Impact of Topiramate on Rat Phrenic Nerve-Hemidiaphragm Preparations

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ABSTRACT

Introduction: Topiramate has a negative modulatory effect on voltage-gated ion channels involved in neuromuscular junction transmission. To investigate the potential impact of topiramate on muscle contraction, phrenic nerve-hemidiaphragm preparations were used as a neuromuscular junction model.

Methods: Phrenic nerve-hemidiaphragm preparations were isolated from rats and were mounted in oxygenated Krebs solution. Preparations were stimulated in the presence of topiramate and phenytoin with a rectangular pulse at 0.1 Hertz, 0.3 milliseconds, and 3 milliseconds of duration, forming indirect, direct single, and tetanic muscle contractions, respectively. The expressed tension was isometrically recorded via a force displacement transducer on a polygraph.

Results: Topiramate and phenytoin directly and indirectly reduced contractions in a time-dependent manner. By contrast, topiramate, but not phenytoin, showed an excitatory effect on contraction in tetanic potentiation.

Conclusion: To our knowledge, our study is the first to show the effects of topiramate on muscle contraction and neuromuscular junction transmission. Topiramate needs to be used with caution in patients with muscle weakness and respiratory problems.

Keywords: Topiramate, phenytoin, hemidiaphragm, neuromuscular junction, tetanic stimulation

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INTRODUCTION

The impact of antiepileptic medications on the central nervous system has been extensively studied; in contrast, their impact on the peripheral nervous system is less well understood. Many antiepileptics such as phenytoin have well-known adverse effects on neuromuscular junction (NMJ) functions and are thus avoided in patients with myasthenia gravis (1). Topiramate is a structurally novel broad-spectrum anticonvulsant that is known to have a negative modulatory effect on AMPA/kainate subtypes of glutamate receptors (2). Additionally, similar to phenytoin, topiramate might also interfere with voltage-gated ion channels that are abundantly found in the NMJ (3,4). Topiramate is used for several disorders including headaches, essential tremors, and psychiatric diseases; therefore, its side effects are of particular interest (5). Nevertheless, no evidence has yet been documented regarding the role of topiramate on NMJ functions. To investigate the potential impact of topiramate on muscle contraction, phrenic nerve-hemidiaphragm preparations were used as an NMJ model and the effects of topiramate and phenytoin (as a control) on the muscle twitch response to electrical stimulation were comparatively evaluated.

METHODS

Male Wistar Albino rats (180-200 g, 6-9 weeks old) were used as the source of phrenic nerve-hemidiaphragm preparations (n=10 for topiramate and n=10 for phenytoin). This study was approved by the Animal Ethics Committee of the institution. Following cervical dislocation, the hemidiaphragm was dissected with the phrenic nerve. Each preparation was mounted in a tissue bath containing oxygenated Krebs solution with 95% O₂ and 5% CO₂ (133 mM NaCl, 4.9 mM KCl, 1.8 mM CaCl₂, 11.9 mM NaHCO₃, 0.7 mM NaH₂PO₄, 11 mM glucose, pH 7.4), pre-treated with 350 µM phenytoin (Sigma-Aldrich, St. Louis, MO) or 250 µM topiramate (Sigma-Aldrich), and was maintained at 37°C. Optimal phenytoin and topiramate concentrations were determined after testing a range of concentrations in preliminary studies.

The preparation was attached to a Grass FT03 force displacement transducer (Grass Technologies; West Warwick, RI), was pre-tensioned to 2 g, and was allowed to stabilize for 30 min in the bath. The phrenic nerve was stimulated with 0.1-Hz square wave impulses of 0.3 ms (indirect single contraction) or 0.1-Hz square wave impulses of 3 ms (direct single contraction) every 10 s for 20 min using a stimulator (Grass S88, Grass Technologies; West Warwick, RI). Muscle contractions were recorded (at baseline and at 2.5, 5, 10, 15, and 20 min) by a Grass model 7400 physiological recorder and digitized using a data acquisition software (PolyView v2.5; Astro-Med, West Warwick, RI). For tetanic responses, the preparations were indirectly stimulated with 50-Hz square wave impulses of 3 ms and

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contractions were recorded at baseline and at every 5 min using the same apparatus.

Statistical Analysis

In the direct and indirect isometric contraction studies, percentage changes at each time point from the baseline value were compared using analysis of variance (ANOVA) and post-hoc Tukey's test. In tetanic contraction studies, values obtained at baseline and at 20 min were compared using Student's t-test. A p-value of <0.05 was considered to be statistically significant.

Figure 1. a, b. Phenytoin (a) and topiramate (b) administration reduces hemidiaphragm muscle contractions in a time-dependent manner following both direct and indirect stimulations. Statistical comparisons were made between contraction changes obtained at each time point and at baseline

*p<0.05, **p<0.01, and ***p<0.001. Vertical bars indicate standard errors

Figure 2. Impact of phenytoin and topiramate administration on tetanic muscle contraction. Statistical comparisons were made between contraction values obtained at 20 minutes and at baseline

*p<0.05 and **p<0.01. Vertical bars indicate standard errors

RESULTS

Phenytoin administration reduced muscle contractions in a time-dependent manner by both direct and indirect stimulations. Statistically significant differences from the baseline value started at 10 and 15 min post stimulation by direct and indirect stimulations, respectively. Topiramate administration also reduced contractions in a time-dependent manner, with statistically significant differences starting at 5 min post stimulation by both direct and indirect stimulations (Figure 1). Phenytoin induced a statistically significant suppression in contraction by tetanic potentiation. By contrast, topiramate showed an excitatory effect on contraction in tetanic potentiation (Figure 2).

DISCUSSION

Phenytoin has previously been shown to cause delayed recovery of neuromuscular blockade following general anesthesia in humans and rats (6,7,8). Further, in experimental animal studies, phenytoin has adversely affected neuromuscular transmission and post-tetanic potentiation (9,10). Our results are therefore in agreement with previous studies and further corroborate that phenytoin has a significant inhibitory effect on NMJ functions. Moreover, phenytoin is known to reduce peripheral nerve excitability, and this action might have potentiated its inhibitory effects on the peripheral nerve in hemidiaphragm preparations (4).

To our knowledge, we are the first to show that topiramate also induces neuromuscular blockade in a similar manner to phenytoin. Both drugs inhibit NMJ transmission presumably through the blockade of voltage-gated sodium channels, which is their main mechanism of action (3). However, in contrast to phenytoin, topiramate appears to enhance contraction by tetanic potentiation. Topiramate might exert this effect through the modulation of potassium currents; however, other mechanisms of action cannot be ruled out (11). Exact mechanisms by which topiramate influences NMJ functions need to be identified.

There are only a few reports focused on the impact of topiramate on locomotor activity and motor functions, and one such study has found reduced muscle strength in mice administered with topiramate (12). This action might, at least partially, be due to the neuromuscular transmission blocking effect of topiramate. Although topiramate is not listed among drugs to avoid in muscle disorders, our results suggest that it should be used with caution in NMJ disorders such as myasthenia gravis and in patients with respiratory problems.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of İstanbul University.

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