



Effects of resveratrol, catechin and epicatechin on rat phrenic nerve hemi-diaphragm

Sıçan frenik sinir hemi-diyafrağına resveratrol, kateşin ve epikateşinin etkileri

Aslı Zengin Türkmen¹, Asiye Nurten¹

Abstract

Aim: Resveratrol, catechin and epicatechin are known for their preventive effects of skeletal muscle atrophy, anti-fatigue effects and enhancement of exercise capacity. In this study, we investigate the effects these antioxidants on muscle contraction by using rat phrenic nerve hemi-diaphragm preparations.

Methods: Twitch responses are investigated by giving direct and indirect and tetanic stimulation to rat phrenic nerve hemi-diaphragm preparations.

Results: Resveratrol and catechin facilitated twitch responses to the indirect stimulus in short duration and suppressed in long duration. The decrease in the responses to the direct stimulation by applying epicatechin ($p=0.010$) was significantly higher than the responses by giving indirect stimulation. Epicatechin significantly suppressed tetanic responses ($p=0.005$), but not resveratrol and catechin.

Conclusion: The results of the study show that these antioxidants have presynaptic and postsynaptic effects on neuromuscular junction. Resveratrol effective on muscle contraction via direct stimulus, catechin and epicatechin effects muscle contraction by direct and indirect stimulus.

Key words: Resveratrol, catechin, epicatechin, rat, antioxidant

¹ Istanbul Yeni Yuzyil University, Faculty of Medicine, Department of Physiology, Istanbul, Turkey.

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Sorumlu yazar / Corresponding author:
Aslı Zengin Türkmen

Adres/Address: Istanbul Yeni Yuzyil University, Faculty of Medicine, Department of Physiology, 34010, Zeytinburnu, Istanbul, Turkey.
e-posta: asli.zengin@yeniyyuzyil.edu.tr
Tel/Phone: +905356589478

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Öz

Amaç: Resveratrol, kateşin ve epikateşin iskelet kas atrofisini engelleyen, yorgunluk karşıtı etkileri olan ve egzersiz kapasitesini artıran etkileriyle bilinmektedirler. Çalışmamızda, sıçan frenik sinir hemi-diyafrağı preparatı kullanılarak bu antioksidanların kas kasılmasına etkisi incelenmiştir.

Yöntemler: Kasılma yanıtları sıçan frenik sinir hemi-diyafrağı preparatına, doğrudan, dolaylı ve tetanik uyarılar verilerek araştırıldı.

Bulgular: Resveratrol ve kateşin uygulanması kısa sürede, dolaylı uyarılarla oluşan kasılma yanıtlarını kolaylaştırdı ancak uzun sürede baskıladı. Epikateşin uygulanması sonucunda doğrudan uyarılarla oluşan kasılma yanıtlarındaki azalma ($p=0,010$), dolaylı uyarılarla oluşan azalmadan anlamlı olarak daha fazlaydı. Resveratrol ve kateşin tetanik yanıtları etkilemezken, epikateşin anlamlı derecede baskıladı ($p=0,005$).

Sonuç: Çalışmanın sonuçları, bu antioksidanların nöromusküler kavşakta presinaptik ve postsinaptik etkileri olduğunu göstermektedir. Resveratrol kas kasılmasını doğrudan etkilemekte, kateşin ve epikateşin ise hem doğrudan hem de dolaylı olarak etkilemektedir.

Anahtar kelimeler: Resveratrol, kateşin, epikateşin, sıçan, antioksidan

Introduction

Resveratrol is a well-known grape and wine polyphenolic component, synthesized in grape skin in response to infection. Numerous studies have reported resveratrol as a preventive agent against vascular disease, cancer, viral infections and neurodegenerative processes [1, 2].

Catechin and epicatechin are tea polyphenols and used in vascular, viral, gastrointestinal diseases [3]. Catechin has protective effects in skeletal muscle atrophy and down-regulate the expression of atrogenes, which are muscle atrophy related enzymes [4]. Another study showed that epicatechin enhances exercise capacity in mice [5].

Effect of different tea extracts were studied on neuromuscular junction [6, 7, 8, 9]. In a study with rat phrenic nerve hemi-diaphragm preparation, teaflavin fraction of black tea has facilitatory effect on indirect twitch responses [6]. Black tea extract also inhibits effect of botulinum neurotoxin in mice [9]. Green tea extract (GTE) has facilitatory effect on indirect twitch responses at the lower concentrations, but its effect was suppressed at the higher concentrations [7].

With contraction of the hemi-diaphragm, free radicals may accumulate in the environment as time elapses. Superoxide and hydrogen peroxide (H_2O_2) are produced and released in skeletal muscle fibers [10]. Reactive oxygen species reduced muscle twitches by indirect stimulation; but, they have no effect by direct stimulation on rat phrenic nerve hemi-diaphragm preparation [11]. Also, recent studies have shown that free radicals cause muscle weakness in frogs [10]. Resveratrol suppress oxidative stress markers in aged mice with dietary administration [12]. Dietary resveratrol also slows age related structural changes in neuromuscular junction (NMJ) [13].

As well as the abundant effects of these polyphenols, there is not much data about the direct effects of catechin and epicatechin on the peripheral nervous system. Therefore, we aimed to investigate the effects of resveratrol and catechins on NMJ using rat phrenic nerve hemi-diaphragm preparation for this purpose. Then, the effects of these antioxidant molecules on muscle twitches were investigated by stimulating the phrenic nerve and hemi-diaphragm electrically.

Material and methods

This study was approved by the Animal Ethics Committees of Institute for Experimental Medicine, Istanbul University (Date: 01.03.2006, No: 10). All animal studies carefully conformed to the guidelines outlined in Interdisciplinary Principles and Guidelines for the Use of Animals in Research and Education from the New York Academy of Sciences.

Experiments were performed on adult male Wistar albino rats weighing 250-300 g provided by the Istanbul University, Aziz Sancar Institute for Experimental Medicine. Antioxidant molecules; resveratrol, (+) catechin and (-) epicatechin were purchased from Sigma and Aldrich Chem. Co., St. Louis, MO.

The phrenic nerve hemi-diaphragm preparations were isolated from decapitated rats, according to the method of Bulbring [14] (Figure 1). The preparation was suspended in a 20 ml organ bath containing Krebs' solution (133 mM NaCl, 4.9 mM KCl, 1.8 mM $CaCl_2$, 11.9 mM $NaHCO_3$, 0.7 mM NaH_2PO_4 , 11mM Glucose) aerated with a mixture of 95% O_2 and 5% CO_2 at 37°C. After resting for 15 minutes, preparation was fixed at the rib, while the hemi-diaphragm was fixed to the arm of isometric force transducer (MAY FDT 10-A, Grass Technologies; West Warwick, RI) with a tension of 2 g. The muscle was placed on

platinum electrodes for direct stimulation and the phrenic nerve pulled into another electrode for stimulating the diaphragm indirectly.

Direct responses were studied on 13 preparations for resveratrol, 10 for catechin and 9 for epicatechin. For investigating indirect responses we used 11 preparations for resveratrol, 12 for catechin and 8 for epicatechin.

Supramaximal square wave pulses with 0.1 Hz frequencies and 0.3 ms duration were used for indirect stimulation, and 0.1 Hz 3 ms pulses were used for direct stimulation. For tetanic responses, the preparation was stimulated at 50 Hz and 3 ms indirectly. The responses were recorded by a polygraph (Grass Mode 7400 Physiological Recorder, Grass Technologies; West Warwick, RI) linked to the computer, and data were observed with "PolyVIEW v2.5 data analyze and acquisition system" (Astro-Med, West Warwick, RI).

All antioxidant molecules were dissolved in Krebs' solution containing 15% ethanol. We applied different doses of antioxidant molecules in a range of 25, 50, 62.5, 75, 100, 150 and 200 μM and found the optimal dose as 62.5 μM for resveratrol, 150 μM for catechin and 50 μM for epicatechin (Table 1). The effects of the antioxidant molecules were denoted as the changing percent of the responses, before and after the treatment. Contractions were recorded as baseline and 2.5th, 5th, 10th, 15th and 20th minutes.

Statistical analysis

All values were expressed as mean \pm standard error of mean (SEM). We first analyzed the responses by one-way ANOVA, and then, statistical significance of the direct and indirect responses were calculated by post-hoc analyses (Tukey's test). Significance of the tetanic responses was calculated by the Student's t-test. $p < 0.050$ was considered statistically significant.

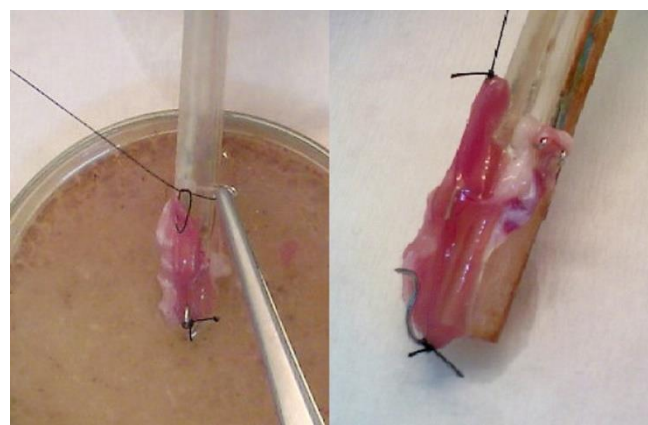


Figure 1. Isolated phrenic nerve hemi-diaphragm preparation placed at platinum electrodes.

Results

When we applied resveratrol, twitch responses gradually decreased in time by direct stimulation. Muscle contraction was reduced to $80.4 \pm 6.8\%$ at 20th minutes, and this decrement was statistically significant from the basal value ($p=0.050$) (Figure 2).

Twitch responses to indirect stimulus increased to $114.2 \pm 7.7\%$ at 2.5th minutes, then, non-significantly decreased to $92.1 \pm 5.3\%$ at 20th minutes by applying resveratrol ($p=0.096$).

As shown in Figure 3, catechin administration significantly reduced muscle contraction by direct stimulation. Twitch responses decreased to $80.6 \pm 18.1\%$ at 10th minutes ($p=0.050$), $80.5 \pm 6.1\%$ at 15th minutes ($p=0.010$) and $76.9 \pm 15.8\%$ at 20th minutes ($p=0.010$). These decrements were significantly different from the basal value.

When we applied catechin, twitch responses to indirect stimulus increased to $106.9 \pm 5.1\%$ at 2.5th minutes, but decreased

to the basal values at 5th minutes. Catechin administration reduced twitch responses to 86.2±3.8% at 20th minutes. This decrement found statistically significant from the value at 2.5th minutes (p=0.010) and 5th minutes (p=0.050).

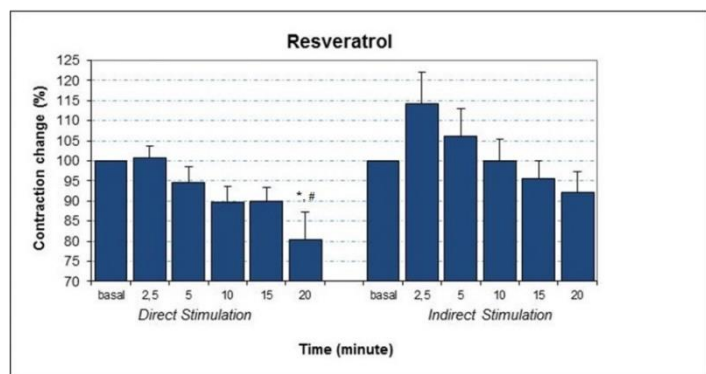


Figure 2. Timecourse of muscle contraction change after administration of resveratrol. Values represented as mean ± SEM. * compared with basal value, p=0.050, # compared with value at 2.5th minutes, p=0.050.

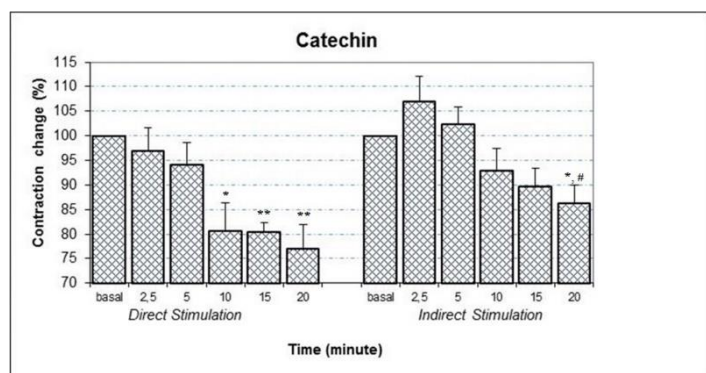


Figure 3. Effects of catechin on muscle contraction. Values represented as mean ± SEM. * compared with basal value, p=0.050, ** compared with basal value, p=0.010, # compared with value at 2.5th minutes, p=0.010.

Epicatechin was significantly decreased the direct responses to 80.6±4.3% at 10th minutes (p=0.050), 76.6±4.3% at 15th minutes (p=0.010) and 70.9±6.7% at 20th minutes (p=0.010) compared to the basal values. Twitch responses to indirect stimulus decreased 88.4± 11.6% by applying epicatechin at 20th minutes. But, this is not statistically significant from the basal value (Figure 4).

When we stimulate the preparation tetanically, epicatechin significantly reduced muscle contraction at 20th minutes (p=0.005), there were no changes after resveratrol and catechin administration (Figure 5).

We used low concentrated ethanol as solvent of resveratrol and catechins. It was ineffective on direct, indirect and tetanic responses at the dose we used.

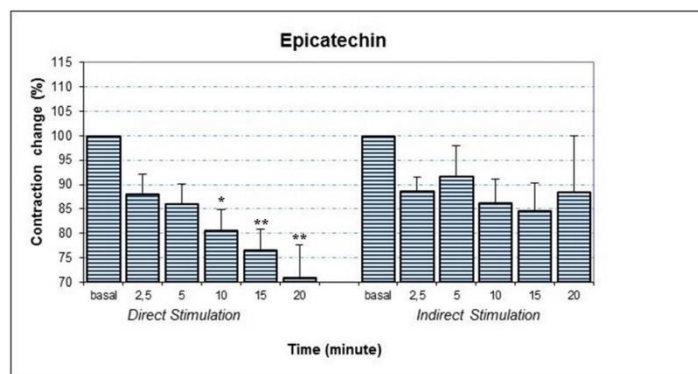


Figure 4. Effects of epicatechin on muscle contraction. Values represented as mean ± SEM. * compared with basal value, p=0.050, ** compared with basal value, p=0.010.

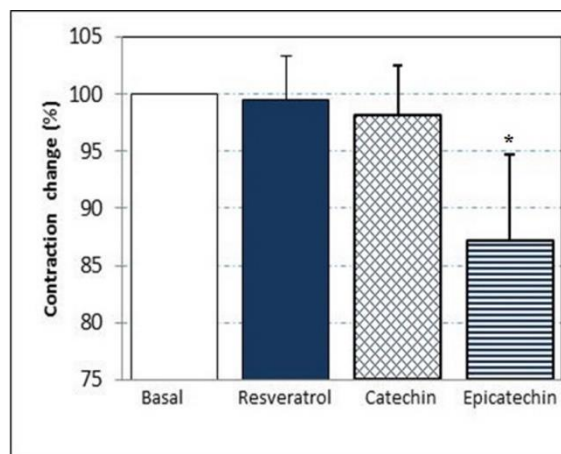


Figure 5. Effects of resveratrol, catechin and epicatechin administration on tetanic muscle contraction at 20th minutes. * compared with basal value, p=0.005.

Discussion

This study is designed to find out the effects of different antioxidants on the phrenic nerve hemi-diaphragm preparation. We showed the effects of resveratrol, catechin and epicatechin on muscle twitches to direct, indirect and tetanic stimulation. With direct stimulus, resveratrol, catechin and epicatechin reduced twitch responses gradually in time. We found no difference on tetanic responses in resveratrol and catechin treated NMJ preparations.

To our knowledge this is the first study that applies resveratrol, catechin and epicatechin on the phrenic nerve hemi-diaphragm preparation.

Resveratrol improved motor performance impairments by long-term application in stroke model in rats [15]. In our study, resveratrol enhanced muscle contraction in short duration, but suppresses in long term application. It can be argued that different results could be obtained in range of resveratrol concentrations.

Table 1. Dose response values of phrenic nerve hemi-diaphragm preparations to resveratrol, catechin and epicatechin.

Time (min) vs dose (µl)	Resveratrol								Catechin								Epicatechin							
	Direct Stimulation				Indirect Stimulation				Direct Stimulation				Indirect Stimulation				Direct Stimulation				Indirect Stimulation			
	5 th	10 th	15 th	20 th	5 th	10 th	15 th	20 th	5 th	10 th	15 th	20 th	5 th	10 th	15 th	20 th	5 th	10 th	15 th	20 th	5 th	10 th	15 th	20 th
25	99	115	98	40	62	78	81	-	66	80	23	20	76	100	44	40	70	72	70	78	85	92	92	103
50	101	100	97	95	92	90	93	106	58	68	50	44	36	49	49	64	70	79	66	82	132	135	116	109
62.5	118	112	102	85	109	116	120	96	68	80	98	98	103	98	98	94	82	89	78	82	105	97	86	112
75	97	105	102	107	97	95	96	91	106	113	106	107	98	-	92	105	69	79	65	85	127	121	112	115
100	100	102	51	110	68	85	87	78	105	107	109	116	105	92	89	90	70	75	70	76	120	113	115	110
150	105	90	96	100	75	84	89	85	114	112	109	115	117	110	110	108	90	95	98	90	100	95	95	-
200	65	65	-	50	70	90	90	95	33	26	32	34	117	107	107	113	88	102	-	-	65	80	50	62

The twitch responses were compared with basal values and denoted as changing percent in time. Selected dose for each antioxidant molecule indicated as bold and italic.

There are no studies showing the effects of catechin and epicatechin directly apply on neuromuscular junction. In a recent study, it has been shown that GTE, which have catechins abundantly, suppress muscle contraction in high doses [7]. GTE facilitates contraction by indirect stimulation in low concentrations, while suppressing the contraction response in high concentrations [7]. In another study, the exercise performance was better in mice, which fed with green tea rich diet [16].

According to results of our study, catechin, in the short term, increased the response to indirect stimuli similar to the effect of low concentrated GTE. However, catechin suppressed the twitch responses in extended time.

We found that catechin suppressed twitch responses by direct stimulation, but GTE has no effect. Different doses of GTE had no effects on contracted muscle, which directly stimulated by acetylcholine administration [7]. Green tea extract had no effect on the direct twitch responses, but the catechin exerted its effect alone. The presence of different compounds in the extracts besides of catechins, can also affect muscle contraction.

In summary, resveratrol suppressed muscle contraction by direct stimulus. Catechin and epicatechin minimize the muscle contraction with direct and indirect stimulus. Epicatechin reduced muscle contraction by tetanic stimulation.

In the light of these findings, we can say that the further studies on the effects of phenolic compounds on neuromuscular junction can give us more comprehensive information.

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