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Title: Inhibition of Hepatic Acyl CoA Oxidase 1 (ACOX1), A Peroxisome B-Oxidation Enzyme, Modifies Brain Fatty Acid Profile And Intrinsic Membrane Properties in Granule Cells of Rat Dentate Gyrus

Running title: ACOX1 inhibition causes neuron hypo-excitability

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Abstract

Peroxisomes are the essential organelles in lipid metabolism. They contain enzymes for β -oxidation of very-long-chain fatty acids, which cannot break down in mitochondria. A reduced expression in hepatic Acyl-CoA oxidase1 (ACOX1), a peroxisome β -oxidation enzyme, followed by modification of the brain fatty acid profile has been seen 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). A specific ACOX1 inhibitor, 10, 12- tricosadiynoic acid (TDYA), 100 μ g/kg (in olive oil) was given by daily gavage administration for 25 days in male Wistar rats. The brain fatty acid composition and electrophysiological properties of dentate gyrus granule cells were determined by gas chromatography and whole-cell patch-clamp, respectively. A significant increase in C20, C22, C18:1, C20:1, and a decrease of C18, C24, C20:3n6 and C22:6n3 were found in TDYA treated rats compared to the control group. The results show 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. The results indicate, systemic inhibition of ACOX1 causes hypo-excitability of neuronal cells. 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 dentate gyrus cells.

Keywords: Peroxisomes, β -oxidation, Acyl CoA oxidase (ACOX1), Fatty acid profile, Neuron intrinsic properties, Dentate gyrus granule cells

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 exclusively be degraded by peroxisomes. They include dicarboxylic acids, bile acid intermediates, very-long-chain fatty acids (VLCFA), and phytanic acid (Poirier, Antonenkov, Glumoff, & Hiltunen, 2006; Wanders & Waterham, 2006). While oxidation of polyunsaturated fatty acids (PUFAs) is slow in mitochondria, in peroxisomes, it is very fast (Hiltunen, Kärki, Hassinen, & Osmundsen, 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 HDAC6 and SIRT1 (Rafiei, Sh., Khodaghali, F., Motamedi, F., Dargahi, L. 2021). Acyl-CoA oxidase-1 is the rate-limiting enzyme of the peroxisome β -oxidation that catalyzes the first step reaction. It can act on dicarboxylic acid, PUFAs, and saturated very-long-chain fatty acids with no effect on branched-chain fatty acids or di or tri hydroxycholestanoic acids (bile acid intermediates). The deficiency of the ACOX1, an autosomal recessive disorder known as pseudo-neonatal adrenoleukodystrophy, is determined by the clinical presentation of hypotonia, seizures, developmental retardation. Its biochemical diagnosis is, 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 the aged rodents, a decline of hepatic ACOX1 expression followed by alteration of brain fatty acid composition has been reported (Yang, Zhang, Wang, Zhang, & Shi, 2014). In addition, growing evidence has suggested the association 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, Chawla, Buonocore, Rapp, & Barnes, 2004; Small, Tsai, DeLaPaz, Mayeux, & Stern, 2002). The brain has the highest lipid content after adipose tissue, and lipids are comprised 50% of brain dry weight (Hamilton, Hillard, Spector, & Watkins, 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 association between brain lipid profile and its electrical properties. They have reported that dietary

fat could 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 (DRG) (Scott, Lew, Clandinin, & Cinader, 1989). Lipid modification of the entorhinal cortex (EC) induced by diet has changed the physiological properties of neurons (Arsenault, Julien, Chen, Bazinet, & Calon, 2012). Moreover, studies show that DHA accumulation, by altering passive properties of EC neurons, improve cognition (Arsenault, Julien, Tremblay, & Calon, 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 condition on rodent hepatic ACOX1 (Zeng et al., 2017).

By designing this study, we aimed to examine the effect of inhibition of ACOX1 activity, a peroxisome β -oxidation enzyme, on cerebral lipid composition and on electrophysiological properties of dentate gyrus granule cells (DG-GCs) in adult rats.

Materials and Methods

Animals

This study was performed on the male Wistar rats (200-250g) provided by a breeding colony in the Neuroscience Research Center (Tehran, Iran). Freely accessed to water and chow, rats in groups of four were housed in each cage. The standard cycle of 12h light/12h dark and temperature of 22-25°C was set in the animal house. 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). The research and ethics committee of the Neuroscience Research Center of Shahid Beheshti University of Medical Sciences approved all experiments under permit number IR.SBMU.MSP.REC.1396.168.

Experimental design

The animals were divided into two groups and received an intragastric gavage administration of 100 μ g/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 the number of cells used in electrophysiological experiments were n=8 in olive oil and n=6 in TDYA groups.

ACOX1 activity assay

ACOX1 activity assay was conducted based on the method of Cable et al. by measuring H₂O₂ production (Cablé, Keding, & Dauça, 1993). The reaction was started by the addition of 0.1 mM palmitoyl-coA into the reaction mixture containing: liver homogenate, 0.01 mM 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 recorded for 30 min and enzyme activity calculated as nmol H₂O₂/min/mg protein by extinction coefficient of $480 \times 10^5 \text{ ml mol}^{-1} \text{ cm}^{-1}$.

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 (60m × 0.25 mm). Fatty acid separation was acquired in a two-stage temperature program by maintaining the initial temperature at 60 °C for 2 min and increasing it to 200 °C at a rate of 10 °C/min for 25 min. Then column reaches the final temperature of 240°C at a rate of 5°C/min and keeping it for 7 min. 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 contained: 206 mM Sucrose, 2.8 mM KCl, 1.25 mM NaH₂PO₄, 26 mM NaHCO₃, 10 mM glucose, 1 mM CaCl₂, 2 mM MgSO₄, pH 7.4, oxygenated with O₂ and CO₂ (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 μ m-thick slices that were then transferred into ACSF solution (124 mM NaCl, 3mM KCl, 1.24mM NaH₂PO₄, 26mM NaHCO₃, 10mM glucose, 2mM CaCl₂, 2mM MgSO₄, pH 7.4, oxygenated with 95% O₂ and 5% CO₂) at 32°C for 30 min. In the recording process, a slice was located into a holding chamber of an upright microscope (Olympus; BX 51WI) stage and continuously superfused with the ACSF solution at room temperature.

Whole-cell patch clamp recording

Dentate gyrus granule cells (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 the borosilicate glass capillary (1.2mm O.D $\times$ 0.9mm ID) to obtain 3-5 M Ω pipette resistance when it was full 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 M Ω and variation below the 20 % during the experiment were selected for analysis. Current-clamp was done using intracellular solution containing 140mM K-Gluconate, 10mM HEPES, 2mM MgCl₂, 2mM Na₂-ATP, 1.1mM EGTA, 0.1mM CaCl₂ and 0.4mM Na₂-GTP adjusted to pH 7.3 by 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 were 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 $\pm$ SEM. The values of $p < 0.05$ were of statistical significance.

Results:

TDYA reduced hepatic ACOX1 activity

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

Inhibition of ACOX1 changed fatty acid composition in the brain

As shown in the table 1, only 13 types of fatty acids (of the total 31 detected types) were compared in our analyses (Table 1). Among all of the analyzed saturated fatty acids (SFAs), 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 ($P < 0.001$, $P < 0.001$). There was no significant difference for C16:1, C22:1, and C24:1 compared to the control group. Among the three analyzed polyunsaturated fatty acids (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.

Following 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. The student's t-test analysis revealed a significant decrease in resting membrane potential (RMP) of DG-GCs in the TDYA group ($-76.98 \pm 1.5\text{mV}$) comparing to the control ($-71.45 \pm 1.08\text{mV}$, $P < 0.01$). In addition, the input resistance (R_{in}) of TDYA treated rats ($169.18 \pm 20.42\text{ M}\Omega$) was significantly lower ($P < 0.01$) in comparison with the control ones ($270.66 \pm 23.37\text{ M}\Omega$). Data analysis also indicated an

increased time constant for the TDYA group (12.19 ± 1.15 ms, $p < 0.05$) compared to the control (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 Fig. 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 lower half-width (1.23 ± 0.052 vs. 1.47 ± 0.059 ms, $P < 0.05$, Fig. 2B), and higher amplitude for AHP compared to the control group (-10.8 ± 0.99 mV vs. -6.96 ± 0.55 mV, $P < 0.01$, Fig. 2C). Rheobase current was determined as the minimum current injected to trigger one AP during one second. As represented in table 2 and Fig. 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.

Fig. 3A shows 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 Fig. 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) has 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, $p < 0.05$; Fig. 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 lower number of APs (0.7143 ± 0.56 vs. 4.375 ± 0.84 , $p < 0.01$) compared to the control (Fig. 3E, F). Together, these data confirm that TDYA administration leads to hypo-excitability in the DG-GCs.

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 brain of young rats. Inhibition of hepatic ACOX1 by TDYA altered the brain fatty acid composition, followed by a drop in DG-GCs excitability. 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, we found a significant increase 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 as they did, except for C16:1, C22:1, C24:0 and C24:1. Analysis of the cortical fatty acid profile in patients from the two inherited peroxisomal disorders of Zellweger syndrome, a peroxisomal biogenesis disorder with absence or non-functional peroxisomes, and X-linked adrenoleukodystrophy, deficiency in metabolism of VLCFAs, exhibited a decrease in C24: 0 and C24: 1 (Martinez, 1992). In addition, we observed a significant decrement of an omega 6 PUFA, eicosatrienoic acid (C20:3n6), which is consistent with another study on aging (Carver, Benford, Han, & Cantor, 2001). The levels of PUFAs decrease and MUFAs increase with aging (Lopez, De Boscher, Castagnet, & Giusto, 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 a decline in the 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, Tayler, & Love, 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, which are categorized into passive and active characteristics of a neuron, can dictate neuronal excitability and affect its input/output gain (Kowalski, Gan, Jonas, & Pernía-Andrade, 2016). DG-GCs in TDYA- treated rats showed a more hyperpolarized RMP with a lower R_{in} and higher time constant (τ_m) than the control cells. Hyperpolarized RMP led to neuron hypo-excitability, and may be attributed to a change of ion gradient or upregulation

of new ion channels active in the resting state (Watari, Tose, & Bosma, 2013). R_{in} , and τ_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 be a determinant of 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, we found no significant difference 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 a decrease in 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 of spike width can influence the gain of a neuron's input/output relationship (Giese et al., 1998; Lin, Hatcher, Wurster, Chen, & Cheng, 2014). Also, the AP duration (width) can be influenced by the repolarization rate of the spike that is commonly regulated by potassium voltage-gated channels (Bean, 2007). For instance, enhancing the density of these channels has been correlated to a decline in RMP, R_{in} , and AP duration observed with maturation for DG-GCs (Spigelman, Zhang, & Carlen, 1992), which is similar to our results. Also, it has been reported that other potassium channels (fast delayed-rectifier K channels or BK channels) control the AHP amplitude (Contet, Goulding, Kuljis, & Barth, 2016; Sesti, Liu, & Cai, 2010). Therefore, alteration of potassium channels could 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, Walsh, & Singer, 1989). Furthermore, there are reports that 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, become 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, Hammarström, & Elinder, 2008; Hoshi, Xu, Hou, Heinemann, & Tian, 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, Schönherr, & Heinemann, 2007). It has to 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, Drzewiecki, Timoney, & Casey, 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, Solomon, & Fonteh, 2020; Ledesma, Martin, & Dotti, 2012; Pakiet, Jakubiak, Czumaj, Sledzinski, & Mika, 2019). On the other hand, brain lipids are the essential determinants of neuron electrical properties. A diet with a low-P/S ratio (supplied 2% of the calories as C18: 2n6) affects dorsal root ganglion cells' electrical properties by reducing AP duration, afterhyperpolarization, R_{in} , and τ_m comparing to a high-P/S diet (supplied 22% of the calories as C18: 2n6) (Scott et al., 1989). Another study, focusing on intrinsic properties of entorhinal cortex cells, has pointed out 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, Börjesson, Lundberg, Elinder, & Fransén, 2012). It seems that ACOX1 inhibition might 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 needs to be studied in detail in future.

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Tables and Figures

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 (Lignoceric acid)	2.82 ± 0.03	2.605 ± 0.02**
Monounsaturated fatty acids (MUFAs)	C16:1 (Palmitoleic 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 (Erucic 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 (Eicosapentaenoic acid)	0.04 ± 0.01	0.095 ± 0.03
	DHA (Docosahexaenoic acid)	10.1 ± 0.1	9.155 ± 0.2*
	Σ	86.65 ± 0.986	84.98 ± 2.043

*P<0.05, **P<0.01, ***P<0.001 versus control group

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

*P<0.05, **P<0.01 versus control group

Fig. 1 In vivo inhibition of ACOX1 by TDYA. ACOX1 activity is significantly lower in the TDYA group compared to the control group (*P < 0.05)

Fig. 2 Active intrinsic characteristics of DG granular cells. (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 $\pm$ SEM. (** $P < 0.01$ compared to the control group)

Fig. 3 Firing properties of DG granule cells evoked by current injection. (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 $\pm$ SEM. (** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ compared to the control group).