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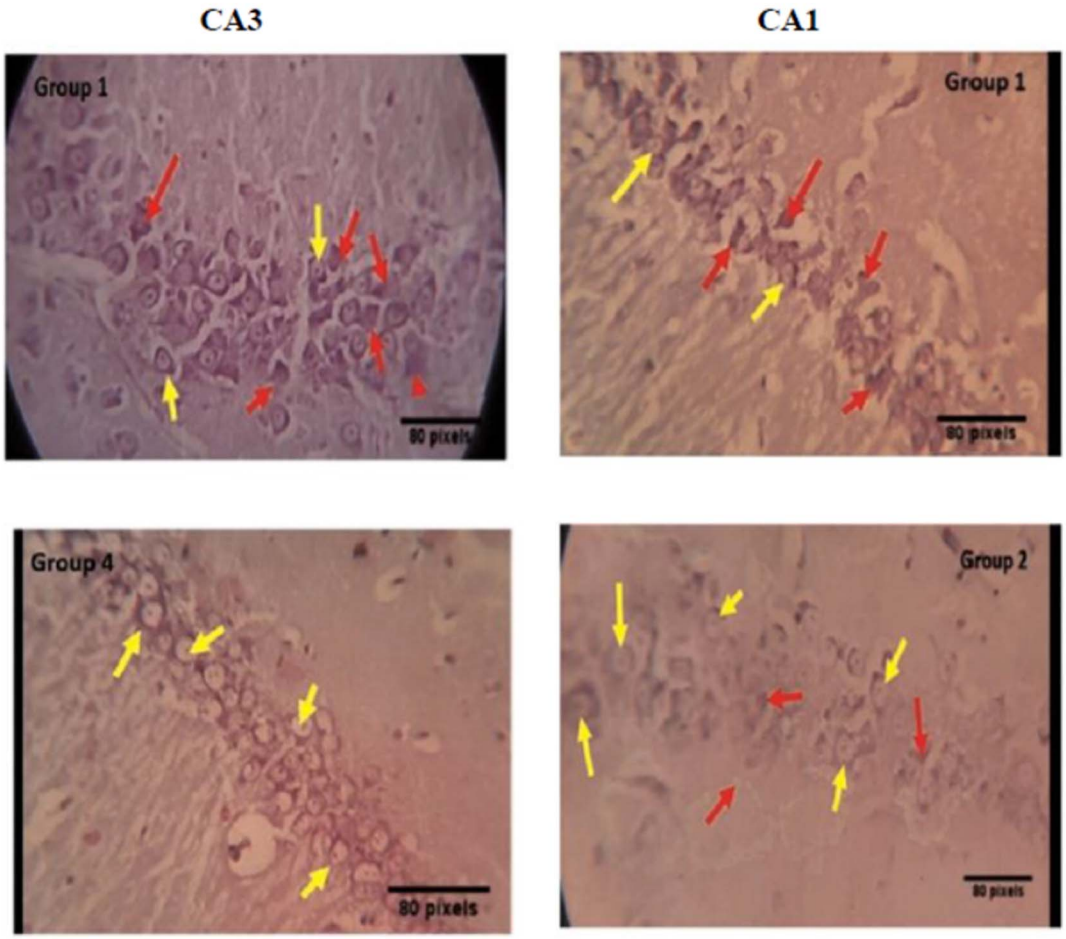

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AIM AND SCOPES
Journal of Cellular Neuroscience and Oxidative Stress is an online journal that publishes original research articles, reviews and short reviews on the molecular basis of biophysical, physiological and pharmacological processes that regulate cellular function, and the control or alteration of these processes by the action of receptors, neurotransmitters, second messengers, cation, anions, drugs or disease.

Areas of particular interest are four topics. They are;

A- Ion Channels (Na^+- K^+ Channels, Cl^- channels, Ca^{2+} channels, ADP-Ribose and metabolism of NAD^+, Patch-Clamp applications)

B- Oxidative Stress (Antioxidant vitamins, antioxidant enzymes, metabolism of nitric oxide, oxidative stress, biophysics, biochemistry and physiology of free oxygen radicals)

C- Interaction Between Oxidative Stress and Ion Channels in Neuroscience
(Effects of the oxidative stress on the activation of the voltage sensitive cation channels, effect of ADP-Ribose and NAD^+ on activation of the cation channels which are sensitive to voltage, effect of the oxidative stress on activation of the TRP channels in neurodegenerative diseases such Parkinson's and Alzheimer's diseases)

D- Gene and Oxidative Stress
(Gene abnormalities. Interaction between gene and free radicals. Gene anomalies and iron. Role of radiation and cancer on gene polymorphism)

READERSHIP

Biophysics	Biochemistry
Biology	Biomedical Engineering
Pharmacology	PhysiologyGenetics
Cardiology	Neurology
Oncology	Psychiatry
Neuroscience	Neuropharmacology

Keywords
Ion channels, cell biochemistry, biophysics, calcium signaling, cellular function, cellular physiology, metabolism, apoptosis, lipid peroxidation, nitric oxide, ageing, antioxidants, neuropathy, traumatic brain injury, pain, spinal cord injury, Alzheimer's Disease, Parkinson's Disease.

RESEARCH ARTICLE

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Effects of vitamin E on calcium signaling and oxidative injury in neutrophils of patients with ischemia/reperfusion (surgical arthroscopy) under sevoflurane anesthesia

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List of Abbreviations;

$[Ca^{2+}]_i$*, intracellular free calcium ion;* ***I/R****, ischemia/reperfusion;* ***fMLP,*** *N- formyl-methionyl-leucyl-phenylalanine;* ***ROS****, free reactive oxygen radicals;* ***TRPM2,*** *transient receptor potential melastatin 2;* ***VGCC,*** *voltage gated Ca^{2+} channels;* ***2-APB,*** *2-aminoethoxydiphenyl borate;* ***V+D,*** *verapamil + diltiazem;* ***LP,*** *lipid peroxidation;* ***GSH,*** *glutathione;* ***GSH-Px,*** *glutathione peroxidase;* ***TBAR,*** *thiobarbituric-acid*

Abstract

Sevoflurane is an anesthetic, and it acts on oxidative activity by activating Ca^{2+} influx. In human neutrophils, oxidative stress activates the voltage-gated calcium channels (VGCC) and the TRPM2 channel; on the other hand, these channels are inhibited by 2-aminoethoxydiphenyl borate (2-APB) and verapamil plus diltiazem (V+D), respectively. Under sevoflurane anesthesia, surgical arthroscopy poses a significant risk to oxidative stress and Ca^{2+} influx-induced neutrophil infiltration and injury of patients. However, vitamin E may inhibit lipid peroxidation (LP) by upregulating reduced glutathione (GSH) and glutathione peroxidase (GSH-Px) but downregulating TRPM2 and VGCC in the neutrophils of surgical arthroscopy patients. This topic was examined in the current study.

We enrolled 20 patients in the current study, separating them into two primary groups: patients and patients plus vitamin E. Ten patients were divided into two groups: preoperative (N1) and postoperative (N2), both of which were not given vitamin E therapy. The remaining ten patients were given 300 IU of vitamin E two hours prior to their surgical arthroscopy (E1), and their blood was again drawn following the procedure (E2). Prior to fMLP stimulation, the isolated neutrophils from each of the four groups were incubated with 10 μM V+D and 100 μM 2-APB.

In the neutrophils, there was an increase in intracellular free Ca^{2+} ($[Ca^{2+}]_i$) concentration and LP levels due to the downregulation of GSH and GSH-Px; however, following vitamin E treatment, GSH concentration and GSH-Px activity increased in the E2 group. While 2-APB and V+D treatment reduced the concentration of $[Ca^{2+}]_i$ in the neutrophils, vitamin E administration had no effect on this measurement.

In summary, vitamin E treatment mitigated the GSH and GSH-Px alterations induced by I/R damage, while

TRPM2 and VGCC inhibition reduced the $[Ca^{2+}]_i$ rise induced by I/R injury. One potential treatment approach for I/R-induced oxidative neutrophil damage is the suppression of TRPM2 and VGCC.

Keywords: Ischemia/reperfusion injury; Neutrophil; Sevoflurane; TRPM2 channel; Vitamin E.

Introduction

An imbalance between the body's oxidation and antioxidation processes is known as oxidative stress, and it results in an excess of free reactive oxygen radicals (ROS) generated or not being scavenged (Averill-Bates 2024). Under typical physiological settings, physiological processes including phagocytosis and the mitochondrial electron transport chain naturally produce ROS. According to ROS serve as signaling molecules and control a number of physiological processes in the human body, including the promotion of cell survival, proliferation, and differentiation (Thapak and Gomez-Pinilla 2024). Neutrophils and phagocytes involved in inflammation and infection produce ROS under healthy and pathological settings. The decrease in the stimulation of the mitochondrial electron transport pathway is generated by many circumstances and might contribute to increased ROS generation (Nazıroğlu 2007). Excessive ROS formation causes damage to the primary cellular components, including proteins, lipids, and DNA (Thapak and Gomez-Pinilla 2024). ROS, including hydroxyl and superoxide radicals, are generated by a variety of physiological and pathological circumstances. Ischemia/reperfusion (I/R) injury is a main pathophysiological function in the brain and neurons (Kounis et al. 2020; Dhalla et al. 2022). Furthermore, after the induction of reperfusion, the I/R results in further damage due to an increased Ca^{2+} influx and oxidative stress (Zhao et al. 2008; Kumar et al. 2014). Enzymatic scavengers of ROS include superoxide dismutase and glutathione peroxidase (GSH-Px), while non-enzymatic scavengers such as vitamin E and reduced glutathione (GSH). I/R-induced damage in vivo is also linked to the activation of phagocytic cells, such as neutrophils (Wu et al. 2023). Consequently, ROS produced by neutrophil activation led to tissue damage by upregulating ROS and downregulating antioxidants such vitamin E, GSH, and GSH-Px (Wallert et al. 2019; Qiu et al. 2023).

Almost all patients receiving general anesthesia have volatile substances, which are a crucial component of perioperative medicine (Kounis et al. 2020). Due to its low-fat soluble solubility, minimal airway damage, and quick reawakening following anesthesia, sevoflurane is one of the primary inhalation anesthetics frequently used in clinical practice. As such, it is frequently used during patient operations for a variety of illnesses (Gascoigne et al. 2022). Sevoflurane has been demonstrated to reduce hypoxia and I/R injury-induced oxidative damage in a variety of organs of animal models by upregulating the antioxidant redox system (Shuai et al., 2023). Studies have revealed that sevoflurane can, however, cause specific toxicity to the hippocampal region, which can result in nerve damage, oxidative stress, and learning and cognitive dysfunction. However, antioxidant treatments like resveratrol and melatonin have been shown to mitigate the negative effects of sevoflurane (Tang et al. 2021; Shuai et al. 2023; Zou et al. 2023). Sevoflurane-induced DNA damage was minimized in lymphocytes of human by the therapy of vitamin C and E combinations (Sardas et al. 2006). It is still unknown how exactly sevoflurane causes neutrophil ROS and Ca^{2+} influx.

The Ca^{2+} influx induces several physiological and pathological actions. N-formyl-methionyl-leucyl-phenylalanine (fMLP) is produced by bacteria and activates leucocytes by increasing the intracellular calcium ion concentration ($[Ca^{2+}]_i$) through the action of transient receptor potential 2 (TRPM2) and voltage-gated calcium channels (VGCC). This increase can lead to the formation of ROS (Nazıroğlu et al. 2014; Ugan et al. 2016; Huang et al. 2024). Ca^{2+} influx mediated by TRPM2 and VGCC activation stimulates human neutrophils (Nazıroğlu et al. 2014). The development of I/R damage triggers the Ca^{2+} influx processes by activating VGCC and TRPM2 (Akyuva et al. 2021; Korte et al. 2022). In pathological circumstances, VGCC stimulation causes an influx of Ca^{2+}, which leads to the generation of ROS (Kim et al. 2020). Consequently, one of the main contributing factors to the development of ROS is the enhanced neutrophil TRPM2 and VGCC-mediated Ca^{2+} influx generated by I/R damage (Kumar et al. 2014; Akyuva et al. 2021). Sevoflurane-mediated cell death leads to neuronal death even if it causes neurotoxicity when VGCC is stimulated (Liu et al. 2014; Zeng et al. 2022).

As far as we know, no research has been done on the modulator action of vitamin E on I/R damage, sevoflurane-

induced lipid peroxidation (LP), and Ca^{2+} influx in human neutrophils via the suppression of VGCC and TRPM2. The current study was designed to investigate the effects of vitamin E on sevoflurane and I/R-induced LP, thiol antioxidants, and Ca^{2+} influx in human neutrophils.

Material and Methods

Patients

The study was carried out in the Biophysics Department Research Laboratory at Suleyman Demirel University (SDU) in Türkiye. Participants in the study were chosen from among the twenty patients at SDU undergoing reanimation and anesthesia. The study was given approval to proceed by the Local Ethics Committee of the Medical Faculty of SDU. Every participant provided written consent confirming their intention to donate blood through the vena brachialis and was made aware of the protocols used in the trial. For every patient who was enrolled in the trial, data were entered on their demographics, anesthetic duration, physical examination results, and clinical information (Table 1). Pregnancy, cancer, rheumatologic or systemic disorders other than surgical arthroscopy, and active infections were excluded.

Patients

The N1 group (n=10) of surgically arthroscopic patients had their neutrophils removed. N2 group (n=10): After the surgical arthroscopy, the patients' neutrophils were collected once more. E1 group (n=10): Patients undergoing surgical arthroscopy received 300 IU of vitamin E intramuscularly prior to two hours (D-Alpha-tocopherol acetate, Evigen, Aksu Inc. Istanbul, Türkiye). E2 group (n=10): After the surgical arthroscopy and vitamin E infusion, the neutrophils of patients were collected once more. We investigated into how VGCC and TRPM2 affected neutrophil Ca^{2+} entrance in Ca^{2+} signaling assays. The neutrophils of four groups were subsequently separated into the following subgroups in order to achieve this goal:

Group A (2-APB group): 30 minutes prior to 1 μM fMLP stimulation, neutrophils were cultured with 2-APB (100 μM).

Group B (V+D): Thirty minutes before 1 μM fMLP stimulation, the group's neutrophils were incubated with V+D (10 μM) (Nazıroğlu et al. 2014; Ugan et al. 2016).

For hematological analysis, we used blood with and anticoagulant (Na-citrate). The non-anticoagulated blood samples were used to separate the neutrophil samples. LP, GSH, and GSH-Px were tested using half of the neutrophils that had been kept at -33 °C for a month. The $[Ca^{2+}]_i$ concentration was determined using the remaining live neutrophil samples.

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Human neutrophil isolation

The phosphate-buffered saline from GIBCO Invitrogen (Istanbul, Türkiye), the 6% hydroxyl ethyl starch solution in isotonic NaCl (Plasmasteril) (Fresenius, Bad Homburg, Germany), and the Ficoll-Paque Plus Solution (GE Healthcare Bio-Sciences, Uppsala, Sweden) were the sterile solutions used for neutrophil isolation (Nazıroğlu et al. 2014; Ekici et al. 2020). In mM, glucose (5.5), $MgCl_2$ (1), $CaCl_2$ (1.6), HEPES (20), and NaCl (138) were the components of the loading buffer. It had a pH of 7.4. With the exception of the lack of serum, the measuring buffers were identical to the loading buffer with extracellular Ca^{2+} concentration (1.2 mM).

Measurement of $[Ca^{2+}]_i$

The concentrations of $[Ca^{2+}]_i$ neutrophils (3 x 10^6 neutrophil per ml) were incubated with 4 μM Fura-2/AM (Promega, Eugene, Oregon, USA) in incubating buffer for 45-60 minutes at 37 ºC in the dark, in accordance with a previously reported procedure (Ekici et al. 2020). After that, they were twice cleaned, incubated for a further half hour at 37 ˚C, and then resuspended in loading buffer. Each of the four groups received fMLP to enhance intracellular Ca^{2+} release. The fluorescence of 2-ml aliquots of a magnetically agitated cellular solution at 37 ºC was measured with a Carry Eclipse spectrofluorometer from Varian Inc., Sydney, Australia. The excitation wavelengths were 340 and 380 nm, and the emission wavelength was 505 nm. The Grynkiewicz et al. (1985) method was utilized to calibrate the Fura-2/AM 340/380 nm fluorescence ratio, which was used to monitor changes in $[Ca^{2+}]_i$ concentration.

The release of Ca^{2+} was measured using the integral of the increase in $[Ca^{2+}]_i$ concentration for a duration of 100 seconds after the administration of **1** μM fMLP. As was previously noted (Ekici et al. 2020), a reading of the Ca^{2+} release was obtained once every second and expressed in nanomoles.

Lipid peroxidation (LP) and total protein analysis

Using the Placer et al. (1966) method, the thiobarbituric-acid (TBAR) reaction was utilized to assess LP in neutrophils. The LP analyses were performed at 532 nm using the spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). In order to quantify the compounds that are reactive to TBAR, the absorption was compared to the standard curve of malondialdehyde equivalents that are induced when 1,1,3,3-tetramethoxypropane hydrolyzes in the presence of acid. LP values in neutrophils were expressed as μM/ gram protein. The Lowry et al. method (1951) was used to assess the protein content of neutrophil samples using albumin of bovine serum as the reference.

Tests for GSH-Px and GSH

At 412 nm, the GSH concentration of neutrophil samples was analyzed using the Sedlak and Lindsay (1968) method. After precipitating the samples with 50% trichloroacetic acid, they were centrifuged for five minutes at 800 x g. The reaction mixture contained Tris-EDTA buffer (2.0 ml and 0.2 M, pH 8.9), cell supernatant (0.5), and 10 μM 5.5-dithiobis-2 nitrobenzoicacid (0.1 ml). The solution was allowed to come to room temperature for five minutes before being measured at 412 nm using the spectrophotometer (UV-1800). Using spectrophotometry at 412 nm and 37 °C, the GSH-Px activity of the frozen cell samples was ascertained by applying the procedure used by Lawrence and Burk (1976). The units of measurement utilized to express the GSH-Px and GSH in neutrophils were IU and μM per gram of protein, respectively (Osmanlıoğlu et al. 2020).

Statistical Analysis

Every single result was presented as means ± SD. For determining the statistical significance, the Kruskal-Walli's algorithm was utilized. An unpaired Mann-Whitney U test was used to examine the significant values of the four groups. SPSS (Chicago, Illinois, USA) was the statistical application used to analyze the data. Significant P-values were those with a value of less than 0.05.

Results

Results of demographic values

Demographic values of patients were shown in the Table 1. The demographic values did not differ between the N and E groups.

The surgical arthroscopy (I/R injury)-mediated upregulation of $[Ca^{2+}]_i$ concentration in the human neutrophils was diminished by the treatments of TRPM2 channel (2-APB) and VGCC (V+D) blockers but not vitamin E.

Figure 1 displayed the line graphics for N1 (Figure 1A), N2 (Figure 1B), E1 (Figure 1C), and E2 (Figure 1D). Nonetheless, column graphics were used in Figure 1E to display the mean values of the changes. The $[Ca^{2+}]_i$ concentration did not differ statistically among the four groups. As a result, we were unable to observe the protective effect of vitamin E therapy on neutrophil $[Ca^{2+}]_i$ concentrations. On the other hand, compared to the N1, N2, E1, and E2 groups, the I/R-induced increases of the $[Ca^{2+}]_i$

Table 1. Demographic characteristics of patients*. (Mean ± SD).*

	Group N (n=10)	**Group E (n=10)**	***p***
Age (year)	37.10 ± 4.76	42.10 ± 2.88	0.165
Height (cm)	167.60 ± 3.63	169.60 ± 2.88	0.684
Weight (kg)	76.30 ± 10.19	84.30 ± 7.55	0.247
Anesthesia time (minutes)	63.40 ± 21.96	54.10 ± 16.51	0.436
Tourniquet time (minutes)	41.60 ± 22.90	32.50 ± 11.74	0.529
Tourniquet pressure (mmHg)	284.00 ± 20.11	277.00 ± 22.63	0.579

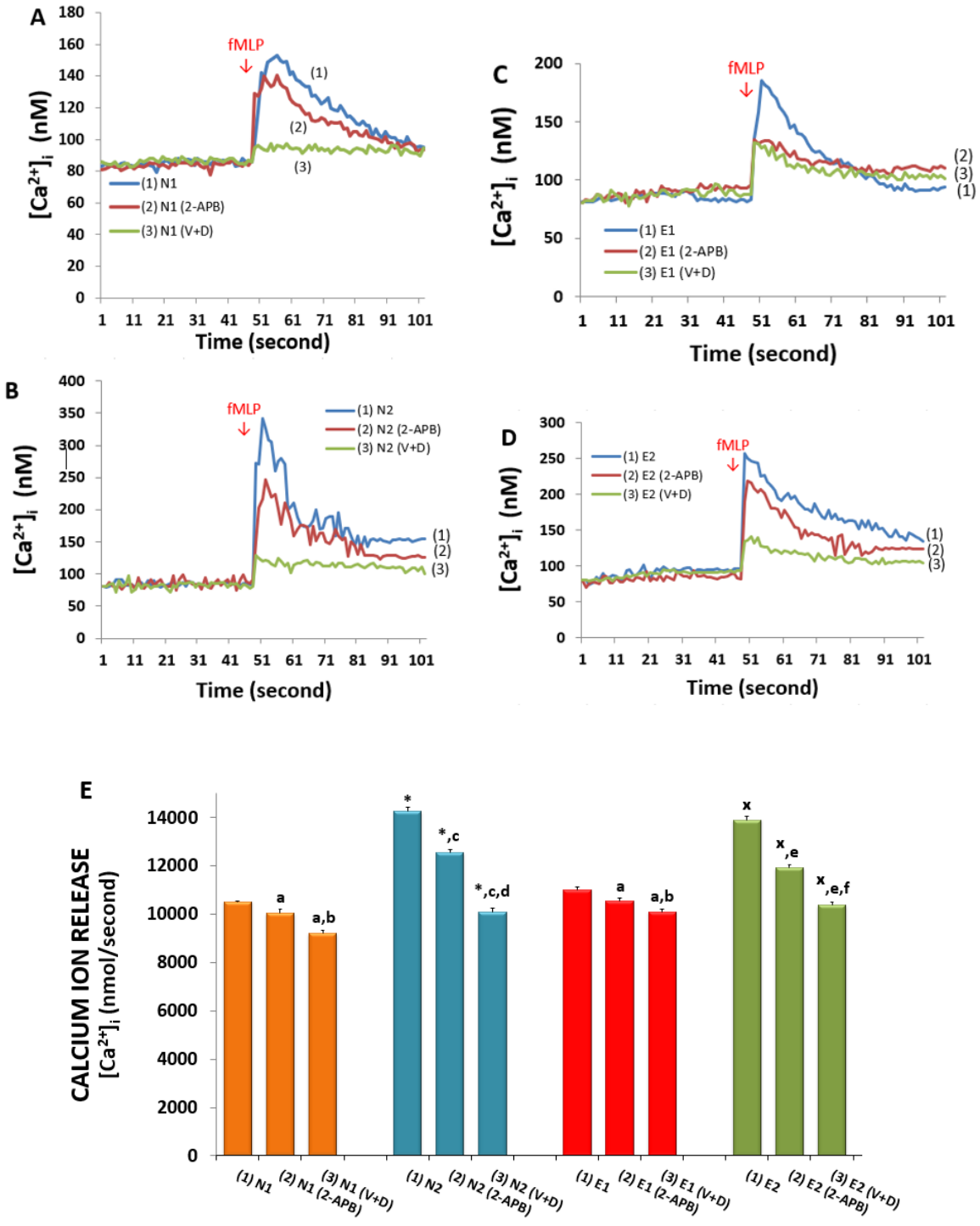

Figure 1. Treatments with TRPM2 channel and VGCC blockers, but not vitamin E, reduced the rise in $[Ca^{2+}]_i$ concentration in human neutrophils caused by surgical arthroscopy (I/R injury). *Neutrophils from pre-operation and post-operation without vitamin E treatment are shown by (**A**) N1 and (**B**) N2, respectively. (**C**) E1 and E2 (**D**) are pre-operative and post-operative neutrophils, respectively, following a 300 IU vitamin E treatment. The resulting neutrophils from four groups were then treated with VGCC inhibitor (10 μM verapamil (V) + diltiazem (D) for 30 minutes) and TRPM2 channel blocker (100 μM 2-APB). N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP and 1 μM) was used to stimulate the neutrophils. [$^{a}p < 0.05$ vs N1 and E1. $^{b}p < 0.05$ vs N1 (2-APB) and E1 (2-APB). $^{c}p < 0.001$ vs N2. $^{d}p < 0.001$ vs N2 (2-APB). $^{*}p < 0.001$ vs N1, N1 (2-APB), and N1 (V+D). $^{e}p < 0.001$ vs E2. $^{f}p < 0.001$ vs N2 (2-APB). $^{x}p < 0.001$ vs E1, E1 (2-APB), and N1 (V+D)]*

concentrations were substantially smaller ($p < 0.05$ and $p < 0.001$) in the N1 (2-APB), N1 (V+D), N2 (2-APB), N2 (V+D), E1 (2-APB), E1 (V+D), E2 (2-APB), and E2 (V+D). Thus, the findings suggested that I/R-induced elevations in $[Ca^{2+}]_i$ concentration in the neutrophils were prevented by blocking the TRPM2 channel and the VGCC. Furthermore, the V+D groups had the greatest declines in comparison to the 2-APB groups. As a result, compared to the 2-APB, the inhibitor roles of V+D were more substantial.

Discussion

As an anesthetic, sevoflurane acts by activating Ca^{2+} influx, which is an oxidative activity in human neutrophils (Wong et al. 2006). The products of ROS activate the TRPM2 and VGCC channels in human neutrophils (Ugan et al. 2016). Surgical arthroscopy under sevoflurane anesthesia presents a serious risk to oxidative stress and Ca^{2+} influx-induced neutrophil infiltration and neuronal injury of patients (Wong et al. 2006). The results of recent studies have indicated that antioxidants such as melatonin and resveratrol can lessen the adverse effects of sevoflurane (Tang et al. 2021; Shuai et al. 2023; Zou et al. 2023). Vitamin C and E therapy reduced the amount of DNA damage caused by sevoflurane in human cells (Sardas et al. 2006). The precise mechanism by which sevoflurane induces Ca^{2+} influx and neutrophil ROS is still unknown. In addition to examining the modulator action of vitamin E on neutrophil Ca^{2+} entry, LP, and the thiol antioxidant system, we also investigated the processes of Ca^{2+} influx-mediated neutrophil stimulation, which are implicated in the etiology of sevoflurane and I/R injury (surgical arthroscopy). Here, we found that vitamin E administration elevated GSH and GSH-Px in patient neutrophils, and regulation of TRPM2 and VGCC regulate Ca^{2+} entry in the human neutrophils.

Oxidative stress activates the TRPM2 channel (Nazıroğlu 2007). ROS levels rise after I/R injury; however, vitamin E therapy reduces oxidative stress and LP in neutrophils caused by I/R injury (Wallert et al. 2019). Vitamin E administration was found to have no effect on oxidative stress-induced TRPM2 stimulation (Naziroğlu and Lückhoff 2008), yet there have also been contradictory

Table 2. Effects of vitamin E treatment (300 IU) on the LP, GSH, and GSH-Px in the neutrophils of patients with I/R injury*. (n=10 and mean ± SD).*

Parameters	Preoperative (N1)	Postoperative (N2)	Preoperative + VE (E1)	Postoperative + VE (E2)
LP (μM/g protein)	3.83 ± 0.43	4.20 ± 0.51[a]	3.85 ± 0.42	4.16 ± 0.42[a]
GSH (μM/g protein)	1.88 ± 0.17	1.61 ± 0.14[b]	1.76 ± 0.31	1.93 ± 0.16[b,e]
GSH-Px (IU/g prot.)	7.95 ± 0.93	3.85 ± 0.48[c]	8.83 ± 0.89	10.60 ± 1.40[d,e]

[a]p<0.05, [b]p<0.01, and [c]p<0.001 vs preoperative groups (N1 and E1). [d]p<0.05, and [e]p<0.001 vs postoperative (N2) group. GSH, reduced glutathione; GSH-Px, glutathione peroxidase; LP, lipid peroxidation.

By upregulating GSH-Px and GSH in the neutrophils of patients with I/R damage, vitamin E therapy reduced lipid peroxidation increases induced by I/R injury

Oxidative stress induces increases of LP but decreases antioxidant values such as GSH and GSH-Px in human neutrophils (Nazıroğlu et al. 2014; Ugan et al. 2016). However, the antioxidant property of vitamin E decreases LP in the neutrophils through the upregulation of GSH and GSH-Px. However, the modulator actions of on the changes of LP, GSH-Px, and GSH in the human neutrophils after I/R injury was not investigated yet. The results of the study indicated the increases of LP ($p<0.05$) but decreases of GSH-Px ($p<0.001$) and GSH ($p<0.01$) in the N2 group as compared to the N1 group. However, the decreases of GSH-Px ($p<0.001$) and GSH ($p<0.05$) were increased in the E2 groups as compared to the E1 groups by the treatment of vitamin E. Hence, we observed the protective action of vitamin E treatment on the I/R injury-mediated decreases of GSH and GSH-Px in the neutrophils.

reports in the dorsal root ganglions of rats (Nazıroğlu and Özgül 2013). L-type VGCC in brain stem cells was activated by vitamin E (Deng et al. 2015). The current analysis found no evidence of any influence of vitamin E on the I/R (arthroscopy) and sevoflurane-induced increase in $[Ca^{2+}]_i$ concentration in the neutrophils, which is consistent with the findings (Naziroğlu and Lückhoff 2008; Deng et al. 2015).

The primary non-protein sulfhydryl molecule in mammalian cells is tripeptide thiol, or GSH, which is known to have a wide range of biological activities. The way that GSH interacts with related enzymes like glutathione reductase and GSH-Px helps it protect against LP and ROS (Adeoye et al. 2018). According to reports, it is an antioxidant therapy that includes vitamin E and inhibits LP by upregulating GSH and GSH-Px in the neutrophils of patients with Behcet's disease (Nazıroğlu et al. 2014), ankylosing spondylitis (Ugan et al. 2016), and endometriosis (Ekici et al. 2020). In accordance with the reports, we observed that I/R injury induced-decreases of GSH-Px and GSH were upregulated by the vitamin E treatment. In accordance with the present data, vitamin E treatment decreased I/R-injury-induced brain edema through the upregulation of GSH in rats (Haghnejad Azar et al. 2017).

Oxidative stress and fMLP stimulate TRPM2 and VGCC, which antioxidants decrease (Nazıroğlu et al. 2014; Ugan et al. 2016; Ekici et al. 2020). To our knowledge, no research has been done on how vitamin E affects the Ca^{2+} entrance through the TRPM2 and VGCC in the neutrophils of patients who have had I/R injury. According to earlier research, vitamin E has preventive properties that prevent mitochondrial injury and preserve cardiac function recovery after ischemia/reperfusion injury by scavenging LP products, reducing ROS generation, and decreasing susceptibility to Ca^{2+} load (Venditti et al. 2011). Contrary to the report, When the postischemic cells were treated with vitamin E, their rate of LP and concentration of $[Ca^{2+}]_i$ increased rather than decreased in the postischemic immature cerebellum of rats (Dyatlov et al. 1998). In the current investigation, vitamin E therapy resulted in higher GSH and GSH-Px levels but no decrease in the lipid peroxidation level in the neutrophils of the patients.

Due to the downregulation of I/R damage stimulation-induced neutrophil injury, vitamin E treatment attenuated VGCC and TRPM2, protecting human neutrophils from I/R-mediated oxidative mediators. The activation of VGCC and TRPM2-mediated excessive Ca^{2+} influx may be taken into consideration as a possible cause of oxidative damage caused by I/R, even if vitamin E treatment did not lessen I/R injury-induced LP-mediated neutrophil injury. One potential treatment approach for I/R-induced oxidative neutrophil damage is the suppression of TRPM2 and VGCC.

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Author contributions

MN prepared the figures and graphics in addition to conceptualizing and designing the study. HÖO carried out antioxidant and lipid peroxidation investigations in addition to vitamin E administration, blood sampling, and anesthesia. BÇ carried out the fura-2 analyses that are included in the text. LY completed the manuscript editing and project application.

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Declarations

Competing Interests There are no relevant financial or nonfinancial interests to reveal.

Ethical Approve The Suleyman Demirel University in Isparta, Türkiye's local human ethics committee accepted the project.

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References

Adeoye O, Olawumi J, Opeyemi A, Christiania O. (2018) Review on the role of glutathione on oxidative stress and infertility. JBRA Assist Reprod. 22(1):61-66. https://doi.org/10.5935/1518-0557.20180003.

Averill-Bates D. (2024) Reactive oxygen species and cell signaling. Review. Biochim Biophys Acta Mol Cell Res. 1871(2):119573. https://doi.org/10.1016/j.bbamcr.2023.119573.

Deng S, Hou G, Xue Z, Zhang L, Zhou Y, Liu C, Liu Y, Li Z. (2015) Vitamin E isomer δ-tocopherol enhances the efficiency of neural stem cell differentiation via L-type calcium channel. Neurosci Lett. 585:166-170. https://doi.org/10.1016/j.neulet.2014.11.031.

Dhalla NS, Shah AK, Adameova A, Bartekova M. (2022) Role of Oxidative Stress in Cardiac Dysfunction and Subcellular Defects Due to Ischemia-Reperfusion Injury. Biomedicines. 2022 10(7):1473. https://doi.org/10.3390/biomedicines10071473.

Dyatlov VA, Makovetskaia VV, Leonhardt R, Lawrence DA, Carpenter DO. (1998) Vitamin E enhances Ca(2+)-mediated vulnerability of immature cerebellar granule cells to ischemia. Free Radic Biol Med. 25(7):793-802. https://doi.org/10.1016/s0891-5849(98)00157-9.

Ekici Eİ, Güney M, Nazıroğlu M. (2020). Protective effect of cabergoline on mitochondrial oxidative stress-induced apoptosis is mediated by modulations of TRPM2 in neutrophils of patients with endometriosis. J Bioenerg Biomembr. 52(3):131-142. https://doi.org/10.1007/s10863-020-09830-y.

Gascoigne DA, Minhaj MM, Aksenov DP. (2022) Neonatal Anesthesia and Oxidative Stress. Antioxidants (Basel). 11(4):787. https://doi.org/10.3390/antiox11040787.

Grynkiewicz C, Poenie M, Tsien RY. (1985) A new generation of Ca2+ indicators with greatly improved fluorescence properties. J Biol Chem. 260:3440-3450.

Haghnejad Azar A, Oryan S, Bohlooli S, Panahpour H. (2017) Alpha-Tocopherol Reduces Brain Edema and Protects Blood-Brain Barrier Integrity following Focal Cerebral Ischemia in Rats. Med Princ Pract. 26(1):17-22. https://doi.org/10.1159/000450648.

Huang P, Qu C, Rao Z, Wu D, Zhao J. (2024) Bidirectional regulation mechanism of TRPM2 channel: role in oxidative stress, inflammation and ischemia-reperfusion injury. Front Immunol. 15:1391355. https://doi.org/10.3389/fimmu.2024.1391355.

Kim SW, Davaanyam D, Seol SI, Lee HK, Lee H, Lee JK. (2020) Adenosine Triphosphate Accumulated Following Cerebral Ischemia Induces Neutrophil Extracellular Trap Formation. Int J Mol Sci. 21(20):7668. https://doi.org/10.3390/ijms21207668.

Korte N, Ilkan Z, Pearson CL, Pfeiffer T, Singhal P, Rock JR, Sethi H, Gill D, Attwell D, Tammaro P. (2022). The Ca2+-gated channel TMEM16A amplifies capillary pericyte contraction and reduces cerebral blood flow after ischemia. J Clin Invest. 132(9):e154118. https://doi.org/10.1172/JCI154118.

Kounis NG, Koniari I, Tzanis G, Soufras GD, Velissaris D, Hahalis G. (2020) Anaphylaxis-induced atrial fibrillation and anesthesia: Pathophysiologic and therapeutic considerations. Ann Card Anaesth. 23(1):1-6. https://doi.org/10.4103/aca.ACA_100_19.

Kumar VS, Gopalakrishnan A, Naziroğlu M, Rajanikant GK. (2014) Calcium ion--the key player in cerebral ischemia. Curr Med Chem. 21(18):2065-2075. https://doi.org/10.2174/09298673216661312282042 46.

Lawrence RA, Burk RF. (1976) Glutathione peroxidase activity in selenium-deficient rat liver. Biochem Biophys Res Commun. 71:952-958. https://doi.org/10.1016/0006-291x(76)90747-6.

Liu A, Li Y, Tan T, Tian X. (2014) Early exposure to sevoflurane inhibits Ca(2+) channels activity in hippocampal CA1 pyramidal neurons of developing rats. Brain Res. 1557:1-11. https://doi.org/10.1016/j.brainres.2014.02.008.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. (1951) Protein measurement with the Folin- Phenol reagent. J Biol Chem. 193:265-275.

Naziroğlu M, Lückhoff A. (2008) Effects of antioxidants on calcium influx through TRPM2 channels in transfected cells activated by hydrogen peroxide. J Neurol Sci. 15;270(1-2):152-158. https://doi.org/10.1016/j.jns.2008.03.003.

Nazıroğlu M, Özgül C. (2013) Vitamin E modulates oxidative stress and protein kinase C activator (PMA)-induced TRPM2 channel gate in dorsal root ganglion of rats. J Bioenerg Biomembr. 45(6):541-549. https://doi.org/10.1007/s10863-013-9524-x.

Nazıroğlu M, Sahin M, Ciğ B, Aykur M, Erturan I, Ugan Y. (2014) Hypericum perforatum modulates apoptosis and calcium mobilization through voltage-gated and TRPM2 calcium channels in neutrophil of patients with Behcet's disease. J Membr Biol. 247(3):253-262. https://doi.org/10.1007/s00232-014-9630-7.

Nazıroğlu M. (2007) New molecular mechanisms on the activation of TRPM2 channels by oxidative stress and ADP-ribose. Neurochem Res. 32(11):1990-2001. https://doi.org/10.1007/s11064-007-9386-x.

Osmanlıoğlu HÖ, Yıldırım MK, Akyuva Y, Yıldızhan K, Nazıroğlu M. Morphine Induces Apoptosis, Inflammation, and Mitochondrial Oxidative Stress via Activation of TRPM2 Channel and Nitric Oxide Signaling Pathways in the Hippocampus. Mol Neurobiol. 2020;57(8):3376-3389. https://doi.org/10.1007/s12035-020-01975-6.

Placer ZA, Cushman L, Johnson BC. (1966) Estimation of products of lipid peroxidation (malonyl dialdehyde) in biological fluids. Anal Biochem. 16:359-364. https://doi.org/10.1016/0003-2697(66)90167-9.

Qiu S, Li X, Zhang J, Shi P, Cao Y, Zhuang Y, Tong L. (2023) Neutrophil membrane-coated taurine nanoparticles protect against hepatic ischemia-reperfusion injury. Eur J Pharmacol. 949:175712. https://doi.org/10.1016/j.ejphar.2023.175712.

Sardas S, Izdes S, Ozcagli E, Kanbak O, Kadioglu E. (2006) The role of antioxidant supplementation in occupational exposure to waste anaesthetic gases. Int Arch Occup Environ Health. 80(2):154-159. https://doi.org/10.1007/s00420-006-0115-6.

Sedlak J, Lindsay RHC. (1968) Estimation of total, protein bound and non-protein sulfhydryl groups in tissue with Ellmann' s reagent. Anal Biochem 25:192-205. https://doi.org/10.1016/0003-2697(68)90092-4.

Shuai P, Hu Z, Li W, You G, Liu Z, Zheng N. (2023) The protective role of circ_0016760 downregulation against sevoflurane induced neurological impairment via modulating miR 145 expression in aged rats. Acta Neurobiol Exp (Wars). 83(4):377-385. https://doi.org/10.55782/ane-2023-2464.

Tang XL, Wang X, Fang G, Zhao YL, Yan J, Zhou Z, Sun R, Luo AL, Li SY. (2021) Resveratrol ameliorates sevoflurane-induced cognitive impairment by activating the SIRT1/NF-κB pathway in neonatal mice. J Nutr Biochem. 90:108579. https://doi.org/10.1016/j.jnutbio.2020.108579.

Thapak P, Gomez-Pinilla F. (2024) The bioenergetics of traumatic brain injury and its long-term impact for brain plasticity and function. Pharmacol Res. 208:107389. https://doi.org/10.1016/j.phrs.2024.107389.

Ugan Y, Nazıroğlu M, Şahin M, Aykur M. (2016) Anti-tumor Necrosis Factor Alpha (Infliximab) Attenuates Apoptosis, Oxidative Stress, and Calcium Ion Entry Through Modulation of Cation Channels in Neutrophils of Patients with Ankylosing Spondylitis. J Membr Biol. 249(4):437-447. https://doi.org/10.1007/s00232-016-9884-3.

Venditti P, Napolitano G, Di Stefano L, Agnisola C, Di Meo S. (2011) Effect of vitamin E administration on response to ischaemia-reperfusion of hearts from cold-exposed rats. Exp Physiol. 96(7):635-646. https://doi.org/10.1113/expphysiol.2011.058289.

Wallert M, Ziegler M, Wang X, Maluenda A, Xu X, Yap ML, Witt R, Giles C, Kluge S, Hortmann M, Zhang J, Meikle P, Lorkowski S, Peter K. (2019) α-Tocopherol preserves cardiac function by reducing oxidative stress and inflammation in ischemia/reperfusion injury. Redox Biol. 26:101292. https://doi.org/10.1016/j.redox.2019.101292.

Wong CH, Liu TZ, Chye SM, Lu FJ, Liu YC, Lin ZC, Chen CH. (2006) Sevoflurane-induced oxidative stress and cellular injury in human peripheral polymorphonuclear neutrophils. Food Chem Toxicol. 44(8):1399-1407. https://doi.org/10.1016/j.fct.2006.03.004.

Wu Y, Lu K, Lu Y, Liao J, Zhang S, Yang S, Zhao N, Dong Q, Chen L, Wu Q, Du Y. (2023) Transient receptor potential vanilloid 4 (TRPV4) in neutrophils enhances myocardial ischemia/reperfusion injury. J Leukoc Biol. 114(3):266-279. https://doi.org/10.1093/jleuko/qiad063.

Zeng S, Zhu R, Wang Y, Yang Y, Li N, Fu N, Sun M, Zhang J. (2022) Role of GABAA receptor depolarization-mediated VGCC activation in sevoflurane-induced cognitive impairment in neonatal mice. Front Cell Neurosci. 16:964227. https://doi.org/10.3389/fncel.2022.964227.

Zou X, Zhang X, Qiang T, Hu X, Zhang L. (2023) Melatonin attenuates sevoflurane-induced hippocampal damage and cognitive deficits in neonatal mice by suppressing CypD in parvalbumin neurons. Brain Res Bull. 204:110809. https://doi.org/10.1016/j.brainresbull.2023.110809.