Some effects of the venom of the Chilean spider *Latrodectus mactans* on endogenous ion-currents of *Xenopus laevis* oocytes

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**A B S T R A C T**

A study was made of the effects of the venom of the Chilean spider *Latrodectus mactans* on endogenous ion-currents of *Xenopus laevis* oocytes. 1 µg/ml of the venom made the resting plasma membrane potential more negative in cells voltage-clamped at −60 mV. The effect was potentially due to the closure of one or several conductances that were investigated further. Thus, we determined the effects of the venom on the following endogenous ionic-currents: (a) voltage-activated potassium currents, (b) voltage-activated chloride-currents, and (c) calcium-dependent chloride-currents *(Tour)*. The results suggest that the venom exerts its action mainly on a transient outward potassium-current that is probably mediated by a K⁺ channel homologous to *shaker*. Consistent with the electrophysiological evidence we detected the expression of the mRNA coding for xKv1.1 in the oocytes.

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α-Latrotoxin (LTX) is the main component of the venom of the euroasiatic black widow spider *Latrodectus mactans* [1]. LTX is a potent synaptic modulator that induces the release of neurotransmitters from vertebrate nerve terminals by inhibiting potassium channels and blocking L-type calcium channels [2–4]. In addition, this toxin assembles in multimeric complexes in the plasma membrane forming a cation permeable channel [5].

In contrast to the high concentration of LTX in spider populations from Europe and Asia, this toxin has not been detected in the Chilean *L. mactans*, although the symptoms of patients after spider biting are very similar. The most common symptoms include muscle contraction, vomiting, and cephalgia. The rate of survival of patients attacked by the South American species is higher [6], most probably due to the low concentration or total absence of LTX.

Although *L. mactans* from Chile apparently does not possess LTX in the venom, this contains several oligopeptides which have been shown to induce muscle contraction and inhibit sperm motility; effects that are suspected to be due to the modulation or blocking of ion channels [7,8]. In order to determine the targets for the modulatory actions of the venom, we report here an examination of the effect of whole extracts of the venom of the Chilean *L. mactans* on several native ion currents of *X. laevis* oocytes.

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**Material and methods**

*Spider retrieval.* Female adult *L. mactans* spiders from Chile were captured during the summer months (December 2005 and January 2006) from the area of Alto Bio-Bio in the VIII region (72°16’51”W, 7°45’24”S). The specimens were maintained separate in individual jars for 30 days without food and given only water in order to stimulate the production and concentration of venom in the glands [7].

*Venom retrieval.* The spiders were immersed in liquid nitrogen and after 1 min transferred to a phosphate buffer saline (PBS: NaH₂PO₄ 0.1 M, Na₂HPO₄ 0.01 M, NaCl 1.35 M, pH 7.4) at 4 °C. The glands were removed and placed in a tube containing PBS (25 pairs of glands for 100 µl of PBS) and homogenized. The homogenate was immediately centrifuged at 1000 g for 15 min and the supernatant was subsequently aliquoted and frozen at −20 °C. The total protein concentration was determined by the method of Bradford [9] and dissolved in Ringer to making the final concentration 1 µg/ml in the experimental solutions.

*Oocyte recordings.* Oocytes were collected from several frogs (Nasco), manually dissected from the ovary and treated with collagenase (0.5 mg/ml, 30 min), then kept at 15–16 °C in Barth’s solution supplemented with gentamicin (0.1 mg/ml). Recordings were performed in oocytes superfused with frog Ringer at room temperature (20–25 °C). Membrane currents were recorded from oocytes voltage-clamped at −60 mV and then voltage stepped using four protocols to activate an hyperpolarization-activated current [25], a calcium-dependent chloride-current [10], an outwardly rectifying
chloride current [16] or a transient-outward potassium-current [12]. In addition, serum was perfused onto some oocytes to see any effect on the calcium-dependent chloride-current activated through the lysophosphatidic acid (LPA) receptor [13]. TEA (0.03–

Fig. 1. Currents gated by *L. mactans* venom. (A) Sample records from an oocyte exposed to the spider venom at 1 or 5 μg/ml. (B) Plot of averaged currents generated by 100 or 500 nM (6 oocytes). (C) Currents generated by 20 mV increments in the absence or presence of 1 μg/ml venom gave the I/V shown in (D) (9 oocytes). Data from experiments using 0.01 and 10 μg/ml venom are also shown. *p* < 0.05 vs control values.

Fig. 2. Voltage-and calcium-activated chloride channels. (A) Taur recorded by stepping the voltage form –100 mV to either 0 or +20 mV in the absence or presence of 1 μg/ml of venom. (B) Oscillatory chloride-currents generated by serum (1:1000) were not affected by the venom.
2 mM), 4-AP (0.03–2 mM) or low calcium Riger were perfused onto several oocytes to observe the potential role of potassium-, sodium- and calcium-channels respectively. Moreover, several oocytes were frozen in liquid nitrogen to isolate RNA [14] and RT-PCR using primers designed to amplify xKv 1.1 (GenBank Accession No. M94258) [15].

Data analysis. All the recordings were acquired using a Warner amplifier (OC-765C) and an AC/DC converter (Digidata 1322A, Axon) and stored in winWCP. Files were analyzed and plotted using pClampfit 9 (Axon, molecular devices) and Origin 6.0 respectively.

Results

Activation of a smooth slow-activating ionic-current

Perfusion of the venom generated a slow-activating non-desensitizing current in oocytes held at −60 mV (Fig. 1A). Those currents were clearly evident from 0.01 µg/ml and saturated at about 5 µg/ml; this concentration gave rise to currents that averaged 60 ± 20 nA from 9 oocytes and 3 frogs. The venom gave an EC50 of about 1 µg/ml and therefore this concentration was used for the following experiments (Fig. 1B).

We tried to define the ion responsible for the current by constructing an I/V curve, stepping the membrane voltage of the oocyte from the holding potential (−60 mV) to −100 mV and then to −40 mV in 20 mV steps while in the absence or presence of 0.01, 0.1 or 10 µg/ml of the venom (Fig. 1C). The current reversed direction at a membrane potential of about −20 mV, which is near the equilibrium potential for chloride in oocytes; furthermore this reversal potential was independent of the venom concentration at all tested concentrations (Fig. 1D). This result was contrary to a previous report [8] suggesting that the venom modulates mainly voltage dependent calcium-channels. Therefore we decided to investigate further other venom effects on endogenous currents.

Calcium-dependent chloride-currents

Frog oocytes normally possess chloride-channels that are activated by either calcium ions permeating through voltage-operated channels (Tout) [11] or calcium released from intracellular stores due to the activation of G-protein coupled receptors such as the LPA receptor that is effectively activated with serum [13].

Perfusion of 1 µg/ml of the venom extract in 9 oocytes and 3 frogs, did not produce any evident effect on the Tout current (962 ± 240 vs 820 ± 216 nA Fig. 2A), thus discounting its ability to block either voltage-dependent calcium-channels or the calcium-dependent chloride-current. In addition, the oscillatory chloride currents elicited by activation of the LPA receptor did not show any indication of being affected by the venom (Fig. 2B).

Voltage-dependent chloride-currents

Two main conductances carried out by chloride ions are known in the oocyte: (1) an outward rectifying current, probably due to the expression of ClC5 [16] and an hyperpolarizing inward current at a membrane potential of about −20 mV, which is near the equilibrium potential for chloride in oocytes; furthermore this reversal potential was independent of the venom concentration at all tested concentrations (Fig. 1D). This result was contrary to a previous report [8] suggesting that the venom modulates mainly voltage dependent calcium-channels. Therefore we decided to investigate further other venom effects on endogenous currents.

![Fig. 3](image-url) Voltage-activated chloride-currents. (A) Sample traces of the outwardly rectifying chloride current. (B) This conductance was not affected by the venom, and was also evidenced by substituting Cl\(^-\) with I\(^-\). (C) Hyperpolarizing currents generated by stepping the oocyte membrane potential from −60 to −140 mV were potentiated by 1 µg/ml of venom with an EC50 of 0.3 µg/ml (D). (E) The potentiation was evident at all tested membrane potentials as shown in I/V curves. *p < 0.05 vs control values.
whose molecular identity is unknown and is a mixture of anionic and cationic conductances [25].

Fig. 3A shows that up to 1 µg/ml the venom did not have an evident effect on the outwardly-rectifying chloride-current. To see more clearly this current, chloride was substituted by iodide in the bathing solution. This did not alter the results found in normal Ringer’s (Fig. 3B).

On the other hand, hyperpolarizing the membrane potential of the oocyte from −60 to −140 mV revealed a current that is mainly due to a mixture of conductances. Fig. 3C shows sample traces of this current in the presence or absence of the venom which potentiates the current up to 100% in a dose-dependent manner with an EC50 of 0.3 µg/ml (Fig. 3D). The potentiation was evident at all the hyperpolarizing voltages tested (Fig. 3E); but when the membrane was depolarized the venom partially blocked the current. This part of the protocol suggested the blocking of conductances other than chloride which could be due to potassium channels.

Blocking of an outward-rectifying potassium-current

Fig. 4A shows sample recordings of an oocyte whose membrane potential was changed from −60 to −80 mV then depolarized for 0.5 s to +40 mV, brought back to −80 mV and finally returned to −60 mV. This protocol of activation evidenced an outward-rectifying potassium current which is sensitive to TEA. Thus, we contrasted the effect of 5 mM TEA with that of the venom during the peaks of hyperpolarization and depolarization and plotted the results in Fig. 4B and C. In addition, the venom is more potent blocking the currents than TEA at all tested voltages in Fig. 4D and E.

Considering the strong blocking of the venom on the potassium current we tested the effect of several concentrations of TEA (0.1–5 mM) to try to learn about the molecular identity of the channel. 1 mM TEA blocked considerably the current, suggesting that a Kv channel may be involved [17]. Therefore, we tried to detect the expression of the xKv 1.1 gene in the oocyte. This channel has some of the properties and TEA sensitivity that we illustrate in Fig. 4F. Fig. 4G shows the result of an RT-PCR detecting the transcript for xKv 1.1 in RNA isolated from defolliculated oocytes and from heart, where it is well know that Kv1.1 is expressed.

Discussion

The venom of the Euroasiatic black widow affects synaptic function due to the effect of the LTX [18–20]. However, some reports
indicate other effects due to several minor components of the venom, in particular those that cause the systemic effects which may be generated by low molecular weight peptides [21,22]. In the Chilean L. mactans, several experiments suggested that the nociceptive effects are also mediated by the impact of low molecular weight components of the venom on the function of ion channels and the results described here explain in part the previously reported modulatory actions of the venom on cells such as the sperm cell [8].

To try to explain the effects of the venom on ion channels, endogenous conductances of the frog oocyte were investigated [11, 12, 23–25]. The voltage-operated calcium-and chloride-currents were not sensitive to the concentrations of venom tested. In addition, the calcium-dependent chloride-currents, which are mainly generated by the bestrophins [26] were not affected when applying up to 5 μg/ml of the venom. Therefore, in the venom extract used for these experiments we have no evidence of blocking or modulatory effects on either voltage gated calcium channels or the calcium gated -chloride currents. In contrast, a previous report explained the blocking of calcium channels with the L. mactans venom [8]. The discrepancy with the present results may be due to differences in the molecular identities of voltage-activated calcium-channels of frog oocytes and sperm cells [8]. Unfortunately, thus far, the calcium channels present in the oocyte have not been cloned.

Potassium currents of the oocyte were also investigated [27]. The genes coding for the channels responsible for these currents have not been fully characterized, but due to their functional properties and TEA sensitivity shown in Fig. 4 we assumed they may be part of the Kv family. The L. mactans venom efficiently and reversibly blocked the transient-outward potassium-current, whereas no apparent effects were found in other potassium conductances. In order to confirm the expression of a K channel in the oocytes, we searched for the mRNA coding for the xKv 1.1 [17] channel which generates currents similar to those of the endogenous potassium channel of the oocyte. RT-PCR indirectly evidenced the expression of this channel (Fig. 4G). In conclusion, the results presented here suggest that the L. mactans venom blocks voltage activated potassium channels. Nevertheless, more studies will be necessary to determine: (a) the active oligopeptide(s) of the venom and (b) their molecular target(s).

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