-chain fatty acids (VLCFA) that cannot be broken down in mitochondria. Reduced expression in hepatic acyl-CoA oxidase 1 (ACOX1), a peroxisome β-oxidation enzyme, followed by modification of the brain fatty acid profile has been observed in aged rodents. These studies have suggested a potential role for peroxisome β-oxidation in brain aging. This study was designed to examine the effect of hepatic ACOX1 inhibition on brain fatty acid composition and neuronal cell activities of young rats (200-250 g).

Methods: A specific ACOX1 inhibitor, 10, 12- tricosadiynoic acid (TDYA), 100 µg/kg (in olive oil) was administered by daily gavage for 25 days in male Wistar rats. The brain fatty acid composition and electrophysiological properties of dentate gyrus granule cells were determined using gas chromatography and whole-cell patch-clamp, respectively.

Results: A significant increase in C20, C22, C18:1, C20:1, and a decrease of C18, C24, C20:3n6, and C22:6n3 were found in 10, 12- tricosadiynoic acid (TDYA) treated rats compared to the control group. The results showed that ACOX1 inhibition changes fatty acid composition similar to old rats. ACOX1 inhibition caused hyperpolarization of resting membrane potential, and also reduction of input resistance, action potential duration, and spike firing. Moreover, ACOX1 inhibition increased rheobase current and afterhyperpolarization amplitude in granule cells.

Conclusion: The results indicated that systemic inhibition of ACOX1 causes hypo-excitability of neuronal cells. These results provide new evidence on the involvement of peroxisome function and hepatic ACOX1 activity in brain fatty acid profile and the electrophysiological properties of dentate gyrus cells.

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Highlights

- Peroxisome β-oxidation is involved in dentate gyrus cell activity in adult rats.
- Hepatic ACOX1 inhibition changes brain lipids in younger similar to older animals.
- Hepatic ACOX1 inhibition remodels passive membrane properties in dentate gyrus.
- Hepatic ACOX1 inhibition reduces firing frequency of dentate gyrus granule cells.

Plain Language Summary

The brain has the highest amount of lipid after the adipose tissue. Brain lipids play a vital role in the function and structure of the brain. Therefore, lipid metabolism, which is mainly performed by the liver, is pivotal for brain function. Fatty acid degradation through the process of β-oxidation is a common metabolic pathway in both peroxisomes and mitochondria. However, certain mammalian fatty acids, especially very long-chain fatty acids (VLCFAs), can exclusively be degraded by peroxisomes. Abnormal lipid metabolism and alteration of fatty acid profiles in the brains of aged and Alzheimer's disease patients have been reported. Peroxisomes are the most important organelles in eukaryotic cells. Impairment of peroxisome function has been reported in aging and Alzheimer's. On the other hand, brain fatty acids can affect the neuron's physiological activity and electrical properties. The main question in the present study is whether the fatty acid composition of the brain would change if the main peroxisome β-oxidation enzyme is inhibited. And, if so, what will happen to the electrical activity of the brain, especially hippocampal dentate gyrus granular cells. Our results showed that inhibition of hepatic Acyl-CoA oxidase 1 (ACOX1), a peroxisome β-oxidation enzyme, could modify the profile of the brain fatty acids in adult male rats. Also, inhibition of this enzyme caused hypo-excitability in the hippocampal cells, the area of the brain involved in learning and memory function. These findings provide a new evidence on the involvement of peroxisome function and hepatic ACOX1 activity in brain fatty acid profile and the electrophysiological properties of hippocampal cells.

1. Introduction

Fatty acid degradation through the process of β-oxidation is a common metabolic pathway in both peroxisomes and mitochondria. However, certain mammalian fatty acids can be exclusively degraded by peroxisomes. They include dicarboxylic acids, bile acid intermediates, very-long-chain fatty acids (VLCFA), and phytanic acid (Poirier et al., 2006; Wanders & Waterham, 2006). While oxidation of polyunsaturated fatty acids (PUFAs) is slow in mitochondria, it is very fast in peroxisomes, (Hiltunen et al., 1986). Additionally, peroxisome β-oxidation contributes to the synthesis of the major brain PUFA, docosahexaenoic acid (DHA) (Ferdinandusse et al., 2001). Peroxisomes have a role in modulating mitochondrial regulatory factors, such as histone deacetylases 6 (HDAC6) and sirtuin-1 (SIRT1) (Rafiei et al., 2021). Acyl-CoA oxidase-1 is the rate-limiting enzyme of the peroxisome β-oxidation, which catalyzes the first step reaction. It can act on dicarboxylic acid, PUFAs, and saturated VLCFA without affecting branched-chain fatty acids or di or tri hydroxycholestanoic acids (bile acid intermediates). The deficiency of the Acyl CoA oxidase (ACOX1), an autosomal recessive disorder known as pseudo-neonatal adrenoleukodystrophy, is determined by the clinical presentation of hypotonia, seizures, and developmental retardation. Its biochemical diagnosis is the plasma accumulation of VLCFA C26:0 (Ferdinandusse et al., 2007). Some studies emphasize the decline of the peroxisome function during aging (Narayan et al., 2016). Moreover, in aged rodents, a decline of hepatic ACOX1 expression followed by altered brain fatty acid composition has been reported (Yang et al., 2014). In addition, growing evidence has suggested the relationship of peroxisome with age-related pathologies, such as neurodegeneration (Cipolla & Lodhi, 2017).

The dentate gyrus, involved in pattern separation, response decorrelation, spatial navigation, and engram formation, has been described as a more vulnerable hippocampal subregion to age-related processes (Small et al., 2004; Small et al., 2002). The brain has the highest lipid content after adipose tissue, and lipids comprise 50% of brain dry weight (Hamilton et al., 2007). Brain lipids

have a crucial role in the development and maintenance of brain structure throughout life. By localizing proteins and allocating their function, brain lipids mainly organize the neuronal cell membrane (Simons & Toomre, 2000). Some studies have focused on the relationship between the brain lipid profile and its electrical properties. They have reported that dietary fat can influence these properties by remodeling brain lipid composition. Variations that occurred in the ratio of PUFAs to saturated fatty acids (P/S) in mice diets have made significant differences in membrane electrical properties of neurons in dorsal root ganglion (Scott et al., 1989). Lipid modification of the entorhinal cortex induced by diet has changed the physiological properties of neurons (Arsenault et al., 2012). Moreover, studies show that docosahexaenoic acid (DHA) accumulation improves cognition by altering the passive properties of EC neurons (Arsenault et al., 2011).

An acetylene derivative of fatty acids, 10, 12-tricosadiynoic acid (TDYA), was proposed as a specific ACOX1 inhibitor in a study in 2017, in which the inhibitory effect of TDYA was assessed in both in vitro and in vivo conditions on rodent hepatic ACOX1 (Zeng et al., 2017).

This study was conducted to examine the effect of inhibition of ACOX1 activity, a peroxisome β-oxidation enzyme, on cerebral lipid composition and electrophysiological properties of dentate gyrus granule cells (DGGCs) in adult rats.

2. Materials and Methods

Animals

This study was conducted on the male Wistar rats (200-250 g) provided by a breeding colony in the Neuroscience Research Center (Tehran City, Iran). Rats in groups of four were housed in each cage with free access to water and chow. The standard cycle of 12 h light/12 h dark and a temperature of 22°C-25°C was set in the animal house.

Experimental design

The animals were divided into two groups and received an intragastric gavage administration of 100 µg/kg TDYA or olive oil as the drug's solvent for 25 consecutive days. Electrophysiological recordings and tissue collection for fatty acid profile analysis (brain hemispheres) were performed on the 25th day. For enzyme assay, five hours after the last administration of TDYA or vehicle, animals were decapitated, and hepatic tissue was removed. The collected tissues were quickly transferred to liquid nitrogen and then stored at -80°C until use. For each experiment, the number of rats was as follows, enzymatic and fatty acid profile experiments (n=3 for each group) and electrophysiological experiments (n=4 for olive oil and n=6 for TDYA groups). In addition, 8 cells were used in electrophysiological experiments in olive oil and 6 cells in TDYA groups.

Acyl CoA oxidase (ACOX1) activity assay

ACOX1 activity assay was conducted based on the method of Cable et al. by measuring H_2O_2 production (Cablé et al., 1993). The reaction was started by adding 0.1 mM palmitoyl-coA into the reaction mixture containing liver homogenate, 0.01 mM flavin adenine dinucleotide (FAD), 0.082 mM 4-aminoantipyrine, 0.8 IU of horseradish peroxidase, 1.06 mM phenol, and 50 mM potassium phosphate, pH 8. Increasing the absorbance at 500 nm was recorded for 30 minutes and enzyme activity was calculated as nmol H_2O_2/min/mg protein by extinction coefficient of 480×10^5 mL mol^{-1} cm^{-1}.

Fatty acid analysis

Brain lipids were extracted by two commonly used solvents, chloroform, and methanol, in a volume ratio of 2:1. The methyl ester fatty acids were derived from a BH3/methanol reagent and analyzed using Shimadzu Nexis 2030 gas chromatography with a detection system of flame-ionization. The chromatography column was Dikmacap-2330 (60 m×0.25 mm). Fatty acid separation was acquired in a two-stage temperature program by maintaining the initial temperature at 60°C for 2 minutes and increasing it to 200°C at a rate of 10°C/min for 25 minutes. Then column reaches the final temperature of 240°C at a rate of 5°C/min and keeps it for 7 minutes. The chromatogram was analyzed by comparing the retention times of each peak with the peaks from the standard fatty acid methyl esters. Data were presented as the relative percentage by calculating the peak area ratio for each fatty acid to the total area from the rest of the peaks by the LabSolutions software.

In vitro whole cell patch clamp

Acute slice preparation

Transcardial perfusion in a deeply anesthetized rat (intraperitoneal ketamine/xylazine) was done with an ice-cold cutting solution containing 206 mM sucrose, 2.8 mM potassium chloride (KCl), 1.25 mM monosodium phosphate (NaH_2PO_4), 26 mM sodium bicarbonate ($NaHCO_3$), 10 mM glucose, 1 mM calcium chloride

($CaCl_2$), 2 mM magnesium sulfate ($MgSO_4$), pH 7.4, oxygenated with molecular oxygen (O_2) and carbon dioxide (CO_2) (95%-5%). After rapidly removing the brain and immersing it into the cutting solution, the hippocampus was dissected out and sliced by a vibratome (752 HA, Campden Instruments Ltd, UK) to obtain 350 µm-thick slices that were then transferred into artificial cerebrospinal fluid (ACSF) solution (124 mM sodium chloride [NaCl]), 3 mM potassium chloride (KCl), 1.24 mM monosodium phosphate (NaH_2PO_4), 26 mM sodium bicarbonate ($NaHCO_3$), 10 mM glucose, 2 mM $CaCl_2$, 2 mM magnesium sulfate ($MgSO_4$), pH 7.4, oxygenated with 95% molecular oxygen (O_2), and 5% carbon dioxide (CO_2) at 32˚C for 30 minutes. In the recording process, a slice was located in a holding chamber of an upright microscope (Olympus; BX 51WI) stage and continuously superfused with the artificial cerebrospinal fluid (ACSF) solution at room temperature.

Whole-cell patch clamp recording

DG-GCs were visualized by infrared video imaging (Hamamatsu, ORSA, Japan) using a 40x water immersion objective lens. A PC-10 vertical puller (Narishige, Japan) was used for two-stage pulling of the borosilicate glass capillary (1.2 mm O.D×0.9 mm ID) to obtain 3-5 MΩ pipette resistance when filled with an intracellular solution. A whole-cell configuration was achieved by applying negative pressure to the gigaseal cell-attached statement. GCs were recorded by Multiclamp 700B amplifier (Axon Instruments, Foster City, CA) connected to an A/D converter of Digidata 1320 (Axon Instruments, Foster City, CA). Recordings with series resistance less than 25 MΩ and variation less than 20% during the experiment were selected for analysis. Current-clamp was done using an intracellular solution containing 140 mM K-Gluconate, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 2 mM magnesium chloride ($MgCl_2$), 2 mM adenosine-5-triphosphate disodium salt (Na_2-ATP), 1.1 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), 0.1 mM $CaCl_2$, and 0.4 mM guanosine-5-triphosphate disodium salt (Na_2-GTP) adjusted to pH 7.3 by potassium hydroxide (KOH), and the osmolality to 290 mOsm. The records were obtained at a sample rate of 20 kHz after applying a low-pass Bessel filter at 10 kHz and analyzed offline. The measured parameters were resting membrane potential (RMP), input resistance, time constant, action potential (AP) duration, rising time and decay time, firing frequency of neurons, AP peak amplitude, afterhyperpolarization (AHP) amplitude, and rheobase current.

Statistics

GraphPad Prism software, version 6.07 was used for analysis. All data were analyzed using student's t-test and expressed as Mean±SEM. The values of $P<0.05$ were statistically significant.

3. Results

Tricosadiynoic acid (TDYA) reduced hepatic ACOX1 activity

Data shown in Figure 1 shows ACOX1 activity in two comparing groups TDYA and olive oil as a control. The analysis revealed a significant 30% reduction in enzyme activity in the TDYA group ($P<0.05$) (Figure 1).

Inhibition of ACOX1 changed fatty acid composition in the brain

As shown in Table 1, only 13 types of fatty acids (of the total 31 detected types) were compared in our analyses (Table 1). Among all saturated fatty acids, C18:0 was significantly lower in the TDYA group ($P<0.05$). VLCFA levels (C20:0 and C22:0) were significantly higher in the TDYA ($P<0.001$, $P<0.01$), and in contrast, C24:0 was significantly decreased ($P<0.01$). The analyzed data for five monounsaturated fatty acids (MUFAs) revealed a significant increase for C18:1 and C20:1 ($P<0.001$, $P<0.001$). No significant difference was observed for C16:1, C22:1, and C24:1 compared to the control group. Among the three analyzed PUFAs, the TDYA group had a significant decrease in C20:3n6 and DHA (C22:6n3) levels ($P<0.001$, $P<0.05$).

Inhibition of hepatic ACOX1 resulted in hypo-excitability of dentate gyrus neuronal cells

After 25 days of TDYA treatment, significant differences in electrophysiological properties in DG-GCs were detected (Table 2). We found several differences in the passive properties of granule cells in the TDYA group compared to the control group. The student's t-test analysis revealed a significant decrease in RMP of DG-GCs in the TDYA group (-76.98±1.5 mV) compared to the control group (-71.45±1.08 mV, $P<0.01$). In addition, the input resistance (R_{in}) of TDYA-treated rats (169.18±20.42 MΩ) compared to the control ones (270.66±23.37 MΩ) was significantly lower ($P<0.01$). Data analysis also indicated an increased time constant for the TDYA group (12.19±1.15 ms, $P<0.05$) compared to the control group (8.44±0.71 ms).

The current clamp was recorded to illustrate how the granule cell spike waveform can be affected by TDYA administration. TDYA had no significant effect on AP peak amplitude, threshold, and rise or decay slope (Table 2). As shown in Figure 2A, the granule cells of TDYA-treated rats fired at a much more depolarized current intensity than the control group. Further, DG-GCs from the TDYA group showed APs with a lower half-width (1.23±0.052 vs. 1.47±0.059 ms, P<0.05, Figure 2B), and higher amplitude for AHP compared to the control group (-10.8±0.99 mV vs. -6.96±0.55 mV, P<0.01, Figure 2C). Rheobase current was determined as the minimum current injected to trigger one AP during one second. As represented in Table 2 and Figure 2D, the rheobase current in the TDYA group was approximately 2-fold higher than the control group, indicating that TDYA induced hypo-excitability in DG-GCs.

Figure 3A shows that TDYA administration decreased the firing rate of DG granule cells at different current injections, indicating that the granule cells in the TDYA group required a larger current to fire an AP. As shown in Figure 3B, current injection higher than 150 pA elicited lower AP numbers in the granule cells from TDYA-treated rats compared to the control group. Furthermore, the depolarizing current injected in the form of a ramp (200 pA, 300 ms) revealed a lower tendency of DG-GCs for firing in TDYA-treated rats (control 144.7±11.75 pA; TDYA 187.2±10.80 pA, P<0.05; Figure 3C, D). They also manifested a greater latency to fire the first AP (280.7±16.22 ms vs. 207.6±16.77 ms, P<0.01) and a lower number of APs (0.7143±0.56 vs. 4.375±0.84, P<0.01) compared to the control (Figure 3E, F). Together, these data confirm that TDYA administration leads to hypo-excitability in the DG-GCs.

4. Discussion

The present study is the first report on intrinsic membrane properties of DG-GCs after inhibition of hepatic ACOX1 and subsequent modified fatty acid profile in the brains of young rats. Inhibition of hepatic ACOX1 by TDYA altered the brain fatty acid composition, followed by decreased excitability of DG-GCs. We found a significant increase in MUFAs (C18:1, C20:1) and a reduction of PUFAs (C20:3n6 and C22:[DHA]) and C18:0 in the statistical analysis. For VLCFAs, a significant increase was found in C20:0 and C22:0 with a decrease in C24:0. A study published in 2014 reported that the expression of hepatic ACOX1 in old rats has been diminished and caused brain fatty acid alteration compared to younger ones (Yang et al., 2014). They saw an increase in VLCFAs C20:0, C24:0 and MUFAs C16:1, C18:1, C20:1, C22:1, C24:1, and a decrease in C18:0, C20:4, and DHA. We had the same trend for fatty acid differences, except for C16:1, C22:1, C24:0, and C24:1. Analysis of the cortical fatty acid profile in patients with two inherited peroxisomal disorders of Zellweger syndrome, a peroxisomal biogenesis disorder with absence or non-functional peroxisomes, and X-linked adrenoleukodystrophy, deficiency in the metabolism of VLCFAs, exhibited a decrease in C24:0, and C24:1 (Martinez, 1992). In addition, a significant decrement of an omega 6 PUFA, eicosatrienoic acid (C20:3n6) was observed, which is consistent with another study on aging (Carver et al., 2001). The levels of PUFAs decrease and MUFAs increase with aging (Lopez et al., 1995). Aberrant lipid metabolism and altered fatty acid composition in the brain have been suggested as an underlying mechanism for pathology involved in brain aging and certain neurodegenerative disorders, including AD and Parkinson's disease (Cunnane et al., 2012; Fabelo et al., 2011). Some studies reported declined levels of C18:0 (stearic acid), arachidonic acid, and DHA in the brain of mild cognitive impairment and AD, with a significant enhancement of C18:1 (oleic acid), C16:0 (palmitic acid), and VLCFAs (Cunnane et al., 2012; Fraser et al., 2010). It can be concluded that ACOX1 inhibition in the present study may change brain lipid components at a younger age, similar to aging and AD.

Intrinsic membrane properties, categorized into passive and active characteristics of a neuron, can dictate neuro-

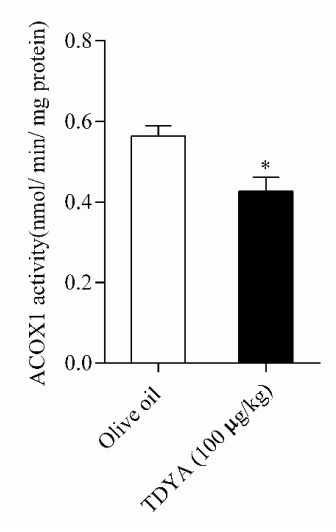

Figure 1. In vivo inhibition of Acyl CoA oxidase (ACOX1) by TDYA

ACOX1 activity is significantly lower in the TDYA group compared to the control group (*P<0.05)

Table 1. The percentage of fatty acids in brain

Fatty Acid Types	Fatty Acid (%)	Olive Oil	TDYA (100 µg/kg)
Saturated fatty acids (SFAs)	C16 (Palmitic acid)	20.44±0.43	19.005±0.87
	C18 (Stearic acid)	19.975±0.1	18.865±0.28*
Very-long-chain fatty acids (VLCFAs)	C20 (Arachidic acid)	0.27±0.006	0.39±0.01***
	C22 (Behenic acid)	0.27±0.01	0.325±0.003**
	C24 (Ligniceric acid)	2.82±0.03	2.605±0.02**
Monounsaturated fatty acids (MUFAs)	C16:1 (Palitoleic acid)	0.49±0.02	0.515±0.02
	C18:1 (Oleic acid)	21.175±0.12	23.595±0.19***
	C20:1 (Gadolic acid)	1.335±0.08	2.29±0.14***
	C22:1 (Eruccic acid)	0.23±0.02	0.32±0.03
	C24:1 (Nervonic acid)	0.65±0.04	0.33±0.12
Polyunsaturated fatty acids (PUFAs)	C20:3n6 (Ecosatrienoic acid)	8.855±0.02	7.49±0.13***
	EPA (Ecosapantaenoic acid)	0.04±0.01	0.095±0.03
	DHA (Docosaheaenoic acid)	10.1±0.1	9.155±0.2*
Σ		86.65±0.986	84.98±2.043

TDYA: Tricosadiynoic acid. *P<0.05, **P<0.01, ***P<0.001 versus control group.

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Table 2. Intrinsic membrane properties of dentate gyrus granule cells

Parameters	Control (n=8)	TDYA (100 µg/kg) (n=6)
Resting membrane potential (RMP) (mV)	-71.45±1.08	-76.98±1.5**
Input resistance (R_{in}) (MΩ)	270.66±23.37	169.18±20.42**
Time constant (τ_m) (ms)	8.44±0.71	12.19±1.15*
Rheobase (pA)	94.12±8.38	158.33±17.21**
Afterhyperpolarization (AHP) (mV)	-6.96±0.55	-10.79±0.99**
Half width (ms)	1.47±0.059	1.23±0.052*
Threshold (mV)	-34.76±2.95	-32.34±1.21
Peak amplitude (mV)	67.68±4.22	65.72±4.49
Rise time (ms)	0.55±0.023	0.51±0.051
Decay time (ms)	1.12±0.056	1.07±0.079

TDYA: Tricosadiynoic acid. *P<0.05, **P<0.01 versus control group.

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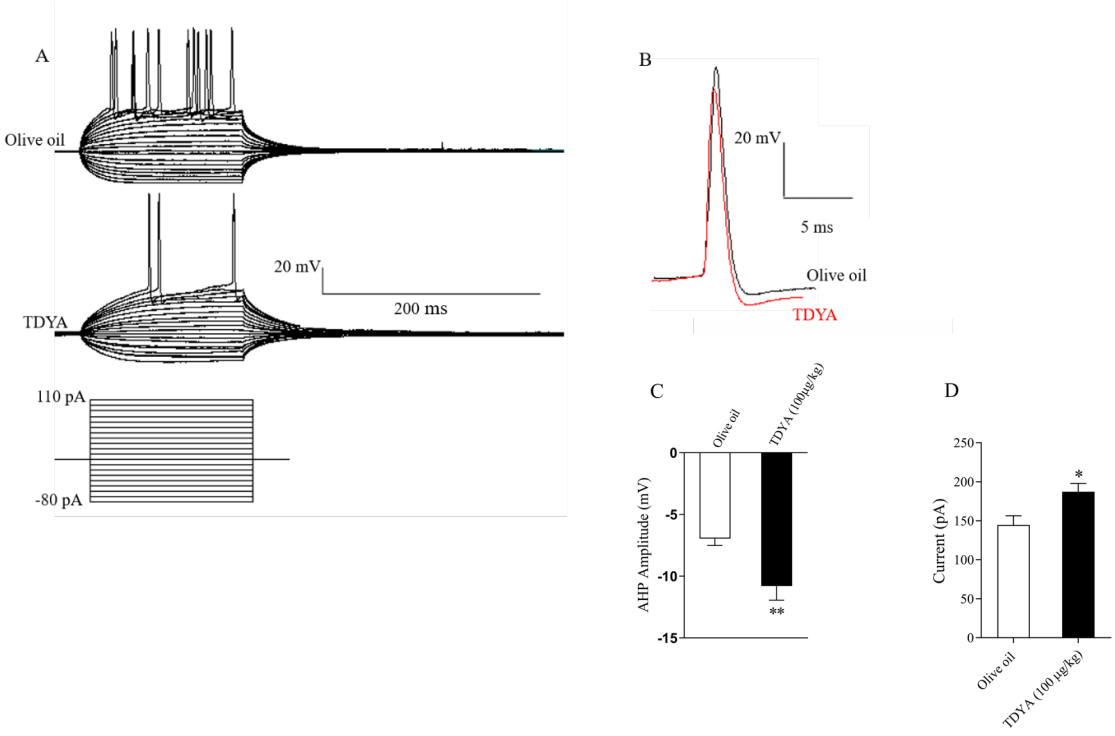

Figure 2. Active intrinsic characteristics of DG-GCs

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A) Granule cells of TDYA-treated rats fired at a much more depolarized current intensity than the control group, B) Granular cells in the TDYA group have narrower action potential repolarization than the control groups, C) Granular cells in the TDYA group have a larger subsequent hyperpolarization amplitude than the control, D) The minimum current intensity required for inducing an AP during 1s in the TDYA group showed a significant increase compared to the control group

Data are reported as Mean±SEM, $^{**}P<0.01$ compared to the control group.

nal excitability and affect its input/output gain (Kowalski et al., 2016). DG-GCs in TDYA-treated rats showed a more hyperpolarized RMP with a lower R_{in} and higher time constant ($_{\tau m}$) than the control cells. Hyperpolarized RMP led to neuron hypo-excitability, and may be attributed to changed ion gradient or upregulation of new ion channels active in the resting state (Watari et al., 2013). R_{in}, and $_{\tau m}$ are two contributing factors in the integration of postsynaptic signals. Diminution of R_{in} can result from R_m reduction, which is inversely related to conductivity, and/or enhanced membrane surface. On the other hand, R_m and C_m, the parameters correlative to the cell surface, determine the τ_m (Young et al., 2009). The granule cells of the TDYA group manifested strongly enhanced τ_m values compared to the control. Since TDYA influenced R_{in} and τ_m in the opposite direction, it can be inferred that C_m was increased by the TDYA administration. Lipid composition can determine this value. In vitro, PUFA application has disclosed their differential effects on membrane capacitance. While eicosapentaenoic acid and arachidonic acid made capacitance increase, docosahexaenoic acid, linoleic acid, oleic acid did not change it (Ong et al., 2006). In analyzing the active properties of the granule cell membrane, no significant difference was observed in some AP parameters, including peak amplitude, threshold, rise time, and decay slope between groups. However, TDYA altered AP properties by enhancing rheobase current, decreasing AP duration, and increasing AHP amplitude with decreased AP firing frequency. The shape of the AP waveform has an essential effect on the firing frequency of a neuron. According to Ohm's law (R=V/I), decreased R_{in} in TDYA-treated rats should reduce excitability. Therefore, enhanced rheobase current in this study was predictable. Variations in spike width can influence the gain of a neuron's input/output relationship (Giese et al., 1998; Lin et al., 2014). Also, the AP duration (width) can be influenced by the rate of spike repolarization, which is commonly regulated by potassium voltage-gated channels (Bean, 2007). For instance, the enhanced density of these channels correlates with declined RMP, R_{in}, and AP duration observed with maturation for DG-GCs (Spigelman et al., 1992), which is similar to our results. Also, other potassium channels (fast delayed-rectifier K channels or BK channels) have

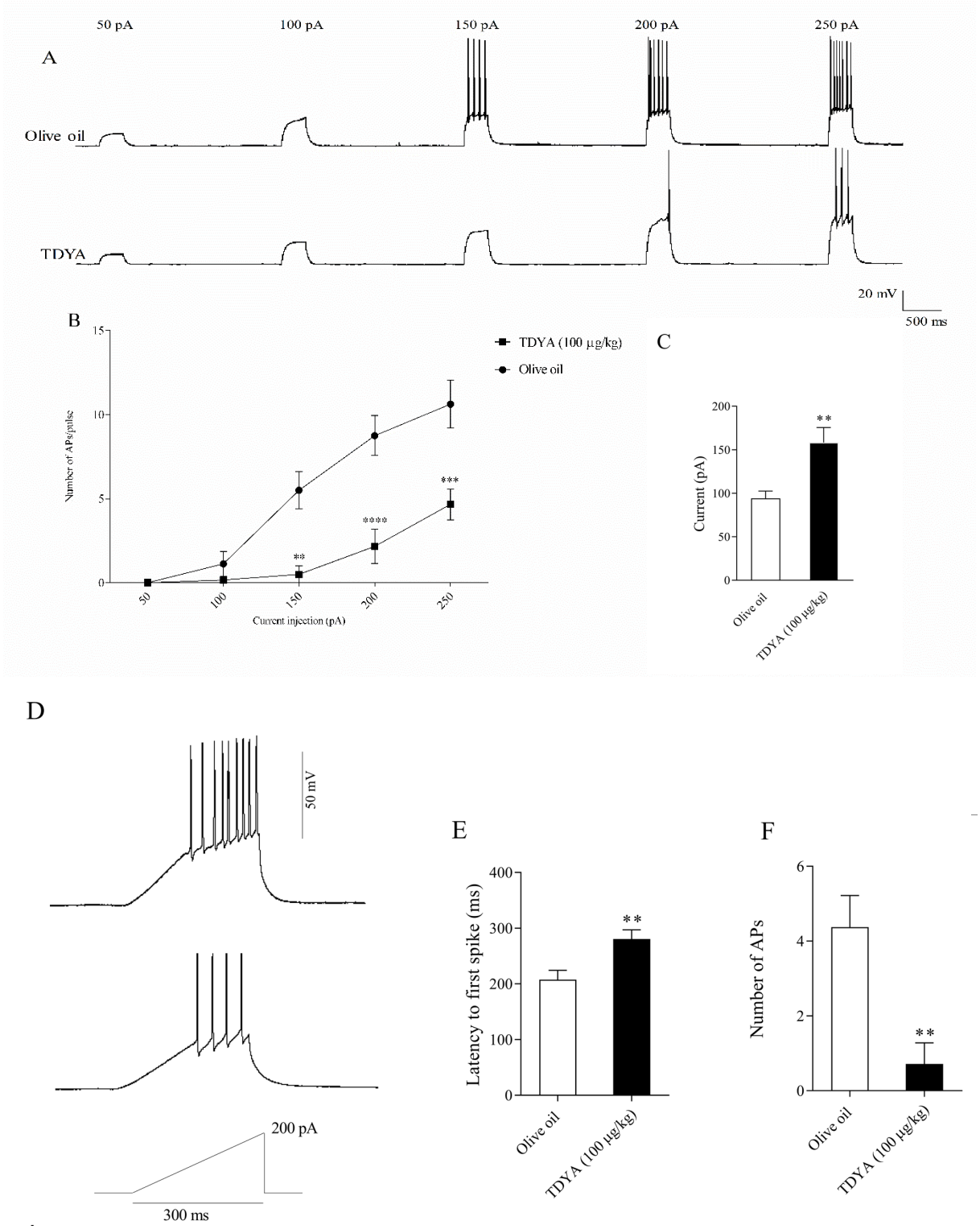

Figure 3. Firing properties of DG-GCs evoked by current injection

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A) TDYA administration decreased the firing rate of DG granule cells at different current injections, B) The number of APs in granular cells of the TDYA group showed a significant decrease after current injection higher than 150 pA compared to the control group, C) The granule cells of TDYA-treated rats required higher current intensity to elicit the first spike of ramp injection, D) Representative spike firings of granule cells in response to ramp injection, E) The latency of the first AP significantly increased in granule cells of TDYA group, F) The frequency of APs in ramp injection was lower for the TDYA group than the control

Data are reported as mean±SEM, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$ compared to the control group.

been reported to control the AHP amplitude (Contet et al., 2016; Sesti et al., 2010). Therefore, alteration of potassium channels can be one of the possible mechanisms for ACOX1 inhibition in our study. The effect of fatty acids on ion channels, including potassium, has been subjected to numerous studies. Arachidonic acid, C14:0, C18:1, C18:3n3, and eicosatetraenoic acid have been reported to activate potassium currents. (Ordway et al., 1989). Furthermore, reports show different effects of PUFAs on different potassium channels. For instance, PUFAs have different effects on the voltage-gated potassium channels. While the K_V1-4 channels are inactivated in the presence of PUFAs, these lipids can activate the slow rectifier K and BK channels, and increase their currents. (Börjesson & Elinder, 2011; Börjesson et al., 2008; Hoshi et al., 2013; Tian et al., 2016). In addition, both activation and inactivation of channels by PUFAs have been reported in the delayed rectifier potassium channels (Gavrilova-Ruch et al., 2007). It should be mentioned that their double bond, cis-geometry of double bond, and carboxyl group with negative charge have been proposed as the required properties of PUFAs in eliciting these effects (Boland et al., 2009; Elinder & Liin, 2017).

Studies have verified that the brain's fatty acid components can be influenced by diet, aging, and age-related disorders (Chew et al., 2020; Ledesma et al., 2012; Pakiet et al., 2019). On the other hand, brain lipids are essential determinants of neuron electrical properties. A diet with a low-P/S ratio (supplied 2% of the calories as C18:2n6) affects the electrical properties of dorsal root ganglion cells by reducing AP duration, after hyperpolarization, R_{in}, and τ_m compared to a high-P/S diet (supplied 22% of the calories as C18:2n6) (Scott et al., 1989). Another study, focusing on the intrinsic properties of entorhinal cortex cells, indicated that canola/soybean oil in the diet decreased AP duration and postsynaptic response duration and enhanced the firing frequency (Arsenault et al., 2012). The ketogenic diet has displayed an anticonvulsant effect by increasing plasma PUFAs. This effect is mediated by modulating A-type potassium and transient Na channels (Tigerholm et al., 2012). It seems that ACOX1 inhibition may change brain activity by modifying its fatty acid ratios. Finally, according to the results of this study, peroxisomes and hepatic ACOX1 are involved in regulating brain fatty acid profile and electrophysiological properties of DG cells, possibly through potassium channels, which require detailed study in the future.

Ethical Considerations

Compliance with ethical guidelines

The Research and Ethics Committee of the Neuroscience Research Center of Shahid Beheshti University of Medical Sciences approved all experiments (No.: IR.SBMU.MSP.REC.1396.168). Animals were treated according to the guide for the care and use of laboratory animals (National Institutes of Health Publication No. 80-23, revised 1996).

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Authors' contributions

Conceptualization, supervision and funding acquisition: Fereshteh Motamedi, Fariba Khodagholi; Methodology: Fereshteh Motamedi, Fariba Khodagholi, Hamid Gholami Pourbadie and Leila Dargahi; Resources: Fereshteh Motamedi, Leila Dargahi and Fariba Khodagholi; Investigation and writing original draft: Shahrbanoo Rafiei; Review & editing: Fereshteh Motamedi, Shahrbanoo Rafiei, Hamid Gholami Pourbadie and Fariba Khodagholi.

Conflict of interest

The authors declared no conflict of interest.

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