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Effects of Ethanol on Contraction Strength of Chick Neck Striatum Muscle Following Electrical Stimulation of the Nerve

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Abstract

Introduction: Ethanol is a suitable solvent for many in vivo and ex vivo studies. However, it can interfere with normal muscle contraction and make variations in the results. Contrary to the present study, previous investigations revealed the suppressant effect of ethanol on muscle contraction.

Methods: This study was based on an isolated chick biventer cervicis nerve-muscle using the twitch tension recording technique. Nerve and muscle complexes were exposed to several concentrations of ethanol (100, 200, 300, 400, and 500 mM), and impulses were recorded.

Results: Twitch height increased in time and dose-dependent manner, and the concentration of 500 mM of ethanol after 30 minutes revealed the most elevation of muscle impulses.

Conclusion: The potential effects of ethanol on striated muscle contraction are important and should be considered in studies using ethanol as a solvent.

Keywords: Ethanol, Chicken biventer cervices, Striatum muscle contraction

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Introduction

The appearance nature of skeletal muscle from different physiological and biological conditions is controversial, and it is important to investigate the effects of various substances, including ethanol, on contractility.[1].Ethanol has been proposed as a suitable solvent for many laboratories and even pharmaceutical materials, which suggests paying more attention to the effects of this material, apart from the effects of the soluble agent on the target tissue.[2] On the other hand, ethanol affects many ion channels and receptors and can have inhibitory or stimulatory effects depending on the type of channel and receptor.[3,4] Alcohol interferes with specific mechanisms by the function of cardiac myocytes, skeletal myotubes, and smooth muscle cells, and clinically, it causes acute and chronic myopathy and muscle weakness.[5]

The acute and chronic effects of ethanol on skeletal muscle are important in cellular and animal studies which use it as a solvent or vehicle.[6] To date, there have been extensive and various studies on the different effects of ethanol, either in the cellular or clinical manner, and they have implicated the functional or structural aspects of cardiac, skeletal, or smooth muscle cells.[7] Studies have shown that ethanol may interfere with the activity of the sarcoma calcium channel and decrease the entry of calcium into cells.[8]

Muscle weakness and atrophy are the main effects of ethanol, but it seems that they have direct and acute effects on skeletal muscle contractility, regardless of the effect of the test. The current study reported the data regarding the effects of ethanol on the contraction strength of the muscle following electrical stimulation of the nerve

Materials and Methods

Animals and Chemicals

Chick double-stranded muscle was used for the electrophysiological study of neuromuscular transmission. The chicks used in this study were about 4-12 days old, weighed 37-47 grams, and were fed and maintained under physiological conditions. All the used chemicals were

pure (more than 99%). Solvents and other reagents were purchased from Merck (Germany) or Sigma Chemical Company (Sigma-Aldrich, USA). Then, experiments were carried out regarding the ethical recommendations of laboratory animal care.[9]

Experimental Procedure

First, chicks were killed by a lethal dose of ether, and then the main veins of the chicks were cut. Immediately, the target muscle, along with its associated nerves, was dissected and placed in a 50 mL tissue bath containing the following physiological solution.[10] Tissue separated by carbogen gas (O_2 95% and CO_2 5%) was continuously aerated. The ambient temperature of the tissue bath was adjusted to 32° C and pH was 7.2 to 7.3. The solute composition in millimoles was NaCl 118.4, KH_2PO_4 1.2, glucose 11.1, $NaHCO_3$ 25, $CaCl_2$ 2.5, $MgSO_4$ 1.4, and KCl, 4.7.[11,12]

The muscle nerve was stimulated by supramaximal square pulses (voltage higher than the required for maximum response) with a frequency of 0.1 Hz and a pulse duration of 0.2 ms to produce single contraction impulses. Afterward, the isotonic contractile responses were recorded and stored by a transducer and a physiograph.[12] After tissue placement under experimental conditions to achieve uniform conditions, the equalization of impulse height, and the stabilization of muscle condition, a 15-30-minute recording of contractile impulses was performed. Then, the pulse height at the last minute was considered as the control to continue the experiment.[13]

Nerve and muscle complexes were exposed to different concentrations of ethanol (100, 200, 300, 400, and 500 mM), and impulses were recorded for at least 60 minutes and evaluated for changes. Then, the groups were compared with each other, and there was no control group.

Statistical Analysis and Data Presentation

Data were expressed as a percentage of impulse height compared to control by two-way analysis of variance in which interventions such as different ethanol concentrations as the between-subjects variable and 5, 10, and 15 to 60 minutes as the within-subjects variables were analyzed. When a two-way analysis of variance showed a significant interaction between time and type, a one-way analysis of variance was performed for each specific time point and, where necessary, a post hoc test (Student Newman-Keuls). All statistical tests were performed using SPSS software (version 20), and *P* values less than 0.05 were considered statistically significant. The number of samples in each case was 6 tissues, and all data were presented as the mean and standard error.

Results

Figure 1 demonstrates the effects of different concentrations of ethanol on the twitch amplitude of the chick neck striatum muscle at different time intervals after exposure. Twitch height was increased in time and dose-dependent manner and the concentration of 500 mM of ethanol after 30 minutes revealed the highest elevation of muscle impulses after electrical neuromuscular stimulation of the chick neck striatum muscle ($P<0.05$). Further, there was no increase in contractile height at any of the tested concentrations after this time.

Discussion

Calcium-dependent stimulation and contraction are the most well-known mechanisms in skeletal muscle

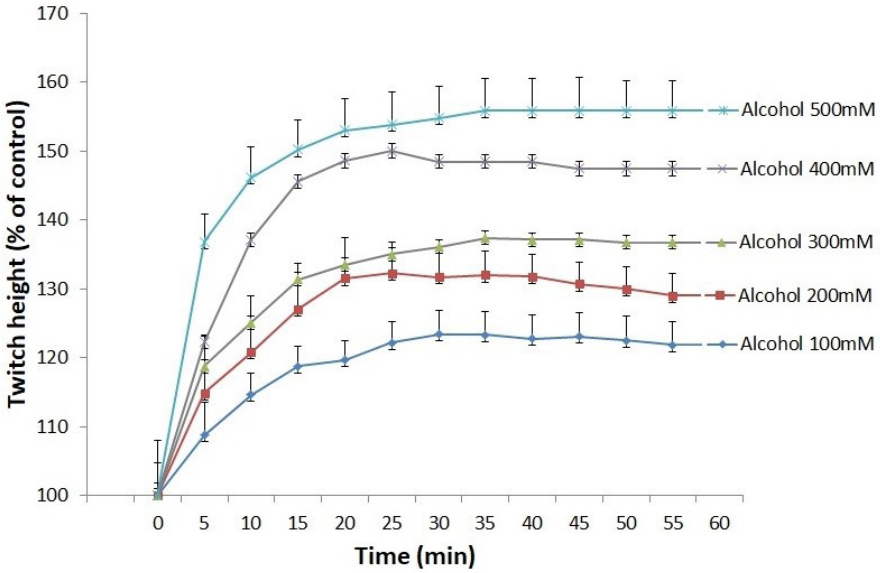

Figure 1. The Time Course Effects of Different Concentrations of Ethanol on Twitch Height. *Note.* Data obtained from the elevation of muscle impulses after electrical neuromuscular stimulation of chick neck striatum muscle are presented as mean ± standard error of twitch height (% of control) after exposure to ethanol (100, 200, 300, 400, and 500 mM). (N = 6) There was no increase in contractile height at any of the tested concentrations after 30 minutes.

contraction, and previous studies on acute exposure to alcohol have shown dose-dependent alcohol-induced interference in the stimulation-contraction couple, particularly by calcium.[14] Muscle contractions and action potentials depend on Ca^{2+}, and since some hormones and drugs are effective on the amount of calcium in and out of the cell, they can affect muscle contractions themselves.[15]

Ethanol itself is a vasomotor suppressant; moreover, its most important metabolite, acetaldehyde, is known as a Ca^{2+}antagonist and vasodilator.[16] Ethanol inhibits Ca^{2+}input current in a time- and voltage-dependent manner, increases calcium-dependent threshold action potentials, and decreases its duration.[17] However, comparing the results of this study with similar investigations is controversial. The evaluation of the effect of ethanol at concentrations of 10 and 100 mM has been found to have similar effects on tetraethyl ammonium, which causes chick neck muscle contraction.[14]

However, such effects on the chick neck muscles were also observed in some anesthetic agents so that, at low concentrations, ethanol increases tetanus impulses and contractions, while at high concentrations, it decreases contractions.[18] In previous studies on different samples, including cardiac myocytes in rats and smooth muscle cells, acute alcohol exposure interfered with dose-dependent contraction-induced stimulation, which was associated with decreased calcium translocation.[19]

The duration of ethanol-attenuating muscle contraction is also important. In one study, ethanol at concentrations of 400-100 mM reduced isometric twitch without a significant effect on the amplitude of the tension stress. These effects were completely reversed after ethanol washing, whereas in our study, the effects of ethanol were not reversible for at least the first few minutes after washing.[20]

In view of the above statements, on the one hand, the effects of ethanol concentration-dependent effects vary on different muscles whether skeletal, smooth, or cardiac. On the other hand, different inhibitory or stimulatory effects on different skeletal muscles were observed in each animal. Furthermore, the type of skeletal muscle is different depending on the type of animal being tested.

What follows is the only statement of observations recorded during a series of experiments, which undoubtedly requires an in-depth investigation of the mechanisms presented in the various studies, the finding of new mechanisms, and their relationship with the researcher's observations. These findings merely act as a guide for interested researchers to consider the effects of solvents such as ethanol apart from the effects of soluble substances on skeletal muscle. Anyway, the potential effects of ethanol on striated muscle contraction are important and should be considered in studies using ethanol as a solvent.

Limitations of the Study

The study was only performed on isolated chick biventer cervices. Further, the investigators did not define a control group, which can be the most important limitation of this study.

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Authors' Contribution

Conceptualization: Mahdi Mashhadi Akbar Boojar.
Data Curation: Gholamreza Poorheidari.
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Funding Acquisition: Gholamreza Poorheidari.
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Supervision: Gholamreza Poorheidari.
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Visualization: Mahdi Mashhadi Akbar Boojar.
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Competing Interests

No conflict or competing financial interests exist.

Ethical Approval

All the safety instructions with laboratory materials and animals (e.g., use of gloves while working, avoiding direct contact with materials, use of masks and hoods when preparing materials, and the like) were implemented before starting work. Moreover, all protocols of the ethics committee of Baqiyatallah University of Medical Sciences were observed in keeping and transporting animals. This study was carried out regarding the ethical recommendations of laboratory animal care at Baqiyatallah University of Medical Sciences and was approved by the ethics committee and supported by the research deputy of Baqiyatallah University of Medical Sciences.

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