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Original Article

Effects of *Buthus eupeus* Venom on Neuromuscular Transmission on Striated Muscle In Vitro

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Abstract

In this study, effects of *Buthus eupeus* venom on chick biventer cervices nerve-muscle preparation were investigated by twitch tension method. The venom, at 1.3 µg/ml, increased contractile responses in indirect stimulations. These effects were milder in direct muscle stimulations. It also caused significant enhancement in postjunctional sensitivity as assessed by responses to exogenous acetylcholine, carbacole and potassium chloride. These results indicate that *B. eupeus* venom can cause irreversible facilitation of neuromuscular transmission by affecting postjunctional excitability in both nicotinic receptors and ion channels of the muscle cells membrane.

Keywords: *Buthus eupeus*; Neuromuscular transmission; Skeletal muscle; Venom.

Introduction

Nowadays, constituents present in the venoms are considered of high value in chemical and pharmacological researches. Iran mainly consists of tropical and subtropical regions and envenomation by scorpion is common in such climates. Scorpions as venomous insects are divided into two groups: *Buthidae* and *Chactoids*. *Buthidae* family includes 40% of known scorpions' species. All human hazardous scorpions belong to the *Buthidae* family (1). *Buthus eupeus* is an Asian scorpion that is found in all tropical, temperate and cold regions of Iran. Thus, envenoming by the scorpion may be scattered all over the country (2). Scorpion venoms contain toxins that can affect channels and synaptic transmission (3, 4). Many studies on the effects of *B. eupeus* venom on ion channels indicate that the venom causes prolonged opening of Na^+ channels (5, 6) and also reversible inhibition of M-type K^+ current (7). However, there is no clarity about postsynaptic or presynaptic activity of the venom. Unclear effects of *B. eupeus* venom on neuromuscular transmission, which may be contributed to some paralytic activities (8), prompted us to determine the effects of the crude venom on neuromuscular junction and to answer whether it acts prejunctionally or postjunctionally. To this end, the effects of the venom on chick biventer cervices (CBC) neuromuscular preparation were detected by using twitch tension method.

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Experimental

Preparation of biventer cervices muscle

Biventer cervices nerve-muscle preparations were isolated from 32 chicks being 3-14 days old. They were sacrificed by ether inhalation

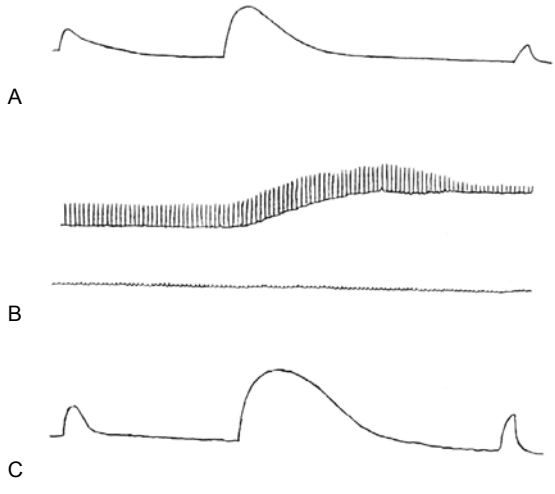

Figure 1. Effect of the venom of *B. eupeus* venom (10 µg/ml) on CBC in vitro preparation: A) contractile responses to exogenous Ach, Cch and KCl before the venom addition, B) indirect electrical stimulation before and after the venom addition, C) contractile responses to exogenous Ach, Cch and KCl after addition of the venom.

and mounted with a resting tension of 0.5-1 g force in 10 ml tissue baths containing tyrode physiological salt solution consisting of KCl, $CaCl_2$, $MgSO_4$, $NaHCO_3$, NaCl and glucose in concentration (mM) of 2.6, 1.7, 0.86, 4.79, 136 and 0.55, respectively. The solution was maintained at 32°C and bubbled with 95% O_2 at pH 7.3.

Chick biventer cervices (CBC) muscle was used because it can be set up in small tissue bath (≤5 ml). It is also robust to toxins, which are often available in small quantities and slow in action. CBC muscle contains both focally innervated twitch fibers and multiply innervated contracture producing fibers. Thus, it can be stimulated by exogenously applied cholinomimetic agonists, as well as by the motor nerve stimulation (9).

Indirect CBC stimulation

Muscular twitches were induced by motor nerve stimulation, using electrical pulses of 0.2 ms duration and a minimal voltage sufficient for producing maximal twitch height. In the absence of nerve stimulation, were recorded prior to the addition of the venom (as control) and also after the venom addition (as test group). Carbachol, potassium chloride and acetylcholine were allowed to bathe the preparations for 30, 30 and 15 s, respectively. Preparations were then washed by overflow of physiological salt at 5-10 ml/s for 15 s. After recording control responses for 15 min, the venom was added to the bath to make concentrations of 1, 3 and 10 µg/ml, the effects in the presence of nerve terminal stimulation or the drugs were observed in the same manner done previously.

Direct CBC stimulation

Neuromuscular transmission was blocked by tubocurarine (1 µg/ml), and then stimulator electrode was moved into contact with the belly of the muscle. Direct electrical stimulations were done at 0.1 Hz with pulses of 2-ms duration and the minimal voltage necessary for producing maximal twitch height. In the absence of direct muscle electrical stimulations, the CBC muscle was exposed to KCl as done before. After washing the bath, the venom was added at concentrations of 1, 3 and 10 µg/ml and its effects on CBC responses were recorded in the presence of direct muscle stimulation in the absence of electrical stimulate in response to KCl.

Twitches and contractures were recorded isometrically on Narco-biosystem polygraphs using F60 force displacement transducer.

The twitch tension measurements were repeated four times and the results were expressed as the mean±standard error of mean (SEM). Differences between groups or treatments were assessed using student t test, with $p<0.05$ indicating significance. Comparing among more than two was performed using ANOVA test.

Results

Indirect CBC stimulation

Buthus eupeus venom, tested on indirectly stimulated preparations, caused a transient augmentation in twitches followed by a contracture, and then increasing in resting tension that coincided with the reduction of twitch height until complete irreversible suppression (Figure 1).

At 1, 3 and 10 µg/ml concentrations of the venom, the maximal twitch augmentations were 129% ±7.3%, 123.4% ±18.3% and

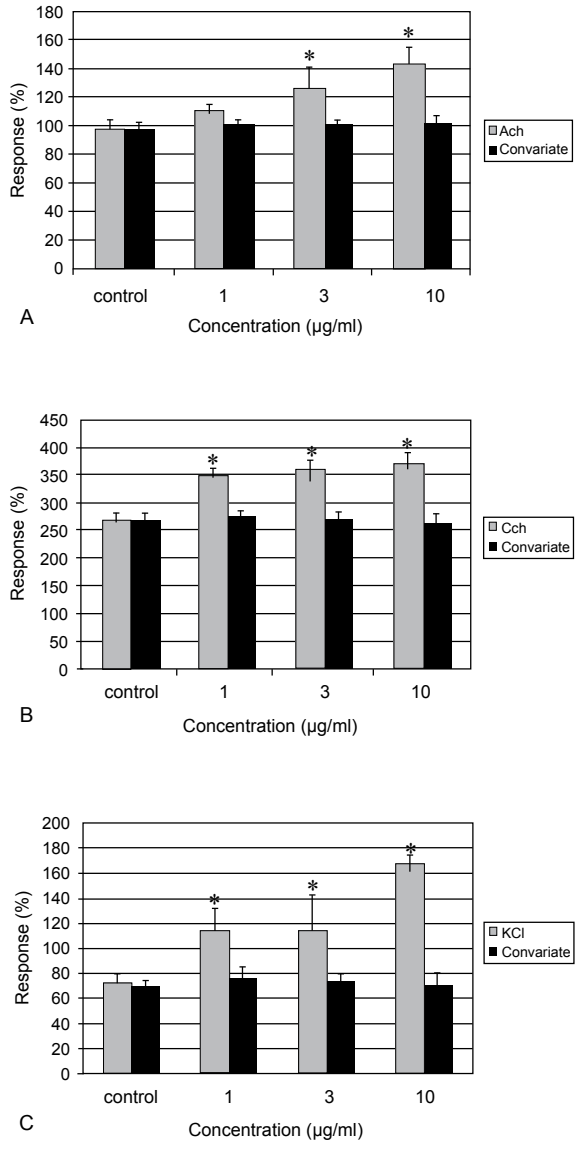

Figure 2. Effects of *B. eupeus* venom (1, 3 & 10 µg/ml) on contractures caused by: A) Ach, B) KCl, C) Cch. Every column represents mean±SD (n=4). The differences with $p<0.005$ are significant.

115.9%±6.1% of control twitch height, respectively. The same values for recorded maximal contractures were 77.5%±28.6%, 148.5%±31.9% and 198.15±14.36% of control twitch height.

Buthus eupeus venom enhanced postjunctinal sensitivity significantly, as assessed by contractures to exogenously applied acetylcholine, carbachol and KCl (Figures 1 and 2).

After suppressing the twitches derived by indirect stimulations by d-tubocurarine, *B. eupeus* venom at 3 µg/ml caused significant maximum contracture as 124.3%±21.5% of control twitch height, which was irreversible by intermittent washing.

Direct CBC stimulation

Effects of the venom (1.3 µg/ml) on twitches and contractures observed in the presence of d-tubocurarine in direct CBC stimulations were similar to indirect stimulation test but with less changes, indicating a milder effect (Figure 3).

Discussion

Envenomation by *B. eupeus* could be threatening because of its wide distribution across the country. Considering the strength and spread of stings by the scorpion, it is critical to determine the mechanisms by which the venom may exhibit some hazardous effects such as paralysis. The present study confirms that the crude venom enhances neuromuscular transmission mainly postjunctionally. In 1980, Orlov et al. investigated some of *B. eupeus* venom effects on autonomic system neurotransmitters (10). The catecholamine content in rat plasma found to be increased, indicating an enhancement in postganglionic nerve mediators (acetylcholine and noradrenalin) release by the venom. However, there was no evidence in the case of somatic nerve fibers. Later, they revealed the generation of cAMP and cGMP in mouse brain and also in isolated guinea-pig heart (11) and then ascertained the relationship between the increased levels of cyclic nucleotides and catecholaminergic and cholinergic effects of the toxin (12).

The present results revealed some of the underlying mechanisms by which the venom facilitates neuromuscular transmission; because of increasing contractile responses to exogenous acetylcholine and carbachol (not metabolized by acetylcholine esterase) nicotinic receptors' sensitivity is suggested to be enhanced to exogenous agonists. This assumption accounts for the twitches and contracture augmentations; however, increasing acetylcholine release from nerve terminals could not be ruled out by the data. With respect to the preparations

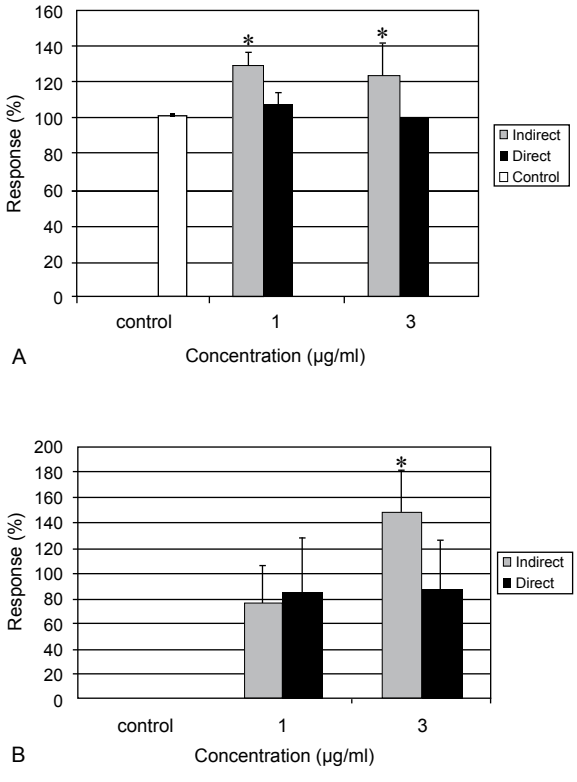

Figure 3. Comparison of the effects of the venom of *B. eupeus* (1 & 3 µg/ml) between direct and indirect CBC stimulations: A) maximum twitch augmentation, B) maximum contracture.

directly stimulated, milder effects of the venom emphasized effects on nicotinic receptors, although the significance of the detected effects probably implicates more over mechanisms i.e, enhancing postjunctional excitability in ion channels other than nicotinic receptors that was also confirmed by increased contractility in both experiments with or without electrical muscle stimulation. *Buthus eupeus* venom may also exert a similar mechanism to the venom from *B. martensi* on contractility of skeletal muscle, which has been shown to stimulate Ca^{++} release channel activity in ryanodine receptors (13). However, this mechanism is yet to be established.

Briefly, postsynaptic effects of the venom could prepare an appropriate target for medicinal approaches against intoxication, in concern with the irreversibility of these effects.

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