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Positive inotropic effects of *Tityus cambridgei* and *T. serrulatus* scorpion venoms on skeletal muscle

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ABSTRACT

Toxins that block voltage-dependent K^+ channels and those that modify Na^+ channel gating exhibit positive inotropic effect on skeletal muscle. We compared the effect of the venom of *Tityus cambridgei* (Tc) and *Tityus serrulatus* (Ts) scorpions on mouse diaphragm force, *in vitro*. In indirect and direct (using D-tubocurarine 7.3 μM) stimulation, Tc, 10μg/mL, increased the contractile force, an effect prevented by tetrodotoxin (TTX) while Ts, 0.5 μg/mL, potentiated only indirectly stimulated diaphragm, thus indicating its activity is mainly mediated through acetylcholine release from nerve terminal. This effect is prevented by TTX and attenuated by the K^+ channel opener cromakalim. In conclusion, our data show that while the positive inotropic effect of both venoms appears associated to the activity of Na^+ and K^+ channels, only Tc venom acts also directly on skeletal muscle. This finding call for further studies on Tc venom to identify the toxin responsible for its direct inotropic activity as it may have clinical applications.

1. Introduction

Diaphragm muscle weakness is common in neurological diseases such as muscular dystrophies (*e.g.* Duchenne's muscular dystrophy), Guillain-Barré syndrome, Eaton-Lambert syndrome and myasthenia gravis and it may result in ventilatory failure (Hughes and Bihari, 1994; Polkey et al., 1999).

Striated skeletal muscles, as diaphragm, are highly ordered structures organized for force generation. Similarly to other excitable cells, also in skeletal muscle the signal transmission depends on electrical currents flowing across the cell membrane, then the duration of the action potential influences muscle contractile performance. As a result, longer action potential duration enhances Ca^{2+} release from the sarcoplasmic reticulum and increases muscle contractile force (Leoty and Leaute, 1982; Peachey and Franzini-Armstrong, 1983; Sandow et al., 1965).

Specific voltage-gated K^+ channels blockers can be potentially useful skeletal muscle positive inotropic agents. Indeed, the aminopyridines, which block many types of K^+ channels, prolong action potential duration and thereby have positive inotropic effects on skeletal muscle (Delbono and Kotsias, 1987; Khan and Edman, 1979; Khan and Lemeignan, 1983; Lin-Shiau et al., 1991; Van Lunteren and Moyer, 1996; Van Lunteren et al., 1995a,b). Unfortunately, their toxic effect on the central nervous system is a limiting factor for clinical use (Van Lunteren and Moyer, 1999).

Scorpion toxins specific against K^+ channels block the ions flow by binding to the channel extracellular face (Goldstein and Miller, 1993). As an example, Tityustoxin Kα, from *Tityus serrulatus* (Ts) scorpion venom, specifically blocks voltage-gated K^+ channels and exhibits a positive inotropic effect on rat diaphragm skeletal muscle *in vitro* (Van Lunteren and Moyer, 1999).

Likewise, also toxins able to modify the Na^+ channel gating mechanism can exhibit positive inotropic action. For example, α-toxins bind to site 3 of Na^+ channels in a voltage dependent mode, slowing or blocking the channel inactivation mechanism; while β-toxins bind to site 4 independently of membrane potential and affect Na^+ channels activation (Jover et al., 1980a; Wheeler et al., 1983; Jover et al., 1980b; Catterall, 1980). Unfortunately, however, their toxicity hampers their use in therapeutic protocols.

Scorpions belonging to the genus *Tityus* are found in Trinidad and Tobago and South America, mainly Brazil, Venezuela, Colombia and Argentina (Bücherl, 1971). While the yellow scorpion *T. serrulatus* (Ts) is one of the most dangerous species for humans in Brazil and is responsible for many clinical cases of envenomation in the southern region of this Country, the black scorpion *T. cambridgei* (Tc) is a rare species restricted to the Amazon region (Brasil, 2001).

An *in vitro* study on isolated mouse phrenic nerve–diaphragm showed that Ts venom facilitated the neuromuscular transmission and increased the contractile force evoked by retrograde injection of acetylcholine (Oliveira et al., 1989). Here, for the first time, we analyze the action of Tc venom on diaphragm force using isolated mouse phrenic

Abbreviations: CRO, cromakalim; Tc, *Tityus cambridgei* venom; Ts, *Tityus serrulatus* venom; TTX, tetrodotoxin; SEM, standard error of the mean.

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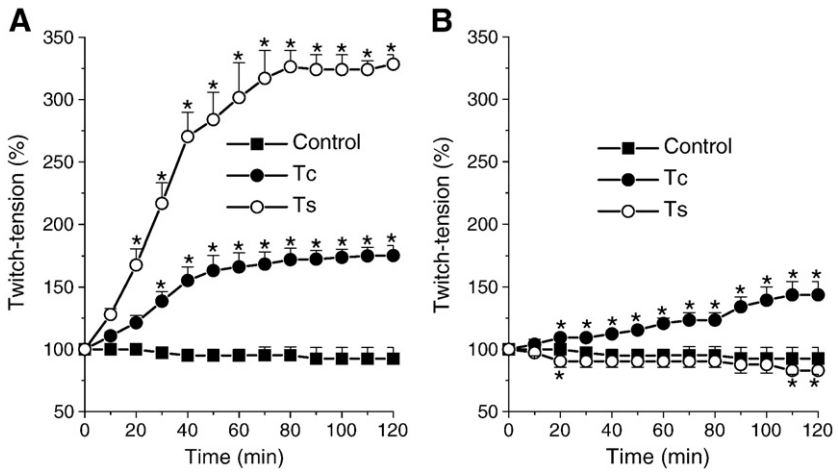

Fig. 1. Effect of the *T. cambridgei* (Tc), 10 μg/mL, and *T. serrulatus* (Ts), 0.5 μg/mL venoms on diaphragm force during indirectly (A) and directly (B) stimulation. The resulting variations in contractile force were compared to control (venom free Tyrode medium) taken as 100%. The muscles stimulated directly (B) were pre-treated with D-tubocurarine. Note that D-tubocurarine prevented the inotropic effect of Ts venom. Each point represents the mean±S.E.M. of 4–6 experiments. The symbol * indicates the result has a significance $p<0.05$ (two-way ANOVA) when compared to control experiments.

nerve–diaphragm and we compare its effect with that of Ts venom. In addition, to highlight the simultaneous or selective involvement of K^+ and Na^+ channels on the effect induced by the venoms, the isolated nerve-muscle has been pre-treated either with the Na^+ channels inhibitor tetrodotoxin (TTX), or with the K^+ channels opener cromakalim (CRO).

2. Materials and methods

2.1. Venom and reagents

Scorpions were captured in Ribeirão Preto (SP, Brazil) and brought to the serpentarium for identification. Scorpions of the species *T. cambridgei* (Tc) and *T. serrulatus* (Ts) were electrically stimulated for venom extraction. For each species, the pooled venoms were dried and kept in a vacuum desiccator. D-tubocurarine chloride was acquired from Abbot Laboratórios do Brasil Ltda (São Paulo, SP, Brazil). 3,4 diaminopyridine, TTX and CRO were purchased from Sigma Chemical Company (St Louis, MO, USA).

2.2. Isolated mouse phrenic nerve–diaphragm

Adult male Swiss white mice (28–35 g) were supplied by the Animal House of the University of Campinas/SP, Brazil. The diaphragm was obtained from mice anaesthetized with chloral hydrate (300mg /kg, i.p.) and sacrificed by exsanguinations. A hemi-diaphragm with its nerve branch (phrenic nerve) was mounted as described (Bülbring, 1946). Briefly, it was immersed in a 5 mL organ bath containing a Tyrode solution (137 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$, 0.49 mM $MgCl_2$, 0.42 mM NaH_2PO_4, 11.0 mM $NaHCO_3$ and 11.1 mM glucose), pH 7.4, 37°C, and continuously aerated with a mixture of oxygen (95%) and carbon dioxide (5%).

2.2.1. Indirect (nerve) electrical stimulation

Phrenic nerve was stimulated by a supra-maximal voltage (0.1 Hz, 0.2 ms) from a bipolar platinum electrode connected to a Grass S4 stimulator (Grass Instruments, Quincy, MA, USA). The resulting isometric muscle twitches were recorded using a force displacement transducer (BG 25 GM, Kulite Semiconductor Products, Inc., Leonia, NJ, USA) coupled to a Gould RS 3400 recorder (Gould Inc., Cleveland, OH, USA). The isolated nerve–muscle was allowed to stabilize for at least 20 min before addition of the venom or of the drug.

2.2.2. Direct (muscle) electrical stimulation

For direct stimulation (50V, 0.1Hz, 2ms) the electrodes were placed in the muscle while the neuromuscular transmission was abolished by the continuous presence of the acetylcholine competitive antagonist 7.3 μM D-tubocurarine. The isometric muscle twitches were recorded as described above.

2.2.3. Isolated nerve-muscle pre-treatment

In order to investigate the possible action of the venom on K^+ channels, the isolated nerve-muscle was pre-treated with cromakalim (CRO), a K^+ channels opener. Alternatively, to highlight the involvement of Na^+ channels, the pre-treatment implied the use of tetrodotoxin (TTX), a Na^+ channels inhibitor.

Table 1
Diaphragm maximum twitch–tension variation measured upon indirect stimulation after 120 min from *T. cambridgei* (Tc) and *T. serrulatus* (Ts) venom addition

	Twitch–tension variation (%)		
	Without pre-treatment	With pre-treatment	
		CRO	TTX
No venom[a]	97.3±3.0	98.0±2.0	97.7±2.3
Tc (10 μg/mL)	175.2±8.1*	135.2±10.8**	99.3±8.1**
Ts (0.5 μg/mL)	328.3±7.6*	268.4±14.8**	124.2±13.2**

Data are the mean±SEM.
[a] Venom free Tyrode medium.
* $p<0.05$ compared with venom free Tyrode medium (two-way ANOVA).
** $p<0.05$ compared with venom alone, without pre-treatment (two-way ANOVA).

Table 2
Diaphragm maximum twitch–tension variation measured upon direct stimulation after 120 min from *T. cambridgei* (Tc) and *T. serrulatus* (Ts) venoms addition

	Twitch–tension variation (%)		
	Without pre-treatment	With pre-treatment	
		CRO	TTX
No venom[a]	98.5±6.9	94.7±2.9	95.5±2.6
Tc (10 μg/mL)	143.7±10.6*	125.3±9.2	89.2±2.1**
Ts (0.5 μg/mL)	97.3±2.7	ND	ND

Data are the mean±SEM.
ND=not determined.
[a] Venom free Tyrode medium.
* $p<0.05$ compared with venom free Tyrode medium (two-way ANOVA).
** $p<0.05$ compared with venom alone, without pre-treatment (two-way ANOVA).