Int. J. Morphol.,
29(3):733-741, 2011.

Molecular Characterization, Electrophysiological and Contraceptive Effect of Chilean *Latrodectus* Venom

Caracterización Molecular, Electrofisiológica y Efecto Anticonceptivo del Veneno de *Latrodectus* Chilena

[,*****]Patricia Navarrete Gómez; [*]David Ormeño; [**]Antonio Miranda;**
[*,*****]Raúl Sánchez Gutiérrez; [*,*****]Fernando Romero Mejía & [*]Jorge Parodi Rivera**

NAVARRETE, G. P.; ORMEÑO, D.; MIRANDA, A.; SÁNCHEZ, G. R.; ROMERO, M. F. & PARODI, R. J. Molecular characterization, electrophysiological and contraceptive effect of Chilean *Latrodectus* venom. ***Int. J. Morphol., 29(3)***:733-741, 2011.

SUMMARY: Since the 1970s, There have been studies of the venom of *Latrodectus sp.* spiders, in particular the latrotoxin (LTX) of *Latrodectus mactans*. Many of the studies were aimed at understanding the action of the venom on the muscular system. Now accepted that LTX is able to generate a calcium-permeable membrane pore and modulate the release of synaptic vesicles that activate a receptor and induce cellular changes. Interestingly, when work began with venom obtained from the *Latrodectus sp* present in Chile, it generated clinical indications similar to the bite of this spider in another country, with some differences in intensity. The purpose of the first studies was to understand the systemic mechanisms of this venom, and other active compounds were studied for biological interest. It was found that these molecules are capable of causing systemic effects such as changes in muscle contraction; of generating vascular relaxation and synaptic and cellular modulation; and of altering potassium conductance channels. Based on this evidence, we suggested biotechnological applications to characterize low molecular-weight compounds obtained from the Chilean *Latrodectus* venom and exploring the effects on the electrophysiology in oocytes and neurons, and the contraceptive effect on spermatozoa.

KEY WORDS: *Latrodectus* venom; Oocytes; Neurons; Spermatozoa; Contraceptive.

INTRODUCTION

The Chilean spider *Latrodectus mactans* (*L. mactans*) belongs to the *Latrodectus* genus which has worldwide distribution (Garb *et al*., 2004). It is known in Chile as "Araña del trigo" (wheat spider) or "black widow", and is present in various regions of the country. Its bite generates a systemic effect known as "latrodectism" or "systemic arachnoidism" in humans, in some cases causing death in both adults and children (Schenone & Correa, 1985).

Venom collected from Chilean *L. mactans* in the VIII and IX Regions of Chile was shown to induce a sustained tonic effect in cardiac and smooth muscle (Romero *et al.*, 2003). In smooth muscle, the mechanism of contraction is related to the permeability of sodium (Na^+) and calcium (Ca^{++}) ions which modulate the contractile response (Nouailhetas *et al*., 1985) that has a fast, phasic component followed by a slower more sustained tonic component (Shimuta *et al.*, 1982). Our studies in the deferent vessel of the rat revealed that the effect induced by the *L. mactans* venom is partially dependent of adrenergic and cholinergic mediators (patent pending).

Moreover, we tested the effect of Chilean venom in hippocampal neuron, and reported the changes in synaptic activity, for have synaptic effects under control and postulate, mechanism over neuromuscular union (Varghese *et al*., 2006).

In a previous report we described that the venom of the Chilean black widow spider *L. mactans* increased intracellular calcium (Ca++i) concentration of the spermatozoa (Romero *et al.*, 2007). Furthermore, this venom is known to block the tetraethylammonium (TEA)-sensitive potassium (K+) currents in neurons (Parodi & Romero, 2008;

*Center of Neurosciences and Peptides Biology- (CEBIOR), BIOREN, Universidad de La Frontera, Temuco, Chile.
**Department of Biophysics University of Sao Paulo, Federal University of Sao Paulo, Sao Paulo, Brazil.
***Centre of Excellence in Biotechnology in Reproduction (CEBIOR), BIOREN, Universidad de La Frontera, Temuco, Chile.
****Department of Preclinics Sciences, University of La Frontera, Temuco, Chile.
*****Doctorate in Sciences Applied Cellular and Molecular Biology, Universidad de La Frontera, Temuco, Chile, CONICYT Scholarship.

Parodi *et al*., 2010) as well as endogenous K+ currents of *Xenopus laevis* oocytes (Parodi *et al*., 2008); this current is similar to that recently described in bovine spermatozoa (Marconi *et al*., 2008). Finally, we analyzed how either depolarization or *L. mactans* venom alters the properties of the sperm cells assessed by microscope imaging, morphology, $Ca^{++}i$ dynamics by fluorometry, acrosome reaction, DNA fragmentation and reactive oxygen species (ROS) production in bovine spermatozoa.

Molecular characterization of Chilean *Latrodectus* venum extract. The venom contains at least 86 unique proteins (Duan *et al*., 2006), including several LTX homologues which play a role in its toxicity in insects and crustaceans (Grishin, 1998), with only one, LTX, specifically targeting vertebrates (Rosenthal & Meldolesi, 1989). LTX is usually isolated from spider venom by conventional chromatography (Frontali *et al.*, 1976; Tzeng & Siekevitz, 1978), but to achieve homogeneity and remove contaminants (Pescatori *et al.*, 1995; Volkova *et al.*, 1995) which may endow the preparation with uncharacteristic properties (Umbach *et al*.,1998), preparative native electrophoresis should ideally be used (Ashton *et al.*, 2000).

The initial work carried out in Chile showed that the effect of a bite of the Chilean spider generated symptoms of latrodectism, similar to, but more moderate than that of their Eurasian relatives (Schenone, 1966). The work of Dr. Romero's group showed effects similar to those reported in muscle preparations (Rauber, 1983-1984). The Chilean spider is harvested in the Eighth and Ninth Regions. The bite induces a sustained tonic effect in heart muscle and smooth muscle tissue (Romero *et al.*, 2003). However, the purification and analysis of the venom by Brazilian groups demonstrated the absence of molecules of high molecular weight (Fig. 1), which could indicate the absence of LTX or similar toxins. Vessel contractility studies done in rats have demonstrated that the effect induced by the venom of the Chilean *L. mactans* spider is partially independent of adrenergic and cholinergic mediators (Romero *et al.*, 2003). These venoms were found to contain only a large group of small peptides, less than 10 kDa, which were considered to be low molecular weight components (Parodi & Romero, 2008). These peptides have been studied and the mixture found to have interesting cellular impacts which may explain the clinical effects found in patients bitten by this spider. This characteristic of the venom of *Latrodectus spp* spiders

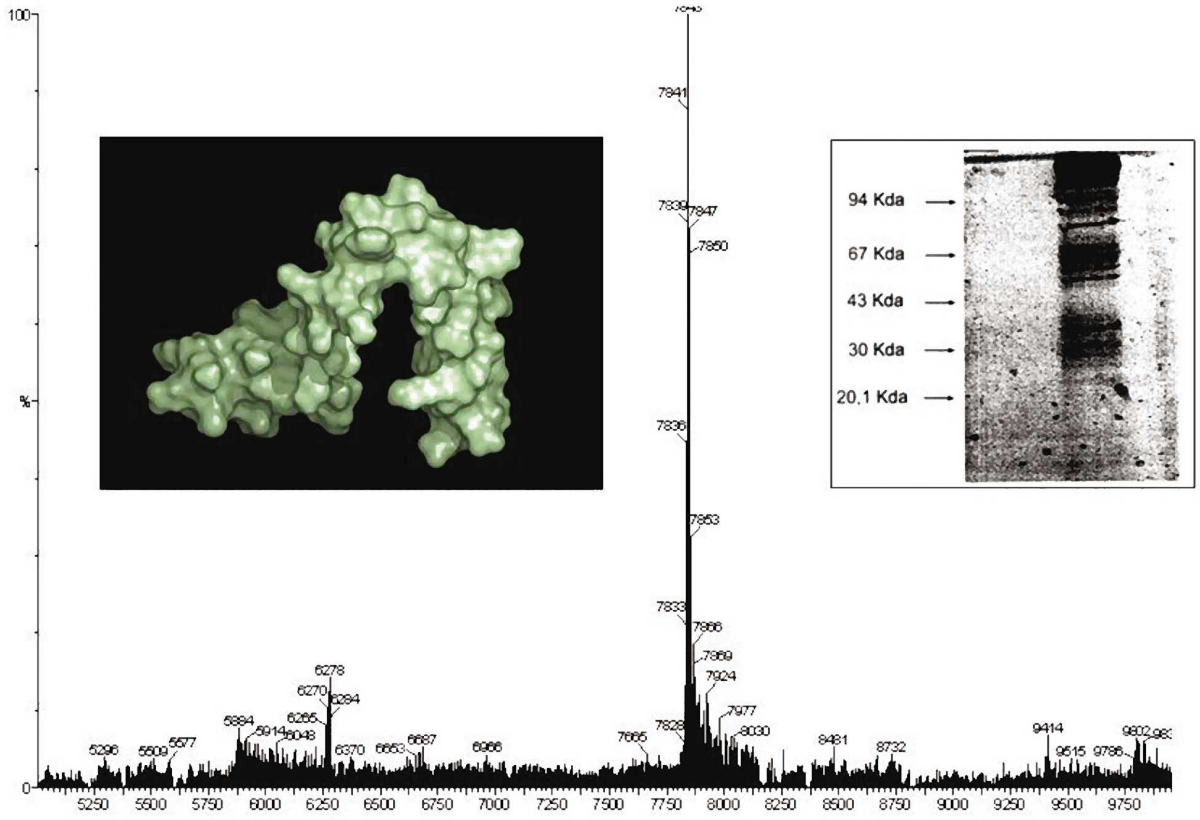

Fig. 1. Total venom. The figure shows typical HPLC-mass obtained by the purification of different peptides present in the Chilean *L. mactans*. The profile indicated the absence of molecules of high molecular weight, like LTX, in the samples. Moreover, shows the molecular simulation of venom and poliacrilamide gel of 10% submitted to electrophoresis in denaturing conditions. The location of the zones of the signal lines of the molecular weight is indicated (94, 67, 43, 30 y 20.1 KDa.).

found in Chile has been exploited to search for peptides of biological interest in which it is easy to purify a natural form of these components.

As previously reported, in our laboratory and work toward development of a patent (Underway in countries PCT) was to provide information about the component of the Chilean venom, *L. mactans*. We described a fractionation of the venom by HPLC–MS, and found the absence of the higher weight component, like a-latrotoxin. This toxin, is the principal component in Black Widow venom and explains most of the systemic effects secondary to this venom. A few reports describe the low weight component, present in the venom but not explored. The Chilean venom, does not present higher component (minor to 10 kDa) (Romero *et al.*, 2000) but, the Chilean venom can induce similar effects to other Black Widow venom in the absence of a-latrotoxin.

Electrophysiological effect of Chilean *Latrodectus* venum extract. The venom from the Chilean Black Widow spider contains several small polypeptides. We have recently demonstrated cellular effects of these peptides at the synaptic level using whole-cell patch clamp techniques. Purified venom from the glands of *L. mactans* was studied in 12 DIV rat hippocampal neuronal cultures (Fig. 2A and B). Our data show that polypeptides present in the venom from Chilean *L. mactans* spiders increase spontaneous synaptic activity in hippocampal neurons and changes the passive properties of the membrane, cells lines. We suggested, K^+ current, for the importance of this current over membrane potential (Gutman *et al*., 2003) and the control in the synaptic activity (Dodson & Forsythe, 2004; Yuan & Chen, 2006). Work by Grider & Makhlouf (1988) demonstrated that the tonic response in smooth muscle is dependent on the influx of Ca^{++} into the cell. Similarly, synaptic activity is dependent

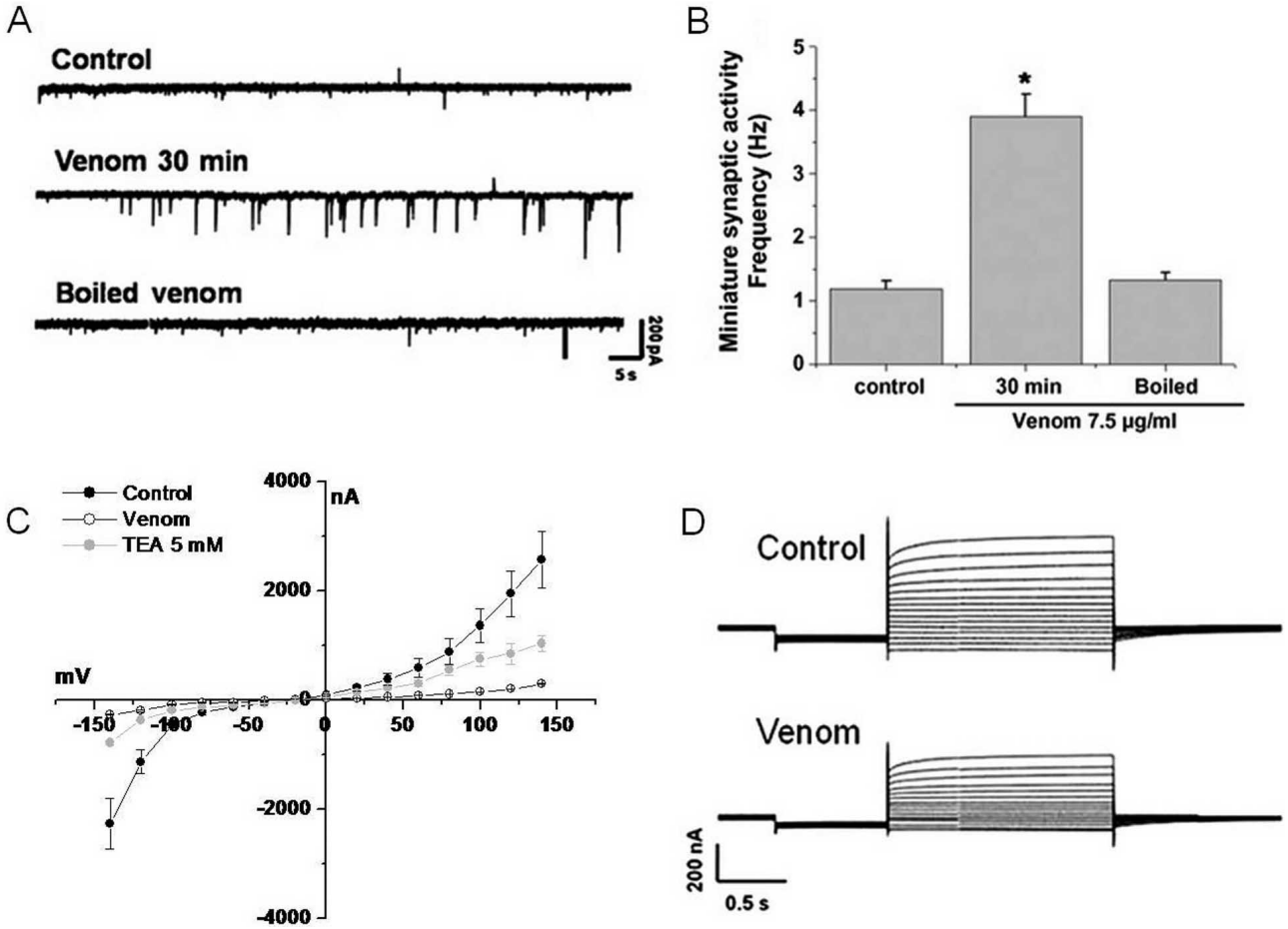

Fig. 2. Effects of Chilean *L. mactans* venom extract on cellular electrophysiology in neurons and oocytes. (A) Shows synaptic currents obtained in the absence and presence of venom or boiled venom in neurons. (B) Shows miniature synaptic current frequency in the presence of the venom. The bars are mean S.E. from 19 different neurons. (C) Shows plot from sample traces control and a venom current from (D) in oocytes. The bars represent means ± SD of 6 different experiments, * was significant $p<0.05$.

on Ca^{++} homeostasis in the presynaptic button (Cousin & Robinson, 2000). Therefore, the neuronal response observed in the presence of total venom could be related to Ca^{++} influx as a result of the change in membrane potential, secondary effect over Ca^{+v}, for regulation of K^+ channels (Pan & Stringer, 1997; Yuan & Chen). These results indicate that venom from Chilean spider *L. mactans* is capable of increasing cell membrane resistance, prolonging the action potential and generating an increase in synaptic activity demonstrating an interesting pharmacological effect of these low molecular weight fragments.

In an attempt to explain the effects of the venom on ion channels, endogenous conductances of the frog oocyte were investigated (Fig. 2C and D) (Lu *et al*., 1990; Miledi, 1982; Parker & Miledi, 1988b; Parker & Ivorra, 1990). The voltage-operated Ca^{++}-and chloride-currents were not sensitive to the concentrations of venom tested. In addition, the Ca^{++}-dependent chloride-currents, which are mainly generated by the bestrophins (Hartzell *et al*., 2005) 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 Ca^{++} channels or the Ca^{++} gated - chloride currents. In contrast, a previous report explained the blocking of Ca^{++} channels with the *L. mactans* venom (Romero *et al*., 2007). The discrepancy with the present results may be due to differences in the molecular identities of voltage-activated Ca^{++}-channels of frog oocytes and sperm cells (Romero *et al*., 2007). Unfortunately, thus far, the Ca^{++} channels present in the oocyte have not been cloned. K^+ currents of the oocyte were also investigated (Weber, 1999). The genes coding for the channels responsible for these currents have not been fully characterized, but due to their functional properties and TEA sensitivity, we assumed they may be part of the Kv family. The *L. mactans* venom efficiently and reversibly blocked the transient-outward K+-current, whereas no apparent effects were found in other K^+ conductances. In order to confirm the expression of a Kv channel in the oocytes, we searched for the mRNA coding for the xKv 1.1 (Gutman *et al*.) channel which generates currents similar to those of the endogenous K^+ channel of the oocyte. RT-PCR indirectly evidenced the expression of this channel.

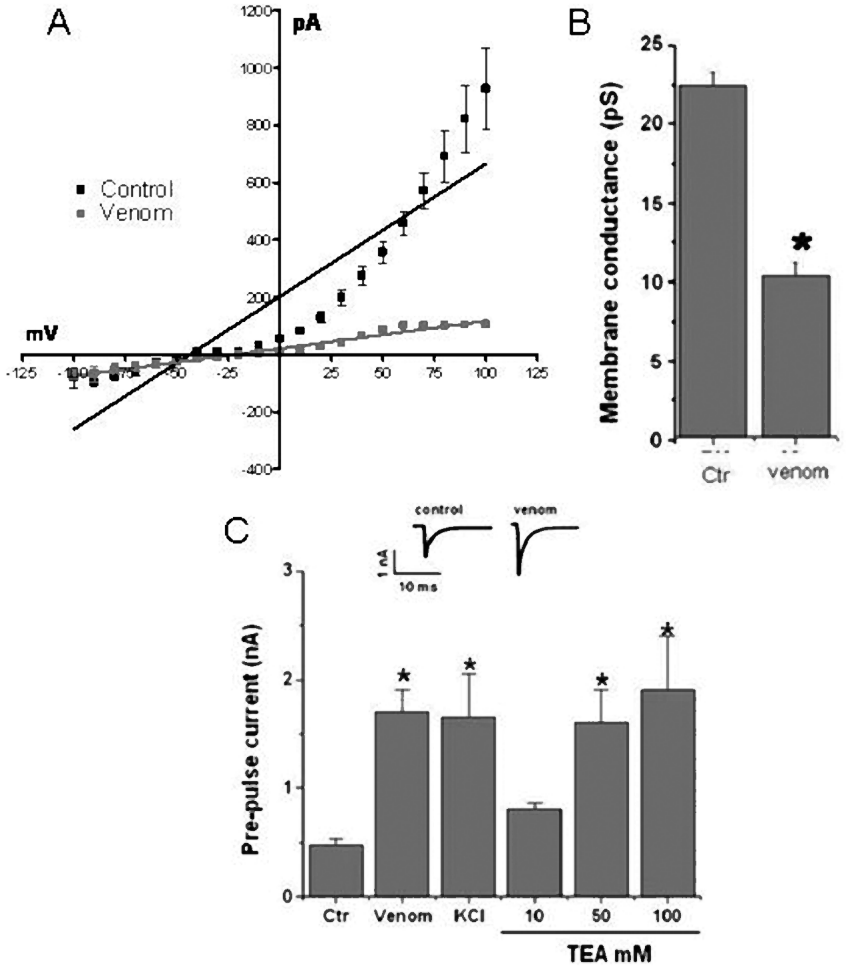

Fig. 3. Effects of Chilean *L. mactans* venom extract on electrophysiology cellular in spermatozoa. (A) I/V relation in the absence or presence of 7.5 µg/mL venom in spermatozoa. (B) Shows the membrane conductance is reduced in spermatozoa exposed to the venom. (C) Shows comparative effect of venom, high K+ and increasing concentrations of TEA (10 to 50 mM) on the pre-pulse current. The bars represent means ± SD of 6 different experiments, * was significant $p<0.05$.

All conductances are crucial to spermatozoa capacitation. After several minutes of exposure of the spermatozoa to the venom (Fig. 3A-C), the change in the membrane conductance after exposure to venom suggests depolarization and Ca^{+v} channel voltage dependent (CCVD) activation in the spermatozoa cells. This may reflect the blockage of voltage-dependent K+ channels leading to the entrance of Ca^{+v} from the extracellular medium in accord with previous studies that suggested that the venom inhibits several K^+ conductances (Parodi & Romero; Parodi *et al.*, 2008). Like the oocyte Kv1.1 s (Parodi *et al.*, 2008), this channel is also present in the spermatozoa midpiece (Darszon *et al.*, 1999). Since Kv1.1 is integral to membrane potential regulation (Gutman *et al.*) we compared the effect with high K^+ added in the medium. In both cases the concentration of free Ca^{++} exhibited a transient rise and sharp fall as detected by spermatozoal midpiece fluorescence. However, arise in Ca^{++} was observed throughout the cell after long periods of time. This is consistent with the view that a CCVD was present (Darszon *et al.*, 2005). The currents blocked by the spider venom appear similar to those found in frog oocytes and neurons in culture (Parodi & Romero; Parodi *et al.*, 2008) although differences in the expression

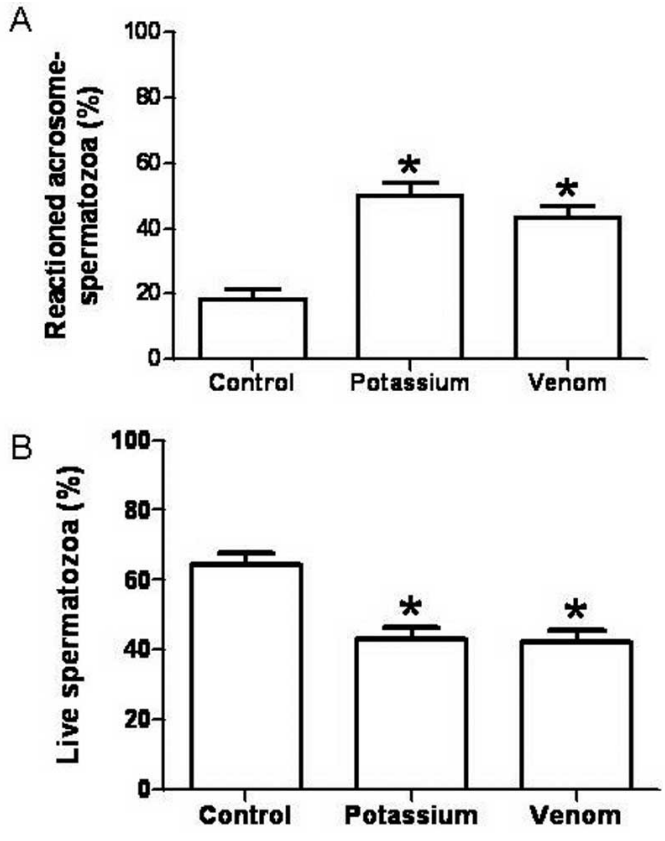

Fig. 4. Venom induced acrosome reaction and decrease of viability spermatic. (A) Shows plot of percent live spermatozoa with intact acrosome in control, high potassium (10 mM) and venom (10 µg/mL) and percent live spermatozoa (B). The bar are means ± E.R. of 5 different experiments, * was significant $p<0.05$.

profiles of Kv channels (Darszon *et al.*, 2006) among species have been observed. Nevertheless the functional data reported here and elsewhere (Darszon *et al.*, 2006; Marconi *et al.*; Navarro *et al.*, 2008) is consistent with the view that Kv channels are integral to the spermatozoa.

Contraceptive effect of Chilean *Latrodectus* venum extract. There is strong experimental evidence that mature mammalian spermatozoa have several ionic-conductances, including those driven by voltage dependent K^+ channels (Darszon *et al.*, 1996; Labarca *et al.*, 1995; Nuccitelli & Ferguson, 1994). In addition, recent reports in which a whole cell patch-clamp was used to study spermatozoa ion-conductance have described the functional properties of Ca^{++} channels (CATsper), which are key components in the capacitation process (Darszon *et al.*, 2005; Wennemuth *et al.*, 2000). Recently other reports have shown a K^+ channel sensitive to TEA in bovine spermatozoa (Marconi *et al.*) which is important for the control of membrane potential (Gutman *et al.*). Mammalian sperm acquire their functional capacity to fertilize an egg during their migration through the male and female genital tract (Boni *et al.*, 2007). In this process the plasma membrane potential is suddenly hyperpolarized by the activation of pH- sensitive K^+ -channels, leading to an increase in Ca^{+v} permeability (Kumar *et al.*, 2000; Linares-Hernandez *et al.*, 1998; Shi & Ma, 1998). Previously we measured the Ca^{++} influx induced by depolarization of the plasma membrane and by venom isolated from the Chilean black widow spider (*L. mactans*), and functional changes in the presence of either high K+ or total venom. Our results indicate that the venom increased the Ca^{++} influx, with an EC50 of 6.1 µg/mL and triggering the acrosome reaction in 43.26% of the cells and decreased the viability in 40% (Fig. 4A and B). The application of potassium (10 mM K+) or total venom (10 µg/mL) did not affect the morphology or DNA stability of the sperm nor the production of reactive oxygen species (ROS) (Fig. 5A and B). We observed a reduction in viability and suggest that this alteration is a consequence of capacitation and the acrosomal reaction (Medeiros *et al.*, 2002). We explain the reduction in viability as methodological condition and cell death mediated by spermatozoa activation and the acrosomal reaction. This notion is supported by the observation of the DNA integrity measured by DNA fragmentation, in the absence or presence of venom. No DNA fragmentation was observed under any condition. The controls with DNase I showed typical fragmentation and support our suggestion that there is no toxic effect of the venom in our model (Navarrete *et al.*, 2010). Previous studies have provided information about the spermicidal properties

of the venom of the Chilean spider *L. mactans*. Other natural sources of spermicidal action have been reported, such as scorpion toxins and plant extracts (Harat *et al.,* 2008; Lopez-Gonzalez *et al*., 2003). All these studies suggest new lines of research to identify the active compound(s) that mediate the modulation of ionic conductances, and that could provide a resource for a new generation of contraceptives. Ca^{++} influx in response to membrane depolarization with K^+ has been reported as one of the key early events leading to the process of sperm capacitation. Therefore, it is possible that components in the venom from the black widow spider may influence membrane properties in sperm leading to capacitation. In conclusion, we find that bovine spermatozoa increases the Ca^{++i} when exposed to aracnotoxin from *L. mactans* and that this phenomenon produces the subsequent acrosome reaction in the spermatozoa, most probably through the blocking of voltage-dependent K^+ channels Thus, molecules derived from venom could be isolated for biotechnological applications, including the design of new contraceptives. New data is presently being gathered that demonstrates the effects of the purified protein and peptides.

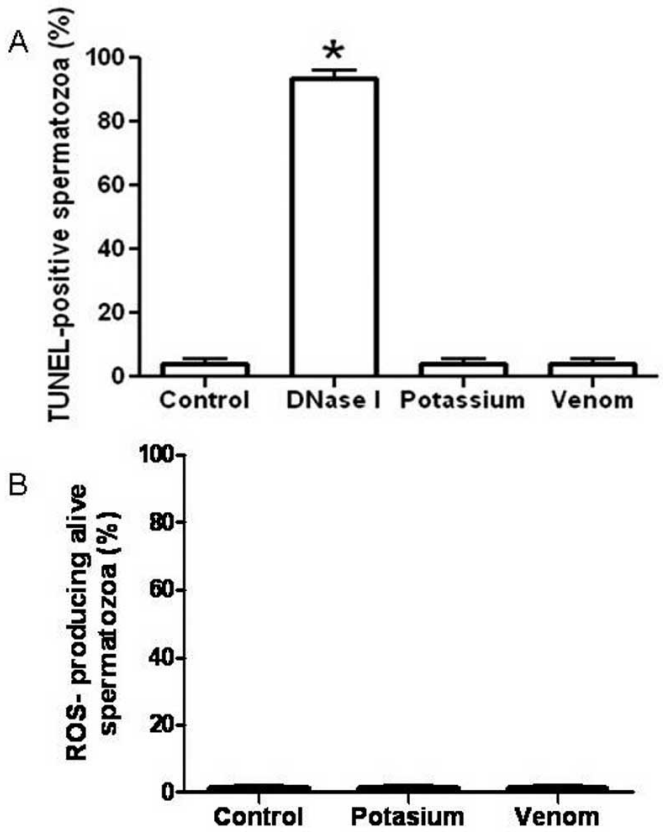

Fig. 5. Venom not induced DNA fragmentation or ROS production in spermatozoa. (A) Shows plot of percent TUNEL positive spermatozoa in control, DNase I, high potassium (10 mM) and venom (10 µg/mL) and percent of live spermatozoa producing ROS (B). The bar are means ± E.R. of 5 different experiments, * was significant $p<0.05$.

Concluding Remarks. In the future, we are looking to find more applications and effects derived from other peptides present in the total venom, or maybe to refine the present cellular effect, to be able to reduce doses and make it the best choice for pharmacological applications. In the end, our experience with venom from Chilean *L. mactans* suggested a new line of application for other peptides, and suggested that we should not look only at the principal component to explain all its toxic effects. In the future we hope to see more biotechnological applications derived from the small peptides present in this venom and others.

ACKNOWLEDGMENTS. Fernando Romero, Raúl Sánchez, Antonio Miranda and Jorge Parodi were supported by the FONDEF-CONICYT Chile N° DO5I10416. David Ormeño has a "Universidad de la Frontera" M. Sc. fellowship. Patricia Navarrete is supported by a CONICYT grant.

NAVARRETE, G. P.; ORMEÑO, D.; MIRANDA, A.; SÁNCHEZ, G. R.; ROMERO, M. F. & PARODI, R. J. Caracterización molecular, electrofisiológica y efecto anticonceptivo del veneno de *Latrodectus* Chilena. ***Int. J. Morphol., 29(3)***:733-741, 2011.

RESUMEN: Desde los años 70`, se han realizado estudios con el veneno de arañas *Latrodectus sp*, en particular la latrotoxina (LTX) de *Latrodectus mactans*. Muchos de estos estudios estuvieron enfocados a entender la acción del veneno sobre el sistema muscular. Hoy en día es aceptado que la LTX es capaz de generar un poro de membrana permeable a calcio y modular la liberación de vesículas sinápticas que activan un receptor e inducen cambios celulares. Interesantemente, cuando comenzamos a trabajar con el veneno obtenido de *Latrodectus sp*. presente en Chile, ésto generó indicaciones clínicas similares a la picadura de esta araña en otros países, con algunas diferencias en su intensidad. El propósito de estos primeros estudios fue entender los mecanismos sistémicos de este veneno y además otros compuestos activos fueron estudiados para interés biológico. Se ha encontrado que estas moléculas son capaces de causar efectos sistémicos así como cambios en la contracción muscular; generar relajación vascular y modulación sináptica y celular; y de alterar los canales de conductancia de potasio. Basados en estas evidencias, nosotros sugerimos usar aplicaciones biotecnológicas para caracterizar los compuestos de bajo peso molecular obtenidos del veneno de *Latrodectus Chilena* y explorar los efectos sobre la electrofisiología en ovocitos y neuronas, y el efecto anticonceptivo sobre los espermatozoides.

PALABRAS CLAVE: Veneno de *Latrodectus*; Ovocitos; Neuronas; Espermatozoides; Anticonceptivo.

REFERENCES

Ashton, A. C.; Rahman, M. A.; Volynski, K. E.; Manser, C.; Orlova, E. V.; Matsushita, H.; Davletov, B. A.; van Heel, M.; Grishin, E. V. & Ushkaryov, Y. A. Tetramerisation of alpha-latrotoxin by divalent cations is responsible for toxin-induced non-vesicular release and contributes to the Ca(2+)-dependent vesicular exocytosis from synaptosomes. *Biochimie, 82(5)*:453-68, 2000.

Boni, R.; Gualtieri, R.; Talevi, R. & Tosti, E. Calcium and other ion dynamics during gamete maturation and fertilization. *Theriogenology, 68(Suppl 1)*:S156-64, 2007.

Cousin, M. A. & Robinson, P. J. Ca(2+) influx inhibits dynamin and arrests synaptic vesicle endocytosis at the active zone. *J. Neurosci., 20(3)*:949-57, 2000.

Darszon, A.; Liévano, A. & Beltran, C. Ion channels: key elements in gamete signaling. *Curr. Top. Dev. Biol., 34*:117-67, 1996.

Darszon, A.; Labarca, P.; Nishigaki, T. & Espinosa, F. Ion channels in sperm physiology. *Physiol. Rev., 79(2)*:481-510, 1999.

Darszon, A.; Nishigaki, T.; Wood, C.; Trevino, C. L.; Felix, R. & Beltran, C. Calcium channels and Ca2+ fluctuations in sperm physiology. *Int. Rev. Cytol., 243*:79-172, 2005.

Darszon, A.; Acevedo, J. J.; Galindo, B. E.; Hernandez-Gonzalez, E. O.; Nishigaki, T.; Trevino, C. L.; Wood, C. & Beltran, C. Sperm channel diversity and functional multiplicity. *Reproduction, 131(6)*:977-88, 2006.

Dodson, P. D. & Forsythe, I. D. Presynaptic K+ channels: electrifying regulators of synaptic terminal excitability. *Trends Neurosci., 27(4)*:210-7, 2004.

Duan, Z. G.; Yan, X. J.; He, X. Z.; Zhou, H.; Chen, P.; Cao, R.; Xiong, J. X.; Hu, W. J.; Wang, X. C. & Liang, S. P. Extraction and protein component analysis of venom from the dissected venom glands of *Latrodectus tredecimguttatus*. *Comp. Biochem. Physiol. B Biochem. Mol. Biol., 145(3-4)*:350-7, 2006.

Frontali, N.; Ceccarelli, B.; Gorio, A.; Mauro, A.; Siekevitz, P.; Tzeng, M. C. & Hurlbut, W. P. Purification from black widow spider venom of a protein factor causing the depletion of synaptic vesicles at neuromuscular junctions. *J. Cell Biol., 68(3)*:462-79, 1976.

Garb, J. E.; Gonzalez, A. & Gillespie, R. G. The black widow spider genus *Latrodectus (Araneae: Theridiidae):* phylogeny, biogeography, and invasion history. *Mol. Phylogenet. Evol., 31(3)*:1127-42, 2004.

Grider, J. R. & Makhlouf, G. M. Contraction mediated by Ca++ release in circular and Ca++ influx in longitudinal intestinal muscle cells. *J. Pharmacol. Exp. Ther., 244(2)*:432-7, 1988.

Grishin, E. V. Black widow spider toxins: the present and the future. *Toxicon., 36(11)*:1693-701, 1998.

Gutman, G. A.; Chandy, K. G.; Adelman, J. P.; Aiyar, J.; Bayliss, D. A.; Clapham, D. E., *et al*. International Union of Pharmacology. XLI. Compendium of voltage-gated ion channels: potassium channels. *Pharmacol. Rev., 55(4)*:583-6, 2003.

Harat, Z. N.; Sadeghi, M. R.; Sadeghipour, H. R.; Kamalinejad, M. & Eshraghian, M. R. Immobilization effect of *Ruta graveolens L.* on human sperm: a new hope for male contraception. *J. Ethnopharmacol., 115(1)*:36-41, 2008.

Hartzell, C.; Qu, Z.; Putzier, I.; Artinian, L.; Chien, L. T. & Cui, Y. Looking chloride channels straight in the eye: bestrophins, lipofuscinosis, and retinal degeneration. *Physiology(Bethesda), 20*:292-302, 2005.

Kumar, S.; Ying, Y. K.; Hong, P. & Maddaiah, V. T. Potassium increases intracellular calcium simulating progesterone action in human sperm. *Arch. Androl., 44(2)*:93-101, 2000.

Labarca, P.; Zapata, O.; Beltran, C. & Darszon, A. Ion channels from the mouse sperm plasma membrane in planar lipid bilayers. *Zygote, 3(3)*:199-206, 1995.

Linares-Hernandez, L.; Guzman-Grenfell, A. M.; Hicks-Gomez, J. J. & Gonzalez-Martinez, M. T. Voltage-dependent calcium influx in human sperm assessed by simultaneous optical detection of intracellular calcium and membrane potential. *Biochim. Biophys. Acta, 1372(1)*:1-12, 1998.

Lopez-Gonzalez, I.; Olamendi-Portugal, T.; De la Vega-Beltran, J. L.; Van der Walt, J.; Dyason, K.; Possani, L. D., et al. Scorpion toxins that block T-type Ca2+ channels in spermatogenic cells inhibit the sperm acrosome

reaction. *Biochem. Biophys. Res. Commun., 300(2)*:408-14, 2003.

Lu, L.; Montrose-Rafizadeh, C.; Hwang, T. C. & Guggino, W. B. A delayed rectifier potassium current in Xenopus oocytes. *Biophys. J., 57(6)*:1117-23, 1990.

Marconi, M.; Sanchez, R.; Ulrich, H. & Romero, F. Potassium current in mature bovine spermatozoa. *Syst. Biol. Reprod. Med., 54(6)*:231-9, 2008.

Medeiros, C. M.; Forell, F.; Oliveira, A. T. & Rodrigues, J. L. Current status of sperm cryopreservation: why isn't it better? *Theriogenology, 57(1)*:327-44, 2002.

Miledi, R. A calcium-dependent transient outward current in Xenopus laevis oocytes. *Proc. R. Soc. Lond. B Biol. Sci., 215(1201)*:491-7, 1982.

Navarrete, P.; Martínez-Torres, A.; Gutiérrez, R. S.; Mejía, F. R. & Parodi, J. Venom of the Chilean *Latrodectus mactans* alters bovine spermatozoa calcium and function by blocking the TEA-sensitive K(+) current. Syst. Biol. *Reprod. Med., 56(4)*:303-10, 2010.

Navarro, B.; Kirichok, Y.; Chung, J. J. & Clapham, D. E. Ion channels that control fertility in mammalian spermatozoa. *Int. J. Dev. Biol., 52(5-6)*:607-13, 2008.

Nouailhetas, V. L.; Shimuta, S. I.; Paiva, A. C. & Paiva, T. B. Calcium and sodium dependence of the biphasic response of the guinea-pig ileum to agonists. *Eur. J. Pharmacol., 116(1-2)*:41-7, 1985.

Nuccitelli, R. & Ferguson, J. E. Fertilization and ion channels. *Science, 263(5149)*:988, 1994.

Pan, E. & Stringer, J. L. Role of potassium and calcium in the generation of cellular bursts in the dentate gyrus. *J. Neurophysiol., 77(5)*:2293-9, 1997.

Parker, I. & Miledi, R. Transient potassium current in native Xenopus oocytes. *Proc. R. Soc. Lond. B Biol. Sci., 234(1274)*:45-53, 1988a.

Parker, I. & Miledi, R. A calcium-independent chloride current activated by hyperpolarization in Xenopus oocytes. *Proc. R. Soc. Lond. B Biol. Sci., 233(1271)*:191-9, 1988b.

Parker, I. & Ivorra, I. A slowly inactivating potassium current in native oocytes of Xenopus laevis. *Proc. R. Soc. Lond. B Biol. Sci., 238(1293)*:369-81, 1990.

Parodi, J. & Romero, F. Synaptic effects of low molecular weight components from Chilean Black Widow spider venom. *Neurotoxicology, 29(6)*:1121-6, 2008.

Parodi, J.; Romero, F.; Miledi, R. & Martinez-Torres, A. Some effects of the venom of the Chilean spider *Latrodectus mactans* on endogenous ion-currents of Xenopus laevis oocytes. *Biochem. Biophys. Res. Commun., 375(4)*:571-5, 2008.

Parodi, J.; Navarrete, P.; Marconi, M.; Sánchez Gutiérrez, R.; Martínez-Torres, A. & Romero Mejía, F. TEA-sensitive K+ current in the bovine spermatozoa and its blocking by the venom of the Chilean *Latrodectus mactans*. *Syst. Biol. Reprod. Med., 56(4)*:303-10, 2010.

Pescatori, M.; Bradbury, A.; Bouet, F.; Gargano, N.; Mastrogiacomo, A. & Grasso, A. The cloning of a cDNA encoding a protein (latrodectin) which co-purifies with the alpha-latrotoxin from the black widow spider *Latrodectus tredecimguttatus* (Theridiidae). *Eur. J. Biochem., 230(1)*:322-8, 1995.

Rauber, A. Black widow spider bites. *J. Toxicol. Clin. Toxicol., 21(4-5)*:473-85, 1983-1984.

Romero, F.; Altieri, E.; Quiñehual, C. & Cayuqueo, A. Contraction activity of the papillar muscle and vas deferens of a rat induced to position from the *Latrodectus mactans* spider of Chile. *Gayana, 64(2)*:161-70, 2000.

Romero, F.; Altieri, E.; Urrutia, M. & Jara, J. Venom of *Latrodectus mactans* from Chile (Araneae, Theridiidae): effect on smooth muscle. *Rev. Biol. Trop., 51(2)*:305-12, 2003.

Romero, F.; Cunha, M. A.; Sanchez, R.; Ferreira, A. T.; Schor, N. & Oshiro, M. E. Effects of arachnotoxin on intracellular pH and calcium in human spermatozoa. *Fertil. Steril., 87(6)*:1345-9, 2007.

Rosenthal, L. & Meldolesi, J. Alpha-latrotoxin and related toxins. Pharmacol. Ther., 42(1):115-34, 1989.

Schenone, H. Latrodectism and loxoscelism incidence in Chile, clinical characteristics, diagnosis, treatment and prevention. *Mem. Instituto Butantan., 33*:207-11, 1966.

Schenone, H. & Correa, L. E. Some practical knowledge of the biology of the spider *Latrodectus mactans* and the latrodectism syndrome in Chile. *Bol. Chil. Parasitol., 40*:18-23, 1985.

Shi, Y. L. & Ma, X. H. Ion-channels reconstituted into lipid bilayer from human sperm plasma membrane. *Mol. Reprod. Dev., 50*:354-60, 1998.

Shimuta, S. I.; Nouailhetas, V. L.; Valero, V. B.; Paiva, A. C. & Paiva, T. B. Effect of sodium concentration and of atropine on the contractile response of the guinea-pig ileum to potassium ions. *Pflugers Arch., 394*:186-90, 1982.

Tzeng, M. C. & Siekevitz, P. The effect of the purified major protein factor (alpha-latrotoxin) of black widow spider venom on the release of acetylcholine and norepinephrine from mouse cerebral cortex slices. *Mol. Brain Res., 139*:190-6, 1978.

Umbach, J. A.; Grasso, A.; Zurcher, S. D.; Kornblum, H. I.; Mastrogiacomo, A. & Gundersen, C. B. Electrical and optical monitoring of alpha-latrotoxin action at Drosophila neuromuscular junctions. *Neuroscience, 87(4)*:913-24, 1998.

Varghese, A.; Tenbroek, E. M.; Coles, J. Jr. & Sigg, D. C. Endogenous channels in HEK cells and potential roles in HCN ionic current measurements. *Progr. Biophys. Mol. Biol., 90*:26-37, 2006.

Volkova, T. M.; Pluzhnikov, K. A.; Woll, P. G. & Grishin, E. V. Low molecular weight components from black widow spider venom. *Toxicon., 33(4)*:483-9, 1995.

Weber, W. M. Endogenous ion channels in oocytes of Xenopus laevis: recent developments. *J. Membr. Biol., 170(1)*:1-12, 1999.

Wennemuth, G.; Westenbroek, R. E.; Xu, T.; Hille, B. & Babcock, D. F. CaV2.2 and CaV2.3 (N- and R-type) Ca2+ channels in depolarization-evoked entry of Ca2+ into mouse sperm. *J. Biol. Chem., 275(28)*:21210-7, 2000.

Yuan, L. L. & Chen, X. Diversity of potassium channels in neuronal dendrites. *Prog. Neurobiol., 78(6)*:374-89, 2006.

Correspondence to:
Jorge Parodi Rivera
Center of Neurosciences and Peptides Biology-
(CEBIOR) BIOREN
Universidad de La Frontera
Temuco
CHILE

Email: jparodi@ufro.cl

Received: 08-04-2011
Accepted: 22-05-2011