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ORIGINAL RESEARCH

Acute Effects of Capsaicin Administration on Diaphragm Muscle Contraction and the Role of the TRPV1 Receptor

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ABSTRACT

Sudden Unexpected Death in Epilepsy (SUDEP) is primarily associated with respiratory failure and complete cessation of respiration caused by seizures. The aims of this study were to investigate basal contraction dynamics and calcium regulation in diaphragmatic muscles of rats with absence epilepsy induced by low-dose pentylenetetrazol (PTZ), and to investigate the dose-dependent effects of capsaicin, an activator of the TRPV1 calcium channel, on TRPV1, RyR1, cellular calcium kinetics, and mechanistic contraction properties ex vivo. Thirty-two male rats (n=8/group) were used to form four groups: Control, PTZ (25 mg/kg), PTZ + 1 μM capsaicin, and PTZ + 10 μM capsaicin. Isolated diaphragmatic muscle strips were placed in an organ bath, and isometric contraction parameters were recorded at a basal frequency of 0.2 Hz and a frequency range of 1–5 Hz. TRPV1 and RyR1 mRNA expression levels in muscle tissues were analyzed using qRT-PCR following contraction recordings. Compared to the control group, the PTZ group showed prolonged contraction and relaxation times at all stimulation frequencies, and the maximum relaxation velocity and total contractile force were significantly reduced ($p<0.05$). RyR1 expression was downregulated at the molecular level in the PTZ group, while TRPV1 expression was significantly increased ($p<0.0001$). Application of a low dose of 1 μM capsaicin to the organ bath restored all disrupted mechanistic contraction parameters and ion channel gene expression levels to normal control levels. While high-dose capsaicin inhibits muscle function, low-dose capsaicin exerts a myoprotective effect by restoring calcium balance through the interaction between TRPV1 and RyR1.

Keywords: Absence Epilepsy. Diaphragm. Capsaicin. TRPV1. RyR1. SUDEP.

Kapsaisin Uygulamasının Diyafram Kas Kasılması Üzerindeki Akut Etkileri ve TRPV1 Reseptörünün Rolü

ÖZET

Epilepside Ani Beklenmeyen Ölüm (SUDEP), öncelikle nöbetlerin neden olduğu solunum yetmezliği ve solunumun tamamen durmasıyla ilişkilidir. Bu çalışmanın amacı, düşük doz pentilenetetrazol (PTZ) ile indüklenen absans epilepsili sıçanların diyafram kaslarında bazal kasılma dinamiklerini ve kalsiyum regülasyonunu araştırmak ve TRPV1 kalsiyum kanalının aktivatörü olan kapsaisinin, TRPV1, RyR1, hücresel kalsiyum kinetiği ve ex vivo mekanistik kasılma özellikleri üzerindeki doza bağımlı etkilerini incelemektir. Dört grup oluşturmak için otuz iki erkek sıçan (n=8/grup) kullanıldı: Kontrol, PTZ (25 mg/kg), PTZ + 1 μM kapsaisin ve PTZ + 10 μM kapsaisin. İzole diyafram kası şeritleri bir organ banyosuna yerleştirildi ve izometrik kasılma parametreleri 0,2 Hz'lik bazal frekansta ve 1-5 Hz'lik frekans aralığında kaydedildi. Kas dokularındaki TRPV1 ve RyR1 mRNA ekspresyon seviyeleri, kasılma kayıtlarının ardından qRT-PCR kullanılarak analiz edildi. Kontrol grubuyla karşılaştırıldığında, PTZ grubunda tüm stimülasyon frekanslarında kasılma ve gevşeme süreleri uzadı ve maksimum gevşeme hızı ile toplam kasılma kuvveti anlamlı derecede azaldı ($p<0,05$). PTZ grubunda RyR1 ekspresyonu moleküler düzeyde azaldı, TRPV1 ekspresyonu ise anlamlı derecede arttı ($p<0,0001$). Organ banyosuna düşük dozda 1 μM kapsaisin uygulanması, bozulan tüm mekanistik kasılma parametrelerini ve iyon kanalı gen ekspresyon seviyelerini normal kontrol seviyelerine geri getirdi. Yüksek doz kapsaisin kas fonksiyonunu inhibe ederken, düşük doz kapsaisin TRPV1 ve RyR1 arasındaki etkileşim yoluyla kalsiyum dengesini geri kazandırarak miyoprotektif bir etki gösterir.

Anahtar Kelimeler: Absans Epilepsi. Diyafram. Kapsaisin. TRPV1. RyR1. SUDEP.

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Epilepsy is a chronic neurological disorder affecting around 1% of the global population, marked by the periodic recurrence of spontaneous seizures and potentially leading to impairments in learning and memory[1]. Absence epilepsy is characterised by increased gene expression of T-type calcium channels (particularly the α1G and α1I isoforms) in the thalamocortical system and by synchronised thalamocortical spike-and-wave discharges[2,3]. The specific ion channel pathologies and disruptions in calcium dynamics within the central nervous system increase cellular excitability, but how this condition affects peripheral tissues, particularly the function of the diaphragm, a crucial skeletal muscle for respiration, and peripheral calcium regulation, remains to be fully elucidated[2,4].

Capsaicin, the primary pungent compound in chili peppers (Capsicum), is a highly specific and potent agonist of transient receptor potential vanilloid 1 (TRPV1) channels, which are non-selective cation channels that exhibit high permeability to calcium ions[5]. TRPV1 receptors are widely expressed in the central nervous system, particularly in neuronal cell bodies, dendrites, astrocytes, and pericytes, and are involved in processes including neurotransmission, cellular excitability, and neuroinflammation[5]. Capsaicin has been reported to exhibit potent neuroprotective and antiepileptic effects in experimental epilepsy models, achieved by reducing oxidative stress (lipid peroxidation and nitric oxide levels), pro-inflammatory cytokines (TNF-α, IL-1β), and neuronal apoptosis[1,5]. Capsaicin has been shown to directly suppress epileptiform activity, cellular excitability, and action potentials through TRPV1-independent mechanisms by increasing the use-dependent blockade of voltage-gated sodium channels in prefrontal cortex pyramidal neurons[6].

Conversely, it has been shown in recent years that TRPV1 receptors, the targets of capsaicin, are functionally present not only in the nervous system but also in skeletal muscle[7,8]. TRPV1 channels in skeletal muscle have been found to function not in the sarcolemma but as a calcium leak channel located directly in the longitudinal region of the sarcoplasmic reticulum membrane, immediately adjacent to the calcium pump SERCA1[7]. Activation of TRPV1 by capsaicin in skeletal muscle cells results in a calcium release from the sarcoplasmic reticulum into the cytoplasm, causing a substantial increase in intracellular calcium ($[Ca^{2+}]_i$) concentration[7,8]. The initial TRPV1-mediated calcium release, which is physiologically crucial, can secondarily activate Ryanodine Receptor 1 (RyR1), a key component of muscle contraction located in the terminal cisternae of the sarcoplasmic reticulum, and, through the calcium-triggered calcium release (CICR) mechanism, thereby extensively regulating skeletal muscle calcium dynamics[7,9].

Activation of TRPV1 leads to a significant increase in intracellular calcium levels, which strongly stimulates the expression of PGC-1α, the primary regulator of energy metabolism, mitochondrial biogenesis, and muscle fiber type conversion, resulting in increased ATP production, cellular respiration, and fatty acid oxidation in skeletal muscle[8]. The impact of the absence of epilepsy pathology on the TRPV1-RyR1 calcium signaling pathway, which reprograms cellular energy capacity, and how it is altered when calcium homeostasis is already genetically disrupted, requires further investigation[7].

Our study investigated cellular calcium homeostasis in diaphragm muscles isolated from rats with an absence epilepsy model, established by administering low-dose PTZ, by applying capsaicin ex vivo. Thanks to the ex vivo experimental design, variables like systemic circulation and the central nervous system will be eliminated, allowing the interaction of capsaicin-induced TRPV1 activation with RyR1 channels and its effects on diaphragm contraction kinetics to be studied in isolation.

Materials and Methods

Experimental Groups

All experimental procedures used in this study were conducted in accordance with the guidelines of the Local Animal Experimentation Ethics Committee (HADYEK) and standards for the care of laboratory animals. The animals were housed in a specific-pathogen-free environment under standard laboratory conditions (23 ± 1 °C temperature, 50 ± 10% relative humidity, and a 12-hour light/12-hour dark cycle) and had *ad libitum* (unrestricted) access to food and water. The study protocol was approved by the Local Ethics Committee for Animal Experiments (ethics committee decision number: 2026/13).

The study was a randomized trial consisting of 4 groups, comprising a total of 32 animals, with 8 rats in each group. Male rats were specifically selected to ensure a homogeneous experimental population and to avoid potential confounding variables associated with the female hormonal (estrus) cycle.

Control (Con): A healthy control group, in which no disease model was established, was perfused solely with Krebs-Henseleit solution.

PTZ: Rats were administered a low-dose PTZ (25 mg/kg) to induce an absence epilepsy profile, and basal seizure responses were recorded in an isolated organ bath without any further interventions.

PTZ + 1 μM Cap: A group in which an absence epilepsy model was established using 25 mg/kg PTZ, with capsaicin applied at a concentration of 1 μM in the *ex vivo* organ bath.

PTZ + 10 μM Cap: An absence epilepsy model was established in a group using 25 mg/kg PTZ, and capsaicin was applied *ex vivo* at a concentration of 10 μM in an isolated organ bath.

Experimental absence epilepsy model using low-dose PTZ

To simulate petit mal seizures, rats in Groups 2, 3, and 4 of the experiment were injected intraperitoneally with a subthreshold dose of 25 mg/kg pentylenetetrazol (PTZ). The rats' sudden cessation of movement, fixed gaze, and occasional mild head-shaking movements were identified as the primary clinical signs of an absence seizure. EEG recordings, the gold standard for confirming the successful establishment of the absence model, were also performed; in our study, Spike-Wave Discharges (SWD) with a bilateral, synchronized frequency of 3–5 Hz (occasionally 7–9 Hz) that began roughly 2–5 minutes after PTZ injection were recorded as electrophysiological evidence of the absence phenotype and of the model's successful establishment (Figure 1).

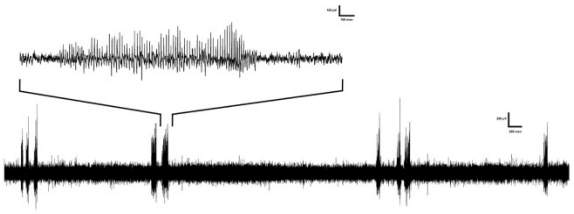

Figure 1:
A representative electrocorticogram (ECoG) recording of a rat with absence epilepsy.

Surgical Procedure and Diaphragm Isolation

A mixture of Ketamine (80 mg/kg, i.p.) and Xylazine (10 mg/kg, i.p.) was administered to the animals on the day of the experiment to induce deep anesthesia. The depth of anesthesia was confirmed by the loss of the pedal reflex, which is a foot withdrawal reflex. The thoracic cavity was quickly opened surgically, and the diaphragm tissue, the main muscle of the respiratory mechanism, was carefully isolated by removing the surrounding connective tissue. Excised diaphragm muscle fibers were preserved by transferring them into a cold (0–4 °C) standard physiological Krebs solution, saturated with carbogen gas (95% O_2, 5% CO_2), to retain cellular viability and receptor function.

Isolated Organ Bath Recording Protocol

Muscle strips prepared from the diaphragm muscle were placed vertically in an isolated organ bath system containing standard physiological solution, which was maintained at 37 °C and continuously aerated with carbogen gas. The lower end of the muscle strips was secured to a stationary hook within the bath, and the upper end was linked to a force transducer to measure isometric contractions (variations in tension). The muscles were allowed to equilibrate for approximately 45-60 minutes until a stable response was obtained at the optimal resting tension, with the bath solution being refreshed by washing it at regular intervals during this process. After the equilibration period, contraction responses were recorded using isometric electrical field stimulation. Capsaicin was added to the organ bath in the relevant experimental groups to achieve the specified final concentrations of 1 μM and 10 μM, and the effects of capsaicin-induced activation of TRPV1 and RyR1 on contraction dynamics were analyzed in real-time using a computer-based data recording system.

RT-PCR Protocol

Diaphragm muscle samples were immediately frozen in liquid nitrogen after recording physiological contractions in the isolated organ bath, and were stored at −80 °C until RNA isolation for gene expression analysis. The total RNA isolated from muscle tissues using standard kits was then used for cDNA synthesis. Quantitative real-time polymerase chain reaction (qRT-PCR) analyses employed specific primers for the TRPV1 and RyR1 proteins, which are involved in cellular calcium dynamics. Relative gene expression (fold change) was calculated using the comparative $2^{-\Delta\Delta Ct}$ method, with GAPDH as the reference gene, after normalization of amplification data.

Statistical Analysis

Data analysis was performed using GraphPad Prism 10.0 software (GraphPad Software, San Diego, CA, USA). A one-way analysis of variance was used to compare the means of group data in a single direction regarding contraction and biochemical parameters. The Tukey post hoc test was used to identify which groups were significantly different from one another. All experimental groups will be represented by their mean ± standard error of the mean (SEM), with $p < 0.05$ being the threshold for statistical significance.

Results

When examining the basic contraction parameters obtained from the isolated diaphragm muscle, contraction time (CT) values were measured as 0.0251±0.0017 seconds in the CON group. PTZ (0.0326±0.0009 s), PTZ + 1 μM Cap (0.0325±0.0012 s), and PTZ + 10 μM Cap (0.0349±0.0005 s) groups, the CT value was found to be statistically significantly different from that of the CON group ($p<0.0001$) (Table I).

Regarding relaxation time (RT), the value of 0.0547±0.0010 seconds in the CON group was found to differ significantly from the CON group in the PTZ (0.0722±0.0030 s) and PTZ + 10 µM Cap (0.0790±0.0035 s) groups (p=0.0033). In the PTZ + 1 µM Cap group (0.0668±0.0071 s), however, no statistically significant difference was observed compared to the CON group. Analysis of contraction force (CF) data revealed that the PTZ + 10 µM Cap group (0.3242±0.0013 g/mg) exhibited a statistically significant difference compared to the other three groups (CON, PTZ, and PTZ + 1 µM Cap) (p<0.001). There was no significant difference found among the CON (1.7830±0.2592 g/mg), PTZ (1.6070 ± 0.2163 g/mg), and PTZ + 1 µM Cap (1.7464 ± 0.3765 g/mg) groups (Table I).

For the maximum contraction rate (+dF/dtmax) parameter, the PTZ + 1 µM Cap group (1.0466 ± 0.1437 g/s·mg) showed a significant difference compared to the PTZ group (0.9434 ± 0.1163 g/s·mg). The PTZ + 10 µM Cap group (0.2145 ± 0.0007 g/s·mg) was found to be statistically significantly different from both the CON and PTZ + 1 µM Cap groups (p < 0.001) (Table I).

When examining the maximum relaxation rate (-dF/dtmax) values, compared to the CON group (-1.2200 ± 0.2932 g/s.mg), the PTZ (-0.4953 ± 0.0714 g/s·mg) and the PTZ + 10 µM Cap group (-0.1418 ± 0.0046 g/s·mg) (p < 0.001) (Table I).

The PTZ group (0.0600 ± 0.0127 g·s/mg) exhibited a significant difference compared to the CON group (0.0908 ± 0.0018 g·s/mg) based on contraction force data, as indicated by the area under the curve (AUC). The AUC value of the PTZ + 10 µM Cap group (0.0095 ± 0.0002 g·s/mg) was significantly different from the AUC values of the CON, PTZ, and PTZ + 1 µM Cap groups (p < 0.0001) (Table I).

At all frequency ranges (1, 2, 3, 4, and 5 Hz), the contraction durations in the PTZ and PTZ + 10 µM Cap groups were found to be statistically significantly longer than those in the CON group (Figure 2). The contraction duration of the PTZ + 1 µM Cap group was found to be significantly longer than that of the CON group only at 1 Hz and 2 Hz frequencies. At 3, 4, and 5 Hz, no significant difference was found between the two groups. Significant differences were found between the PTZ and PTZ + 1 µM Cap groups at all frequencies except 1 Hz, namely 2, 3, 4, and 5 Hz. The PTZ + 10 µM Cap group exhibited significant differences compared to the PTZ + 1 µM Cap group at 2, 4, and 5 Hz, and to the PTZ group at 4 Hz.

When compared to the relaxation time of the CON group, statistically significant increases were observed in both the PTZ and PTZ + 10 µM Cap groups across all frequencies from 1 Hz to 5 Hz. Notably, the relaxation times of the PTZ + 1 µM Cap group were found to be statistically significantly different from those of the PTZ group at all frequencies (1–5 Hz). Between the PTZ + 1 µM Cap and PTZ + 10 µM Cap groups, a significant difference was detected only at 1 Hz and 3 Hz.

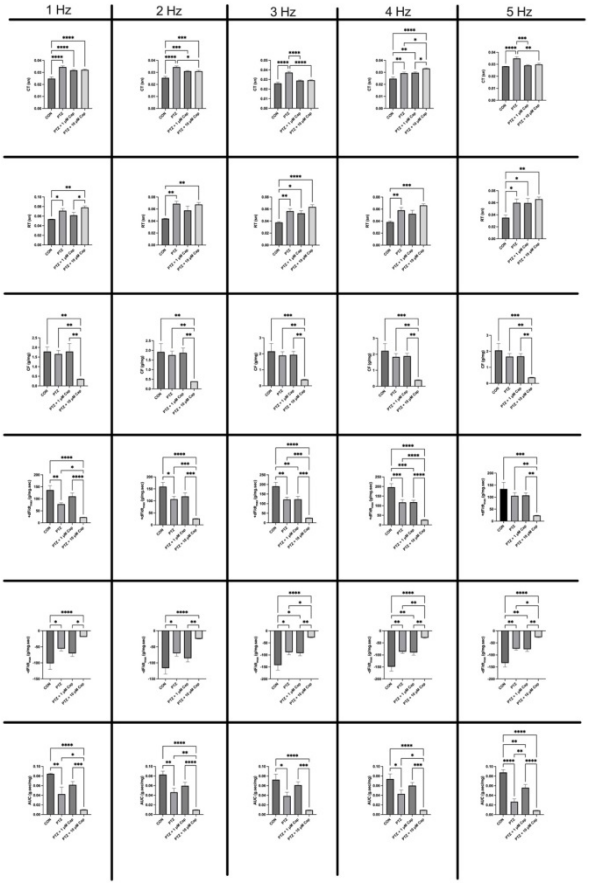

Figure 2:

*Effects of the absence epilepsy model and capsaicin (1 and 10 µM) application on contraction dynamics in isolated diaphragm muscle at increasing stimulation frequencies (1, 2, 3, 4, and 5 Hz). The graphs show changes in the contraction time (CT), relaxation time (RT), contraction force (CF), maximum contraction velocity (+dF/dtmax), maximum relaxation velocity (-dF/dtmax), and area under the curve (AUC) for the isolated diaphragm muscle at stimulation frequencies ranging from 1 Hz to 5 Hz. All data are presented as Mean ± Standard Error of the Mean (SEM) (n=8/group). The levels of statistical significance between groups are indicated by asterisk symbols in the graph: * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001. Abbreviations: CON: Control; Cap: Capsaicin.*

Analysis of contraction force data revealed no statistically significant differences among the CON, PTZ, and PTZ + 1 µM Cap groups at any of the frequencies tested from 1 Hz to 5 Hz. In contrast, the contraction force of the PTZ + 10 µM Cap group was

Table I. Effects of capsaicin application on contraction parameters in isolated diaphragm muscle under basal stimulation (0.2 Hz) across groups.

	CON (n=8)	PTZ (n=8)	PTZ + 1 µM Cap (n=8)	PTZ + 10 µM Cap (n=8)	p
Contraction Time (s)	0.0251 ± 0.0017	0.0326 ± 0.0009[a]	0.0325 ± 0.0012[a]	0.0349 ± 0.0005[a]	<0.0001
Relaxation Time (s)	0.0547 ± 0.0010	0.0722 ± 0.0030[a]	0.0668 ± 0.0071	0.0790 ± 0.0035[a]	0.0033
Contraction Force (g/mg)	1.7830 ± 0.2592	1.6070 ± 0.2163	1.7464 ± 0.3765	0.3242 ± 0.0013[a,b,c]	< 0.001
+dF/dtmax (g/s·mg)	1.0913 ± 0.2222	0.9434 ± 0.1163	1.0466 ± 0.1437[b]	0.2145 ± 0.0007[a,c]	< 0.001
-dF/dtmax (g/s·mg)	-1.2200 ± 0.2932	-0.4953 ± 0.0714[a]	-0.6359 ± 0.1026	-0.1418 ± 0.0046[a]	< 0.001
Area Under the Curve (g·s/mg)	0.0908 ± 0.0018	0.0600 ± 0.0127[a]	0.0629 ± 0.0085	0.0095 ± 0.0002[a,b,c]	<0.0001

significantly lower than that of the other three groups (CON, PTZ, and PTZ + 1 µM Cap) at all frequencies.

In terms of maximum contraction rate parameters, the PTZ group show ed statistically significant differences compared to the CON group at frequencies of 1, 2, 3, and 4 Hz, whereas no significant difference was observed between the two groups at 5 Hz. The PTZ + 10 µM Cap group, how ever, exhibited statistically significant differences compared to the CON group at all tested frequencies (1–5 Hz). While a significant difference was found between the PTZ + 1 µM Cap group and the PTZ + 10 µM Cap group at all frequencies, the only significant difference between the PTZ and PTZ + 1 µM Cap groups was observed at the 1 Hz frequency.

The maximum relaxation rates of the PTZ and PTZ + 10 µM Cap groups were significantly higher than the CON group at all stimulation frequencies from 1 Hz to 5 Hz. Significant differences were observed between the PTZ + 1 µM Cap and PTZ + 10 µM Cap groups at all frequencies, except 2 Hz, at 1, 3, 4, and 5 Hz.

Statistically significant differences in AUC values, which indicate contraction strength, were observed between the PTZ and PTZ + 10 µM Cap groups and the CON group at all frequencies from 1 Hz to 5 Hz. The AUC value of the PTZ + 1 µM Cap group was found to be significantly different from that of the PTZ group at all tested frequencies, ranging from 1 to 5 Hz. Statistically significant differences were observed between the PTZ + 1 µM Cap and PTZ + 10 µM Cap groups at all frequencies, namely 2, 3, 4, and 5 Hz, but not at 1 Hz.

When the expression levels of the TRPV1 and RyR1 genes in isolated diaphragm tissue were analyzed ($2^{-\Delta\Delta Ct}$), statistically significant differences ($p<0.0001$) were detected between groups for all genes (Figure 3). The TRPV1 mRNA expression level was found to be statistically significantly higher in the PTZ group compared to the CON group. It was observed that the expression level in the PTZ + 1 µM Cap group was significantly lower than that in the PTZ group and approached the levels of the CON group. In the PTZ + 10 µM Cap group, TRPV1 expression was found to be statistically significantly lower than in the CON, PTZ, and PTZ + 1 µM Cap groups.

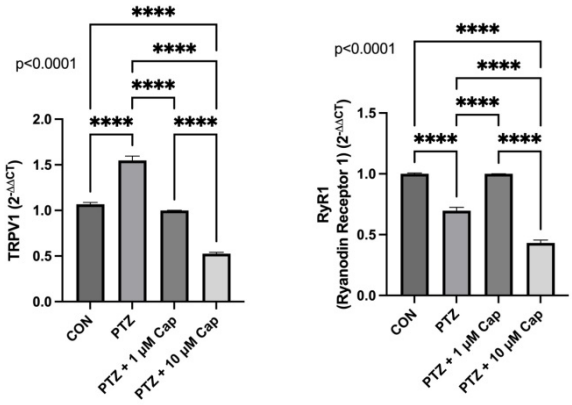

Figure 3:

*Effects of the absence epilepsy model and capsaicin (1 and 10 µM) treatment on TRPV1 and RyR1 gene expression levels in isolated diaphragm tissue. The graphs show relative mRNA expression levels obtained by qRT-PCR and calculated using the $2^{-\Delta\Delta Ct}$ method (ANOVA, $p<0.0001$). Statistical differences between groups are indicated by the **** symbol ($p<0.0001$).*

The RyR1 expression level was lower in the PTZ group than in the CON group. RyR1 expression was found to be statistically significantly higher in the group treated with PTZ + 1 µM Cap compared to the PTZ group. In contrast, RyR1 expression in the PTZ + 10 µM Cap group was found to be statistically significantly lower than in the CON, PTZ, and PTZ + 1 µM Cap groups.

Discussion and Conclusion

This study discovered that cellular calcium regulation and contraction dynamics were disrupted in diaphragm muscle isolated from rats with an absence epilepsy model, and that the expression profiles of

genes involved in cellular calcium homeostasis, specifically TRPV1 and RyR1, were pathologically altered. Capsaicin at a low dose (1 μM) administered in an ex vivo setting has a restorative effect on this dysfunction, whereas high-dose (10 μM) administration disrupts muscle function and gene expression.

Sudden unexpected death in epilepsy (SUDEP) is a major cause of mortality in patients with seizures, and severe seizure-induced respiratory dysfunction and terminal apnea have been identified as the key underlying mechanisms[10,11]. Central apnea and respiratory arrest, occurring after generalized tonic-clonic seizures, are the key common features in SUDEP cases[12]. Our findings of prolonged contraction/relaxation times and significantly reduced contraction force (AUC) in the diaphragms of epileptic rats directly align with the weakening of the mechanical-e in the diaphragm, a key muscle responsible for respiratory function, and sheds light on the peripheral mechanisms of SUDEP[13].

The disrupted contraction dynamics in the absence epilepsy model are closely linked to the suppression of RyR1 gene expression, which regulates the primary calcium release from the sarcoplasmic reticulum (SR)[14]. Epileptic processes are known to cause severe neuroinflammation and oxidative stress at the cellular level[5,6]. Increased reactive oxygen species (ROS) cause irreversible oxidative modifications, including malondialdehyde (MDA) and 3-nitrotyrosine (3-NT), on RyR1 channels, a crucial component of the E-C (excitation-contraction) coupling in skeletal muscle[15]. The oxidative changes cause the dissociation of the FKBP12 protein, which normally stabilizes the channel, thereby pathologically increasing the likelihood of RyR1 remaining open (leakage)[15]. Continuous leakage of calcium from SR stores leads to severe muscle weakness and fatigue due to depletion[16]. In our study, the RyR1 suppression observed in the PTZ group is considered to be a cellular 'down-regulation' strategy that develops to limit oxidative stress-induced pathological calcium leakage. In contrast to the suppressed RyR1 gene, a substantial increase in TRPV1 expression was found in epileptic rats. In skeletal muscle, TRPV1 receptors act as calcium leak channels, located in the longitudinal membrane of the sarcoplasmic reticulum, rather than the sarcolemma, and are situated immediately adjacent to SERCA pumps[7]. The pathogenesis of absence epilepsy is based on the increased expression of T-type calcium channels (especially α1G and α1I) in the thalamocortical system[2]. Excessive TRPV1 expression in the diaphragm muscle may be an extension of systemic calcium channel abnormalities in epilepsy genetics, or an adaptation attempting to reorganize calcium homeostasis to compensate for dysfunctional RyR1.

Capsaicin, applied at a concentration of 1 μM in an isolated organ bath, increased the suppressed maximum contraction rate, reduced prolonged contraction durations, and restored TRPV1 and RyR1 expression to normal control levels. The restorative effect can be attributed to mild TRPV1 activation by capsaicin, which triggers a controlled release of calcium from the sarcoplasmic reticulum into the cytoplasm, subsequently activating RyR1 receptors through the calcium-induced calcium release mechanism[7,17]. TRPV1 activation also induces a mild increase in intracellular calcium signals, which stimulates the PGC-1α gene, the primary regulator of energy metabolism, thereby supporting mitochondrial biogenesis[18] and enhancing exercise and respiratory endurance by increasing the proportion of fatigue-resistant (oxidative) type I muscle fibers[8]. Capsaicin, a potent antioxidant, may have restored contraction dynamics by reducing oxidative damage (MDA/3-NT load) on RyR1 through e modifications[5,19].

In contrast, a high dose of capsaicin (10 μM) dramatically reduced all mechanical parameters (CF, AUC) and gene expressions (TRPV1, RyR1) of the diaphragm muscle to the lowest levels. Prolonged and high-concentration application of capsaicin causes TRPV1 channels to close in a calcium-dependent manner, entering the "desensitization" phase[5,20]. The primary destructive effect arises from high-dose capsaicin's ability to strongly block voltage-gated sodium channels in muscle cells in a use-dependent manner, completely independent of the TRPV1 receptor[6,21]. This cellular electrical paralysis, which halts the generation of action potentials, has completely suppressed the excitation of the diaphragm muscle.

Significant cellular fatigue/damage was demonstrated in the calcium regulation and contraction kinetics of the diaphragm, a key muscle in the SUDEP mechanism, in the absence epilepsy model. The potential of low-dose capsaicin to prevent respiratory fatigue and SUDEP risk through its myoprotective and antioxidant effects via the TRPV1 and RyR1 calcium pathways is pharmacologically promising, but the toxic sodium channel blockade caused by high doses underscores the importance of a narrow therapeutic window for TRPV1 modulators.

This study is the first to show significant cellular disruption in the calcium release kinetics (RyR1/TRPV1 axis) and mechanical contraction dynamics of the diaphragm—the primary respiratory muscle—in an absence epilepsy model. Low-dose (1 μM) capsaicin stimulation of TRPV1 receptors in the diaphragm muscle restored functional recovery (myoprotection) by correcting the disrupted ion balance (CICR). High-dose (10 μM) applications of this molecule paralyze contraction capacity due to sodium channel blockade and desensitization,

highlighting the narrow and critical therapeutic dose range for potential pharmacological interventions. Furthermore, to definitively isolate TRPV1-specific mechanisms from other oxidative stress-sensitive ion channels such as TRPA1, TRPM8, and TRPM2, the use of a specific TRPV1 antagonist like capsazepine should be considered in future studies. Moreover, while our *ex vivo* approach allowed for the isolated investigation of direct muscle interactions, future *in vivo* studies are necessary to evaluate how systemic capsaicin administration and subsequent TRPV1 activation within the central nervous system—known for its neuroprotective properties—might modulate these peripheral respiratory responses. Additionally, such investigations will provide a more holistic understanding of capsaicin's therapeutic potential in the context of SUDEP.

Although male rats were used to provide a controlled experimental environment, future studies incorporating female rats are warranted to assess potential sex-specific differences in TRPV1 expression and seizure thresholds.

These findings provide a strong mechanistic basis for peripheral TRPV1-RyR1 calcium modulation as a potential new target for preventing respiratory dysfunction and SUDEP risk associated with epilepsy.

Researcher Contribution Statement:
Idea and design: O.U., N.A.U, E.E.E.O., E.G.M., M.A.; Data collection and processing: O.U., N.A.U., M.A.; Analysis and interpretation of data: O.U., E.G.M.; Writing of significant parts of the article: N.A.U, O.U., E.E.E.O.
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Non-applicable
Conflict of Interest Statement:
The authors of the article have no conflict of interest declarations
Ethics Committee Approval Information:
Approving Committee: Animal Ethics Committee (HADYEK) of Ondokuz Mayıs University
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Decision No: 2026/13

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