Iranian Journal of Pharmaceutical Research (2006) 2: 101-105
Received: January 2005
Accepted: October 2005

Original Article

The Pharmacological Effects of *Odonthobuthus doriae* Scorpion Venom and Its Extracted Fractions on Neuro-Muscular Transmission

Amir Jalali[a], Zahra Hossininasab[b], Zynab Ehsani[b], Amir-Abbas Zare[c], Afshin Zarghi[d] and Hossein Vatanpour[b]*

[a]*Dept. of Toxicology and Pharmacology, Faculty of Pharmacy, Jundishapour University of Medical Sciences, Ahwaz, Iran.* [b]*Dept. of Toxicology and Pharmacology, Faculty of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, Iran.* [c]*Razi Vaccine and Serum Research Institute, Karaj, Iran.* [d]*Dept. of Pharmaceutical chemistry, Faculty of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, Iran.*

Abstract

The effect of *Odonthobuthos doriae* (O.d) scorpion venom at 0/3, 1 and 3, 10 µg/ml concentrations were investigated on nerve-muscle transmission, using the Twitch tension technique. A concentration of 0.3 µg/ml caused a small change in the twitch height in response to indirect muscle stimulation, but higher concentrations (1, 3, 10 µg/ml) caused a transient augmentation in twitch response followed by a large contracture in the chick biventer cervices (CBC) preparation. This effect could be defined as a complex action of the venom, predominately presynaptic, in which its' effects on postjunctional synapses is also maintained.

In order to find out which bioactive fraction could explain the venom effects, the soluble crude venom was partially separated by the gel filtration method, using a Sephadex G_{50} column, and four fractions were separated. Two of the four purified fractions (O.d F_3, O.d F_4) were characterized as toxic and their LD_{50} values were lower than the crude venom. Unlike the O.d F_1 and O.d F_2 fractions, O.d F_3 and O.d F_4 fractions caused a significant block in the twitch and contracture, in comparison to the control sample . in conclusion, fractions O.d F_3 and O.d F_4 are supposed to be as the biological active components of the O.d. venom.

Keywords: Scorpion venom; *Odonthobuthus doriae*; Pharmacological responses; Neuro-muscular.

Introduction

Scorpion toxins have had instrumental effects in defining both structural and mechanistic properties of ion channels and various conductances normally present in cells involved in its blocking activity (1).

Up to now, more than 1500 genus of scorpions are known word wide, and the most hazardous scorpions to human are those belonging to the *Buthidae* family. *Odonthobuthus* belongs to the *Buthidae* family and *Buthus* genus which has two species, *doriae* and *odonthurus*. This scorpion has an abundant geographical distribution in Iran. Its' sting can cause various effects ranging from pain, inflammation, and necrosis at the site of sting, to muscle paralysis, hematuria and occasionally death in children.

Pharmacological characterizations of this

* Corresponding author:
E-mail: vatanpour@hotmail.com

venom, as well as its' sub-fractions has not yet been reported. In this study, the *in vitro* pharmacological effects of the venom and its' toxic fractions extracted by gel filtration have been investigated on the neuromuscular transmission, using the Chick Biventer Crevices (CBC) preparation.

Experimental

Materials

Crude scorpion venom was gifted by Dr. Zare and Dr. Akbary, Department of Poisonous animal, Vaccine and Serum Research Institute of Razi, Karaj, Iran. Crude venom was obtained by electrical stimulating telson of scorpion and stored after freeze-drying at –50°C, until use.

Krebs physiological solution salts, CO_2/O_2 cylinder, HEPES physiological buffer, O_2 cylinder and other materials were also used.

Methods

Twitch tension recording

Twitch tension recording experiments were performed on the isolated CBC nerve-muscle preparation. This model is suitable for studying the nerve-muscle transmission effects of drugs or venom. It is small enough to be mounted within a 2-5 ml tissue organ bath and retain its' normal function *in vitro*, over a few hours.

Chick biventer cervices preparations

Biventer cervices muscles and associated nerves were dissected from 7-10 days old chicks, sacrificed by exposure to ether as described by Ginsborg and Warriner (2). Two muscles were mounted, with a resting tension of approximately 1 g; within a 5 ml tissue bath containing modified physiological solution (mM) as follows: 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. Twitches were evoked by stimulating the motor nerve at 0.1 Hz with pulses of 0.2 ms duration and a voltage greater than that which produced a maximal response, using ring electrodes and a Grass S88 B stimulator. To detect any changes in postsynaptic sensitivity, responses to sub-maximal concentrations of exogenously applied acetylcholine (1-2 mM), carbachol (30-40 μM) and KCl (20-40 mM) were recorded in the absence of nerve stimulation, prior to the addition of venom and at the end of the experiment. The preparations were exposed to acetylcholine and KCl for 30 sec, and carbachol for 1 min (3). After the wash-out stage of these drugs, the preparations were allowed to stabilize for 15-20 min before the addition of the venom.

Twitches and contractures were recorded isometrically, using the Washington Grass model 79B and Grass model 79 polygraphs and SRI or Grass FT03 force transducers.

Purification Procedure

Fractioning of the soluble venom was accomplished using a Sephadex G_{50} column (3.5×230 cm, 1 ml/min flow) equilibrated and eluted with a pH 8.3 0.1 M ammonium acetate buffer, as previously described (4). Protein content was estimated spectrophotometrically at 215, 260 and 280 nm. All the procedures were carried out at room temperature.

Lethality tests and LD_{50} determination in mice

The mice (white, of both sexes, weighing 20 g) used in this research were treated in compliance with the US Public Health Service policy on human care and the use of animals. The mouse lethality of various protein fractions was observed after i.v. injections to mice (5). The Reed and Muench method, which provides a moderately accurate estimate of the LD_{50} based upon data smoothened by counting cumulative number of survivals, was used (6). Two designations of "toxic" and "nontoxic" were used to express venom fractions. A fraction or peptide was considered non-toxic, if during the first 24 h of its injection the mouse lived or showed no more toxicity signs (heperexcitability, lachrymation, apnea, partial paralysis or respiratory failure). Toxic fraction means a fraction that immediately caused death of the test animal. The control group received an injection of normal saline at pH 7.4**.**

Data analysis was performed using the one-way (repeated) analysis of variance (ANOVA) for multiple comparison and student's t-ttest for comparing two set of data as Mean ± SEM ($p<0.05$).

Results and Discussion

Effects of the venom on chick biventer cervices preparations

Figure 1 shows that the twitch responses of CBC evoked by nerve stimulation were reduced in the presence of *Odonthobuthus* scorpion venom (0.3-10 µg/ml), in a time-dependent manner. In contrast to other concentrations, the effect of 10 µg/ml venom was extremly strong and without any transient increase in the twitch height. It blocked muscle twitching within 13 min. As could be seen in figure 1, there was a direct relation ship between concentration and the maximum contracture effect.

With 3 and 10 µg/ml concentrations, the effect of venom was much faster and stronger in the indirectly stimulated preparation than the direct preparation (Figure 2).

Effects of the venom on acetylcholine, carbachol and potassium chloride responses, without electrical stimulation

Figure 3 shows a significant ($P<0.05$) decrease of Acetylcholine (1-2 mM) for 30 sec, Carbachol (30-40 µM) for 1 min and KCl (30-40 mM) for 30 sec CBC contracture responses, after exposure to 1, 3, and 10 µg/ml concentrations of the venom. Meanwhile, the responses to a concentration of 0.3 µg/ml of venom in acetylcholine and carbachol was not significant. As shown in Figure 3, the decreasing pattern of acetylcholine and potassium chloride is similar at different concentrations of venom.

The effect of O.d F_3 fraction on twitch height in indirect electrical stimulation

Figure 4 shows a gradually significant reduction in the twitch height responses of CBC preparations stimulated indirectly at different concentrations of 1, 3, and 10 µg/ml of O.d F_3, which was followed by complete blockage.

The effect of O.d F_4 fraction on twitch height in indirect electrical stimulation

Figure 5 shows a gradually significant reduction in twitch height responses of CBC preparations stimulated indirectly at different concentrations of 1, 3, and 10 µg/ml of O.d F_4,

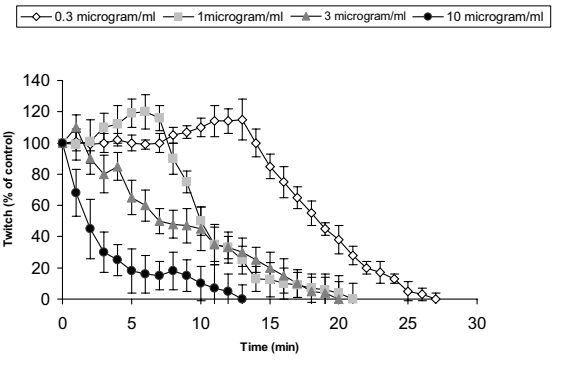

Figure 1. The effect of Odonthobuthus scorpion venom at 0.3 µg/ml ; 1 µg/ml ; 3 µg/ml ; 10 µg/ml concentrations, in response to indirectl stimulation of CBC preparations. Each point represents the maximum response for that concentration (mean ± S E M; n=4).

in comparison to the control, leading to muscle paralysis at 1 and 10 µg/ml concentrations. A complete blockage at 1 and 10 µg/ml concentrations was observed after 21 and 18 min, respectively.

Skeletal muscle paralysis could occur due to either a presynaptic action via blockade/ acceleration of acetylcholine release, or to a postsynaptic effect through blockage of acetylcholine receptors or direct muscle damage. In this study, experiments were performed by the indirect, direct and without electrical stimulation of CBC preparation, in order to investigate the pre- or post-synaptic effects of the O.d venom and its' toxic fractions.

According to the data obtained, it seems that the effects of the venbom are mostly mediated through presynaptic effects on

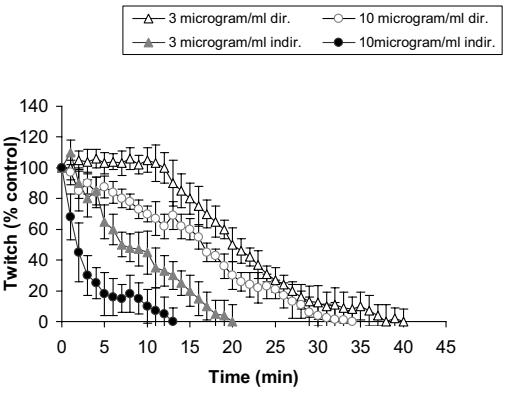

Figure 2. Comparison of the venom at 0.3 µg/ml and 1 µg/ml concentrations, in response to directly and indirectly stimulated CBC preparations. Each point represents the maximum response for that concentration (mean ± SEM; n=4); directly (dir.) ; indirectly (indir.).

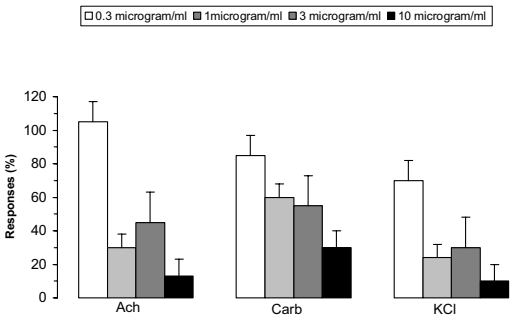

Figure 3. The effect of Odonthobuthus scorpion venom at 0.3 µg/ml ; 1 µg/ml ; 3 µg/ml and 10 µg/ml concentrations in response to exogenous Acetylcholine (Ach.) (1-2 mM) for 30 sec, Carbachol (Carb.) (30-40 µM.) for 1 min and Potassium chloride (KCl) (20-40 mM) for 30 sec in the CBC preparation. Each column represents the maximum response for that concentration (Mean ± SEM; n=4).

nerve terminals. Our results are in agreement with the *Pandinus imperator* scorpion effects on CBC preparation (7). Furthermore, the same effects were noted earlier with *Pandinus exitialis* and *Tityus serrulatus* scorpion venoms on mouse hemi-diaphragm preparation (8, 9).

At higher concentrations, the inhibitory responses of CBC to Ach and Carb were boosted. This effect may be due to the blockage of neuro-muscular transmission, arising from the effects of the venom on nicotinic receptors of the postsynaptic membrane (10). The inhibitory effects of venom in response to Ach and Carb, suggest the curar–mimetic effects of venom. However, the contracture response observed as a result of direct stimulation, after the addition of d-Tubocurarine, can rule out this suggestion (11). Further more, the significant reduction in

Table 1. Comparison of the LD_{50} values of the crude, dialysed venom, as well as and its' isolated fractions, by Reed and Muench method (n=3).

Fraction	LD_{50} µg/ per mouse
Crude venom	13.98
Dialysed venom	12.5
O.d F_1	Non-toxic till 40 µg/ per mouse
O.d F_2	Non-toxic till 70 µg/ per mouse
O.d F_3	6.6
O.d F_4	5.8

response to KCl at a venom concentration of 0.3 µg/ml, contributes to the direct effect of venom on muscle (12).

The influence of 1, 3 and 10 µg/ml concentrations of two non-toxic fractions, O.d F_1 and O.d F_2, were also studied on CBC preparations (table 1). There were no significant pharmacological changes in response to indirect stimulation. In contrast, O.d F3 and O.d F4 fractions result in significant changes in twitch height and yield in a complete blockage. Comparison of different concentrations of O.d F3 and Od F4, show a similar potency in paralysis of twitch tension responses (64% and 63%, respectively).

Finally, according to our obtained data, O.d venom acts mostly prejunctionally to inhibit the neuromuscular transmission. Further studies to find out which bioactive molecule is responsible for the venom action, showed that O.d F_3 and O.d F_4 fractions, which have a similar potency, are the active components of this venom. These findings may warrant research into the development of purification and extraction of a specific pharmacological agent against channels, from the *Odonthobuthus* venom.

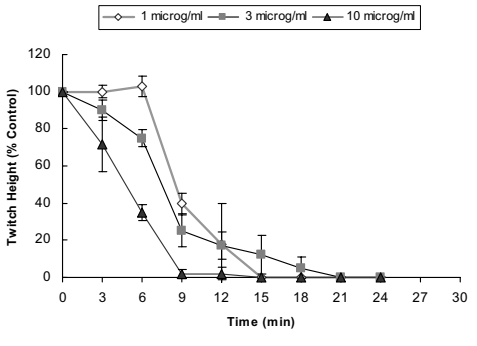

Figure 4. The effect of O.d F_3 fraction of Odonthobuthus scorpion venom at 1 µg/ml ; 3 µg/ml ; 10 µg/ml concentrations, in response to indirect stimulation of CBC preparations. Each point represents the maximum response for that concentration (Mean ± SEM; n=4).

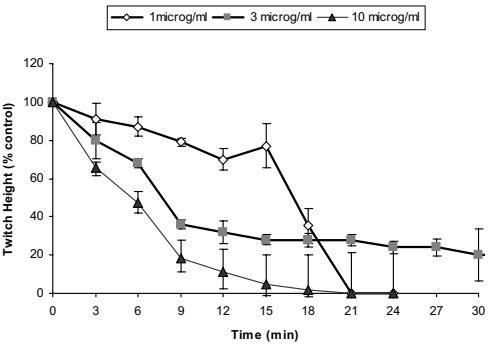

Figure 5. The effect of O.d F_4 fraction of Odonthobuthus scorpion venom at 1 µg/ml ; 3 µg/ml ; 10 µg/ml concentrations, in response to indirect stimulation of CBC preparations. Each point represents the maximum response for that concentration (Mean ± SEM; n=4).

Refrences

(1) Nieto RA, Gurrola GB, Vaca L and Possani, DL. Noxiustoxin2, a novel K^+ blocking peptide from the venom of the scorpion *Centruroides noxius* Hoffmann. *Toxicon* (1996) 34: 913-922

(2) Ginsborg. Warriner. The isolated Biventer Cervices nerve-muscle preparation. *Br. J. Pharmacol*. (1960) 15: 410-11

(3) Crachi TM, Hammer LW and Hodgson WC. The effects on the *in vitro* neurotoxicity of venoms from the taipans *Oxyuranus scutellatus*, *Oxyuranus microlepidotus* and *Oxyuranus scutellatus* canni. *Toxicon* (1999) 37: 1771-1778

(4) DSuze G, Sevcik C and Romas M. Presence of curarizing polypeptides and a pancreatititis–inducing fraction without muscarinic effects in the venom of the Venezuelan scorpion *Tityus discrepans* (Karsch). *Toxicon* (1995) 33: 333-345

(5) Dsuze G, Crona F, Possani LD and Sevcik C. High performance liquid chromatography purification and amino acid sequence of toxins from the muscarinic fraction of *Tityus discreans* scorpion venom. *Toxicon* (1996) 34: 591-598

(6) Reed L and Muench H. A simple method of estimating fifty percent in eukaryotic end point. *Am. J. Hyg.* (1938) 27: 493-497

(7) Mashall DL and Harvey AL. Block of potassium channels and facilitation of acetylcholine release at the neuromuscular junction by the venom of the scorpion *Pandinus imperator*. *Toxicon* (1989) 27: 493-8

(8) Ismail M, Osman OH, Gumaa KA and Karrar MA. Some pharmacological studies with scorpion *Pandinus exitialis* venom. *Toxicon* (1974) 12: 75-82

(9) Oliveira MJ, Fontana MD, Giglio JR, Sampaio SV, Corrado AP and Prado WA. Effects of the venom of the brazilian scorpion *Tityus serrulatus* and two of its fractions on the isolated diaphragm of the rat. *Gen. Pharmac.* (1989) 20: 205-10

(10) Rowan EG, Harvey AL, Takasaki C and Tamiya N. Neuromuscular effects of three phospholipase A_2 from the venom of the Australian king brown snake *Pseudechis australis*. *Toxicon* (1989) 27: 551- 60

(11) Goularte FC, Cruz-Hofing MA, Cogo JC, Gutierrez JM and Rodrigues-Simioni L. The ability of specific antivenom and low temperature to inhibit the myotoxicity and neuromuscular block induced by *Micrurus nigrocinctus* venom. *Toxicon* (1995) 33: 679-89

(12) Bowman WC and Rand MJ. Striated muscle and neuromuscular transmission. In: *Text Book of Pharmacology.* 2nd ed. Black Well Scientific Publications, Great Britain (1988) 1-40

This article is available online at http://www.ijpr-online.com

Give us feed-back
Visit http://www.ijpr-online.com