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Crotamine pharmacology revisited: novel insights based on the inhibition of K_v channels

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Running Title: Crotamine pharmacology revisited through K_V channel inhibition

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Text pages: 23

Tables: 0

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Abbreviations: TTX, tetrodotoxin; DRG, dorsal root ganglion; RP, HPLC, high-performance liquid chromatography; TFA, trifluoroacetic acid; VGPC, voltage-gated potassium channel

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Abstract

Crotamine, a 5KDa peptide possesses a unique biological versatility. Not only its cell-penetrating activity has become of clinical interest but moreover, its potential selective anti-tumor activity is of great pharmacological importance. Before, several studies have attempted to elucidate the exact molecular target responsible for the crotamine-induced skeletal muscle spasm. The aim of this study was to investigate whether crotamine affects voltage-gated potassium (K_V) channels in an effort to explain its *in vivo* effects. Crotamine was studied on ion channel function using the two-electrode voltage clamp technique on 16 cloned ion channels (12 K_V channels and 4 Na_V channels), expressed in *Xenopus laevis* oocytes. Crotamine selectively inhibits $K_V1.1$, $K_V1.2$ and $K_V1.3$ channels with an IC_{50} of ~300 nM and the key amino acids responsible for this molecular interaction are suggested. Our results demonstrate for the first time that the symptoms which are observed in the typical crotamine syndrome may result from the inhibition of K_V channels. The ability of crotamine to inhibit the potassium current through K_V channels unravels it as the first snake peptide with the unique multifunctionality of cell penetrating and antitumoral activity combined with K_V channel inhibiting properties. This new property of crotamine might explain some experimental observations and opens new perspectives of pharmacological uses.

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1. Introduction

Crotamine is a 42 amino acids peptide present in the venom of *Crotalus durissus terrificus* rattlesnake (Laure, 1975). This molecule belongs to the small basic myotoxins family because of the high primary sequence identity, but also its fold and potential surface resemble the structural features of β -defensins-like peptides, including sea anemone Anthopleurin toxins, despite their different biological activities and low primary sequence similarity (~30%) (Nicastro et al., 2003; Siqueira and Nicolau, 2002). Nonetheless, it possesses a wide spectrum of biological activity, such as membrane penetration into different cell types and mouse blastocysts *in vitro*, anti-tumoral agent against several aggressive tumorigenic cell lineages but inactive against normal cells, irreversible membrane depolarization and spontaneous repetitive firings of mammalian skeletal muscle (Chang and Tseng, 1978; Rizzi et al., 2007). When investigating the depolarizing action of crotamine in diaphragm muscle of mouse and rat, Chang and Tseng (1978) concluded that it was due to effects on sodium channels, since this depolarization could be reversed non-competitively by tetrodotoxin (TTX), procaine and high calcium and low sodium media (Chang and Tseng, 1978). After observing that TTX prevents and also restores the depolarization of membrane potential caused by crotamine, but it did not impede its irreversible binding, Hong and Chang (1983) inferred that the interaction site of TTX is distinct from that of crotamine (Chang et al., 1983).

After several studies showing indirect evidence that crotamine acts on Na⁺ channels, Rizzi et al. (2007) demonstrated that it actually did not affect directly mammalian voltage-dependent sodium channels. The authors employed

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transfected HEK cells expressing the α subunits of Na_v 1.1-1.6, as well as DRG neurons (Rizzi et al., 2007). Those results were also supported by an envenoming behaviour comparison between crotamine and toxins with proven sodium channel activity (tetrodotoxin, μ -conotoxin-GIIIA, BcIII, Tx2-6, and α - and β -pompilidotoxins), which were unable to mimic the hind-limb paralysis caused by crotamine. Later it was proposed that crotamine could act as a voltage-dependent potassium channel blocker, based on its three-dimensional structure resemblance with human antibacterial β -defensins using computational docking (Yount et al., 2009). These results suggested crotamine interacts with specific residues of the channel pore.

In order to finally elucidate the exact target through which crotamine exerts its observed activity, we submitted it to a detailed and straightforward electrophysiological screening on a wide range of 16 ion channels expressed in *Xenopus laevis* oocytes. It was observed a potent and selective K_v channel inhibiting properties of crotamine, confirming the suggestion by Yount et al. (2009) and the inability of interaction with sodium channels as shown by Rizzi et al. (2007) (Rizzi et al., 2007; Yount et al., 2009). Our findings do not contradict the pharmacological results with crotamine as discussed herein.

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2. Material and Methods

2.1 Purification and biochemical characterization of crotamine

Crotalus durissus terrificus venom was extracted from snakes maintained at the Faculdade de Medicina de Ribeirão Preto serpentarium, Universidade de São Paulo, and dried under vacuum. Crude venom (600 mg) were dissolved in 5 ml of 0.25 M ammonium formate buffer, pH 3.5, and the bulk of crotoxin, the major venom component, was eliminated by slow-speed centrifugation as a heavy precipitate that formed upon slow addition of 20 ml of cold water to the solution. Tris-base 1 M was then added drop-wise to the supernatant to raise the pH to 8.8, and the solution was applied to a CM-Sepharose FF (1.5 x 4.5 cm; Amersham-Biosciences, Buckinghamshire, UK) column equilibrated with 0.04 M Tris-HCl buffer, pH 8.8, containing 0.064 M NaCl. After the column was washed with 100 ml of equilibrating solution, crotamine was recovered as a narrow protein peak by raising the NaCl concentration of the diluting solution to 0.64 M. The material was thoroughly dialyzed against water (benzoylated membrane, cut off MW = 3000) and lyophilized, as described (Kerkis et al., 2004). ESI-MS analysis was done by using the LCQ Deca XP ion trap mass spectrometer (Thermo-Finnigan, San José, USA). The crotamine peptide was subjected to Edman degradation using an automated peptide sequencing instrument (PPSQ-33A, Shimadzu, Japan).

2.2 Expression of voltage-gated ion channels in *Xenopus laevis* oocytes

For the expression of the VGPCs (rK_V1.1, rK_V1.2, hK_V1.3, rK_V1.4, rK_V1.5, rK_V1.6, *Shaker* IR, rK_V2.1, hK_V3.1, rK_V4.2, rK_V4.3, hERG) and VGSCs (rNa_V1.2, rNa_V1.3, hNa_V1.5 and the insect channel DmNa_V1) in *Xenopus*

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oocytes, the linearized plasmids were transcribed using the T7 or SP6 mMESSAGE-mMACHINE transcription kit (Ambion, USA). The harvesting of stage V-VI oocytes from anaesthetized female *Xenopus laevis* frog was previously described (Liman et al., 1992). Oocytes were injected with 50 nl of cRNA at a concentration of 1 ng/nl using a micro-injector (Drummond Scientific, USA). The oocytes were incubated in a solution containing (in mM): NaCl, 96; KCl, 2; CaCl₂, 1.8; MgCl₂, 2 and HEPES, 5 (pH 7.4), supplemented with 50 mg/l gentamycin sulfate.

2.3 Electrophysiological recordings

Two-electrode voltage-clamp recordings were performed at room temperature (18-22°C) using a Geneclamp 500 amplifier (Molecular Devices, USA) controlled by a pClamp data acquisition system (Axon Instruments, USA). Whole cell currents from oocytes were recorded 1-4 days after injection. Bath solution composition was ND96 (in mM): NaCl, 96; KCl, 2; CaCl₂, 1.8; MgCl₂, 2 and HEPES, 5 (pH 7.4) or HK (in mM): NaCl, 2; KCl, 96; CaCl₂, 1.8; MgCl₂, 2 and HEPES, 5 (pH 7.4). Voltage and current electrodes were filled with 3M KCl. Resistances of both electrodes were kept between 0.5-1.5 MΩ. The elicited currents were filtered at 1 kHz and sampled at 500 Hz using a four-pole low-pass Bessel filter. Leak subtraction was performed using a -P/4 protocol. K_v1.1-K_v1.6 and *Shaker* currents were evoked by 500 ms depolarizations to 0 mV followed by a 500 ms pulse to -50 mV, from a holding potential of -90 mV. Current traces of hERG channels were elicited by applying a +40 mV prepulse for 2 s followed by a step to -120 mV for 2 s. K_v2.1, K_v3.1 and K_v4.2, K_v4.3 currents were elicited by 500 ms pulses to

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+20mV from a holding potential of -90 mV. Sodium current traces were, from a holding potential of -90 mV, evoked by 100 ms depolarizations to V_{\max} (the voltage corresponding to maximal sodium current in control conditions). In order to investigate the current-voltage relationship, current traces were evoked by 10 mV depolarization steps from a holding potential of -90 mV. To assess the concentration dependency of the crotoxin induced inhibitory effects, a dose-response curve was constructed, in which the percentage of current inhibition was plotted as a function of toxin concentration. All data represent at least 3 independent experiments ($n \geq 3$) and are presented as mean \pm standard error.

2.4 Sequence alignment, molecular visualization and dipole moment calculation

Protein sequences were aligned using Muscle in JalView 2.0 bioinformatic workbench and were shaded by similarity score using BLOSUM62. Crotoxin (PDB code 1Z99); ShK (1ROO) and BgK (1BGK) from the sea anemones *Stichodactyla helianthus* and *Bunodosoma granulifera*, respectively; κ -Hefutoxin (1HP9) and Charybdotoxin (2CRD) from the scorpions *Heterometrus fulvipes* and *Leiurus quinquestriatus hebraeus*, respectively, were visualized and graphically manipulated using the publicly available software Chimera (Petersen et al., 2010) The crotoxin dipole moment was calculated using the default parameters with the "Protein Dipole Moments Server", available at <http://bioinfo.weizmann.ac.il/dipol/>.

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3 Results

3.1 Purification and biochemical characterization of crotamine

After purification, crotamine was evaluated in order to verify its purity. The purified peptide shows an ESI-MS experimental mass of 4885.6 Da which corresponds well with theoretical mass of 4886.32 Da. The N-terminal automated Edman sequencing showed the sequence YKQCHKKGGHCFPKE KICLP, the same residues described by Laure (1975).

3.2 Electrophysiological recordings

Crotamine was subjected to a screening on a wide range of 16 ion channels. Its activity was investigated on 12 cloned voltage-gated potassium channels ($K_V1.1$ - $K_V1.6$, $K_V2.1$, $K_V3.1$, $K_V4.2$ - 4.3 , *Shaker* IR and *hERG*) (Figure 1) and 4 cloned voltage-gated sodium channels ($Na_V1.2$, $Na_V1.3$, $Na_V1.5$ and the insect channel *DmNa_V1*) (data not shown). Crotamine showed no activity on Na_V channels at 3 μ M concentration ($n=4$) (Supplemental Figure 1). Interestingly, 3 μ M toxin could block $K_V1.1$ - 1.3 channels while the same concentration had no effect upon other K_V channel isoforms from the *Shaker* ($K_V1.4$ - $K_V1.6$ and *Shaker* IR), *Shab* ($K_V2.1$), *Shaw* ($K_V3.1$), *Shal* ($K_V4.3$ and $K_V4.3$), *erg* ($K_V11.1$) subfamilies. Due to its cytolytic effects, concentrations higher than 5 μ M could not be tested. Concentration response curves were constructed in order to determine the values at which half of the channels were blocked by crotamine. The IC_{50} values yielded 369 ± 56 nM, 386 ± 11 nM and 287 ± 92 nM for $K_V1.1$, $K_V1.2$ and $K_V1.3$, respectively. (Figure 2A). Since crotamine had the highest affinity for $K_V1.3$, this isoform was used to further investigate the characteristics of inhibition. The inhibition of $K_V1.3$ channels induced by the

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toxin was not voltage-dependent as in a range of test potentials from -20 mV to +40 mV no difference in the degree of block could be observed (Figure 2B). In order to investigate if the observed current inhibition is due to pore blockage or rather to altered channel gating upon toxin binding, the IV curves in ND96 and HK solution were constructed (Figure 2C and D). One micromolar (μM) of crostamine caused $71 \pm 3\%$ and $77 \pm 3\%$ inhibition of the potassium current in ND96 and HK, respectively ($n=4$). In ND96, the IV curves in control and in the presence of $1 \mu\text{M}$ toxin were characterized by $V_{1/2}$ values of 23 ± 2 mV and 27 ± 3 mV ($n=4$) respectively. It can be concluded that no significant shift in the midpoint of activation occurred ($*p>0.05$). Crostamine does not significantly influence the reversal potential E_K , as can be concluded from the IV relationship in HK solution ($*p>0.05$; $n=8$), showing that ion selectivity is not changed. E_K values yielded -10 ± 3 mV in control and -8 ± 3 mV after application of toxin. All together, these experiments imply that current inhibition upon crostamine binding does not result from changes in the voltage-dependence of channel gating. The inhibition of $K_V1.3$ channels occurred rapidly ($\tau_{\text{on}} = 35.2$ s) and its binding was reversible since the current recovered quickly ($\tau_{\text{off}} = 104.8$ s) and completely upon washout (data not shown).

3.3 Dipole moment calculation

The anisotropic moment of crostamine was calculated and it is represented by the purple trace pointing from the most negative towards the most positive region of the molecule (Figure 3A). Based on proposal of Ferrat et al. (2001)

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and Jouirou et al. (2004) for scorpion toxins and Chagot et al. (2005a,b), which electrostatic anisotropy acts as an orientating force of the ligand within the electrostatic field of the membrane receptor (Chagot et al., 2005a; Chagot et al., 2005b; Ferrat et al., 2002; Jouirou et al., 2004). We can infer that the Tyr1 and Lys2 residues, together with Arg31 and Trp32, might be involved in the interaction surface of crostamine towards Kv1.1-3 channels, as these basic and hydrophobic patches are exposed in a surface in the vicinity of the dipole moment emergence (Figure 3A-B).

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4 Discussion

4.1 Inhibition of K_V channels by crotamine

Here we report for the first time a thorough investigation of crotamine on 16 different ion channels. This broad screening not only reveals a significant activity against $K_V1.1$, $K_V1.2$ and $K_V1.3$, but also points out an interesting selectivity for these channels as none of the other isoforms tested were affected under the conditions of this study. So this peptide represents the newest member of snake toxins acting on K_V channels.

Even though a wide variety of peptides targeting voltage-gated potassium channels have been isolated from scorpions and spiders, the number of comparable toxins identified in the venom of snakes remains scarce (Mouhat et al., 2008; Rodriguez de la Vega and Possani, 2005). Up to date, only the dendrotoxins (DTXs) have been very well studied and characterized in depth (Harvey and Robertson, 2004). α -DTX and δ -DTX, isolated from the venom of the green mamba, *Dendroaspis angusticeps* and their respectively homologous, DTX-I and DTX-K from the black mamba, *Dendroaspis polylepis*, are all highly potent inhibitors of K_V channel isoforms of the *Shaker* subfamily (Harvey, 2001). Besides these dendrotoxins, only one other snake peptide capable of inhibiting K_V channels has been reported (Wang et al., 2006). Natrin is a cysteine-rich secretory protein (CRISP) isolated from the venom gland of the snake *Naja naja atra* that could block $K_V1.3$ channels within the nM range.

Crotamine belongs to the β -defensin-like superfamily (Figure 4), whose members exhibit relatively diverse biochemical and biological functions. Although, a first glance in the overall secondary structure suggests also a

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resemblance of crostamine as a small peptide stabilized by three disulfide bonds belonging to the structural cysteine-stabilized α -helix and β -sheet (CS $\alpha\beta$) superfamily. However, in most cases these peptides share a common function in innate immunity of animals, plants and microorganisms. The extensive distribution of this common motif throughout diverse organisms highlights that this relatively stable and versatile scaffold has the potential to tolerate insertions, deletions and substitutions within the structure (Zhu et al., 2005). Interestingly, the CS $\alpha\beta$ resemblance might suggest some similarity to scorpion toxins that block potassium channels.

Further electrophysiological characterization showed that crostamine does not modulate the voltage dependence of gating of K_V1.3 channels. The IV relationship in HK solution showed that ion selectivity was not changed after toxin binding. The observation that there is no difference on the percentage induced block either in ND96 or HK leads to the conclusion that channel blockage is independent of the direction of the potassium current flux and is not influenced by the extracellular concentration of K⁺ ions. Moreover, this toxin did not show voltage dependence in its blockage of channels in a wide voltage range. The inhibition of current through K_V1.3 channels occurred rapidly and the binding was reversible upon washout.

4.2 A functional dyad contributes to K_v channel inhibition

All the electrophysiological data suggest that the binding site of crostamine is presumably located at the extracellular side. It has been proposed that most toxins that block K_V channels possess a conserved functional core composed of a key basic residue (Lys or Arg) associated with a 6.6 ± 1 Å distant key

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hydrophobic or aromatic residue (Leu, Tyr or Phe). Such a functional dyad can be found in a broad range of structurally unrelated peptides from various animals such as scorpions, cone snails, snakes and sea anemones (Dauplais et al., 1997; Jouirou et al., 2004). However, it has been reported that besides this dyad, other determinants are required for a high affinity interaction between the toxin and its target (MacKinnon et al., 1998). Examples of toxins lacking a dyad but still capable of blocking K_V channels strongly suggest that the functional dyad on its own cannot represent the minimal pharmacophore or prerequisite for K_V1 binding (Shon et al., 1998). In general, it is assumed that toxins recognize the K_V1 subtypes through the interaction of their residues, among which the basic ring, with certain residues of the K_V1 channel turret. These interactions can be sufficient to inhibit the potassium current. Moreover, these specific molecular contacts determine toxin selectivity towards particular K_V1 channel isoforms. The functional dyad can then be viewed as a secondary anchoring point, providing a higher toxin affinity without altering its selectivity. The side chain of the basic key residue enters the ion channel pore and is surrounded by four Asp residues of the P-loop selectivity filter. The key hydrophobic residue of the dyad will interact through both hydrophobic forces and hydrogen bonding with a cluster of aromatic residues in the P-loop.

Using the solution structure of crotoxin and its electrostatic anisotropy represented by the dipole moment (Figure 3A), a surface of basic and aromatic residues is displayed (Arg31 Trp32 and Tyr1 Lys2). The residues in the vicinity of the emerging dipole moment may be considered as involved in the direct contact surface of a toxin towards its ligand (Chagot et al., 2005a;

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Chagot et al., 2005b; Jouirou et al., 2004). In this way, it would be possible that the Arg31 and Tyr1 might fulfill the requirements to function as a possible dyad, which is in concordance with its previously reported docking model of crotamine with K_V1.2 (Yount et al., 2009).

However, the calculated distance between the Arg31 and Tyr1 amino acids is 9.5 Å, which is larger than the ideal 6.6 ± 1 Å between the two key residues of the functional dyad as described above. The other possible dyad would be formed by Arg31 Trp32, and their distances are 3.8 Å apart. Furthermore, the overall comparison of crotamine Arg31 Trp32 putative dyad fits well with other dyads from known potassium channel blockers, as represented by their superimpositions (Figure 3C-F). However, these crotamine possible dyads supposed here are only hypothesized, thus requiring further confirmation from site-directed mutagenesis studies and structure analysis in order to define if crotamine is exhibiting its K_V channel inhibiting activity through a functional dyad and if so, which residues are definitely composing it.

4.3 K_v channel inhibiting properties of crotamine and its biological versatility

Previous works have clearly demonstrated that crotamine will serve as a lead compound in the development of diagnostic probes and delivery systems in proliferative cells (Kerkis et al., 2004). Furthermore, crotamine is considered as a very promising cell-penetrating peptide-mediated delivery drug and anti-cancer agent (Kerkis et al., 2010). In fact, the potent inhibition of K_V1.3 channels, as demonstrated in this work, contributes to the anti-tumor properties of crotamine. The enhanced expression of K_V1.3 channels and their critical role in the proliferation of several types of carcinogenic cell types has

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been well established (Bielanska et al., 2009). Over-expression of K_V channels has been reported for diverse cancers, such as breast cancer ($K_V1.1$ and $K_V1.3$), prostate cancer ($K_V1.3$) and melanoma ($K_V1.3$). Evidence indicates that K_V channel activity is a critical regulator of tumor cell proliferation by membrane polarization (Pardo et al., 2005; Wang et al., 2004). Thus, if crotamine acts on $K_V1.3$ in nontoxic concentration to humans, it might also be involved with the previously reported efficacy as an anti-tumoral agent against several aggressive tumorigenic cell lineages, such as, murine melanoma cells (B16-F10), human skin melanoma cells (SK-MEL-28) and pancreatic carcinoma cell line (MIA PaCa-2) (Pereira et al., 2011).

Also, crotamine should be evaluated as a potential 'tool' for treatment of autoimmune diseases (e.g. multiple sclerosis, rheumatoid arthritis and type-1 diabetes mellitus) (Beeton et al., 2001). Since $K_V1.3$ has been identified as the channel that sets the resting membrane potential of peripheral human T lymphocytes, they are responsible for activation of the cells involving an increase of cytosolic Ca^{2+} necessary for mitogen-induced activation that normally occurs following receptor-ligand coupling. Studies using selective blockers of $K_V1.3$ channel have proven that depolarization of the T cell membrane potential attenuate Ca^{2+} entry, and suppress the signaling cascade leading to cytokine production and cell proliferation (Beeton et al., 2008; Chi et al., 2012; Leonardi et al., 1992).

4.4 Conclusions

We have demonstrated the unique multiple function of crotamine, which is not only a cell penetrating peptide and anti-tumoral agent as reported before, but

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also a potent K_V channel inhibitor. Moreover, several amino acid residues have been suggested to play a functional and critical role in the potent K_V channel inhibition of this toxin. Also, the *in vivo* effects of crostamine and some of the previous contradictory electrophysiological results shown in the literature might be explained based on its novel K_V channel blockage activity. Crostamine is together with the dendrotoxins one of the few snake K_V channel toxins known up to date. Furthermore, it is, to our knowledge, the first snake K_V channel toxin isolated from a non-mamba species. The fact that crostamine acts upon K_V channels as shown here, raises the interesting possibility that other defensin-like peptides present in the venom of snakes and other venomous animals might also exert some affinity towards voltage-gated potassium channel.

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Acknowledgements

We would like to thank O. Pongs for sharing the rK_V1.2, rK_V1.4, and rK_V1.5 and rK_V1.6 cDNA. We are grateful to M.L. Garcia for sharing the hK_V1.3 clone and to DJ Snyders for sharing the rK_V2.1, hK_V3.1, rK_V4.2 and rK_V4.3. The *Shaker* IR clone was kindly provided by G. Yellen. We thank M. Keating for sharing h*ERG*, A.L. Goldin for sharing rNa_V1.2, rNa_V1.3, G, R.G. Kallen for sharing hNa_V1.5, S.H. Heinemann for sharing the ratβ1 subunit, S.C. Cannon for sharing the hβ1 subunit and Martin S. Williamson for providing the Para and tipE clone.

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Authorship Contribution

Participated in research design: Orts, Prieto da Silva, de Freitas

Conducted experiments: Peigneur, Orts

Contributed new reagents or analytic tools: Prieto da Silva, Boni-Mitake, Oguiura, de Oliveira, Tytgat

Performed data analysis: Peigneur, Orts, Prieto da Silva, Zaharenko

Wrote or contributed to the writing of the manuscript: Peigneur, Orts, Prieto da Silva, Zaharenko, Oguiura, Oliveira, Boni-Mitake, de Freitas and Tytgat

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Footnotes

Steve Peigneur and Diego Jose Belato y Orts contributed equally to this work.

This research was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo [2009/07128-7], Programa de Apoio à Pós-graduação-Coordenação de Aperfeiçoamento de Pessoal de Nível Superior 2010 (Brazilian Government – grants to D.J.B.O.) and Conselho Nacional de Desenvolvimento Científico e Tecnológico [490194/2007-9] to J.C.F. J.T. was supported by the following grants: [G.0257.08] and [G.0330.06] (Fonds Wetenschappelijk Onderzoek Vlaanderen), Onderzoeks Traject K.U. Leuven [05-64], Universitaire Attractie Pool 6/31 (IAP).

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Figures Legends

Figure 1: Activity of crotamine on K_V channels.

The current were measured in *X. laevis* oocytes expressing several cloned voltage-gated potassium channel isoforms. Traces shown are representative of at least three independent experiments ($n \geq 3$). The dotted line indicates the zero-current level. The asterisk (*) distinguishes the steady-state current after application of 3 μM of crotamine.

Figure 2: Effects on $K_V1.3$ channel gating.

A: Concentration-response curve on $K_V1.1$, $K_V1.2$ and $K_V1.3$ channels obtained by plotting the percentage blocked current as a function of increasing toxin concentrations.

B: The plot shows the degree of inhibition at a broad range of potentials. No voltage dependence of inhibition was observed.

C and D show IV curves in ND96 and HK, respectively of $K_V1.3$. The potentials were tested in a range from -30 mV to +70 mV. Closed circles shows the $V_{1/2}$ in control and the open circles the addition of 1 μM crotamine.

All data represent at least 3 independent experiments ($n \geq 3$) and are presented as mean \pm standard error.

Figure 3: Crotamine putative 'functional dyad' and K-toxins from sea anemones and scorpions.

Previous work has shown that, based on differences in topography of key residues, K_V channel toxin can be classified in distinct classes (Srinivasan et al., 2002). Therefore crotamine putative 'functional dyad' (Arg31 Trp32) was

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determined by superimposition for best fit with well described 'functional dyads' of several sea anemone and scorpion toxins acting on voltage-gated potassium channel. The data in the parenthesis indicate the number of residues, disulfide bridges, followed by the Protein Data Bank accession number. Secondary structure: α -helix (Vargas et al., 2002), β -sheet (yellow), loops (Shon et al., 1998), SS-bonds (green).

A: Crotonamine dipole and presumed functional dyads (Tyr1 Lys2 and Arg31 Trp32), dipole momentum is depicted in purple

B: Crotonamine presumed 'functional dyad' Arg31 Trp32.

C-F (Upper panel): K-toxins structures and 'functional dyads': BgK from the sea anemone *Bunodosoma granulifera* (Lys25 Tyr26); ShK from *Stichodactyla helianthus* (Lys22 Tyr23); κ -Hefutoxin from the scorpion *Heterometrus fulvipes* (Tyr5 Lys19); Charybdotoxin from *Leiurus quinquestriatus hebraeus* (Lys27 Tyr36).

C-F (Lower panel): Close-ups of K-toxins 'functional dyads' superimposition with crotonamine functional dyad (Arg31 Trp32). Crotonamine is depicted in gold and K-toxins in blue (ribbon representation with transparency, respectively). The 'functional dyads' are also depicted in gold and blue, respectively.

Figure 4: Tridimensional scaffold of crotonamine and β -defensin-like peptides.

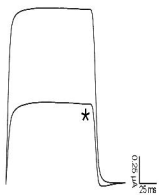
The orientation of β -defensin-like structures was determined by superposition for best fit with the cysteine heavy atoms involved in disulfide bonds. PDB models: 1Z99 - Crotonamine from *Crotalus durissus terrificus*; 1FD3 - Human β -defensin; 1WQK - APETx-1 from the sea anemone *Anthopleura*

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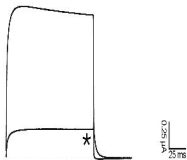
elegantissima. Secondary structure: α -helix (Vargas et al., 2002), β -sheet (yellow), loops (Shon et al., 1998), SS-bonds (green).

Figure 1

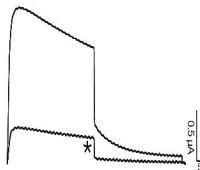
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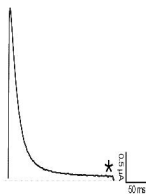
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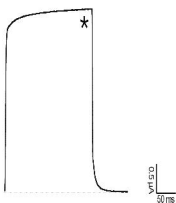
$K_V1.3$



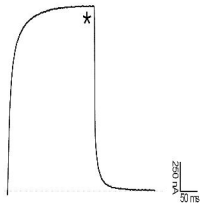
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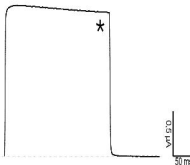
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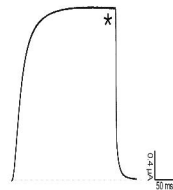
$K_V1.6$



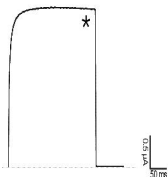
Shaker IR



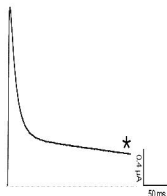
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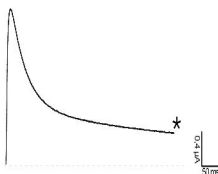
$K_V3.1$



$K_V4.2$



$K_V4.3$



hERG

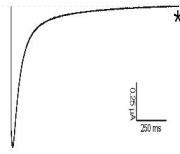
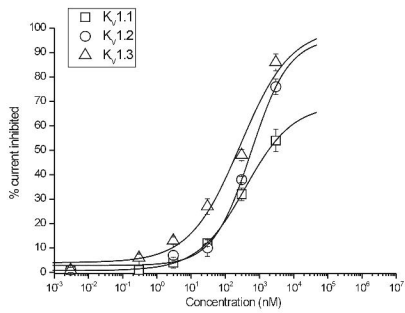
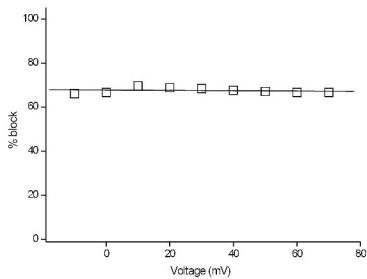


Figure 2

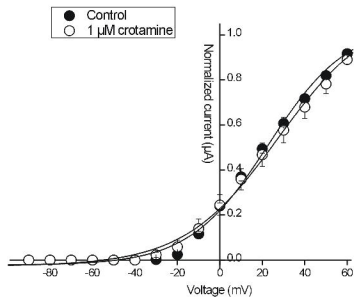
A



B



C



D

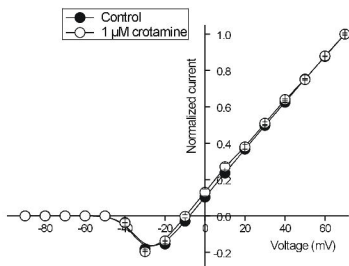


Figure 3

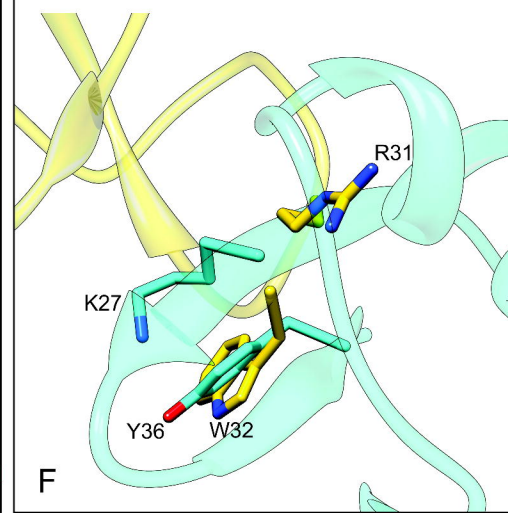
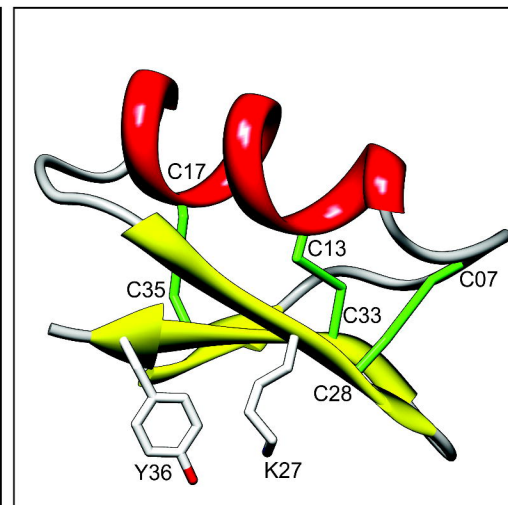
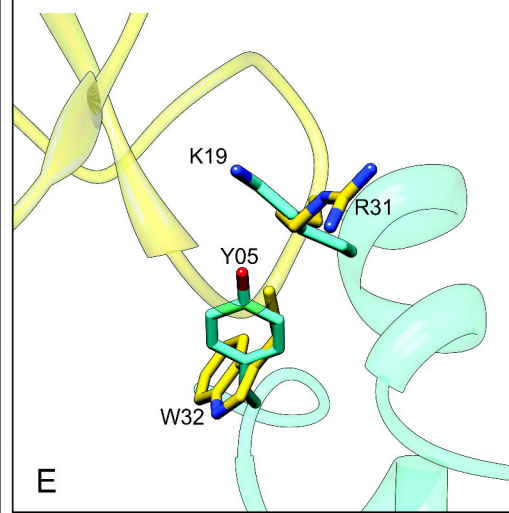
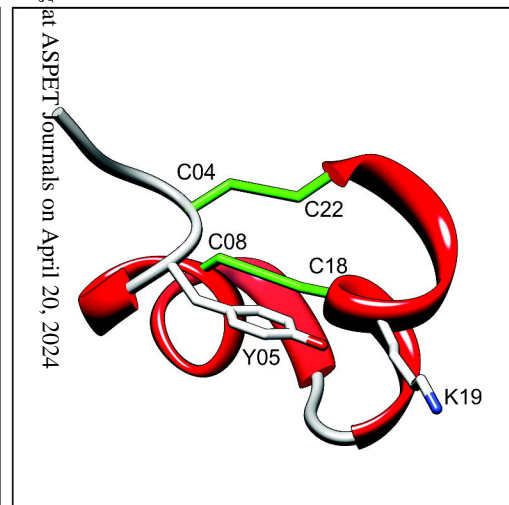
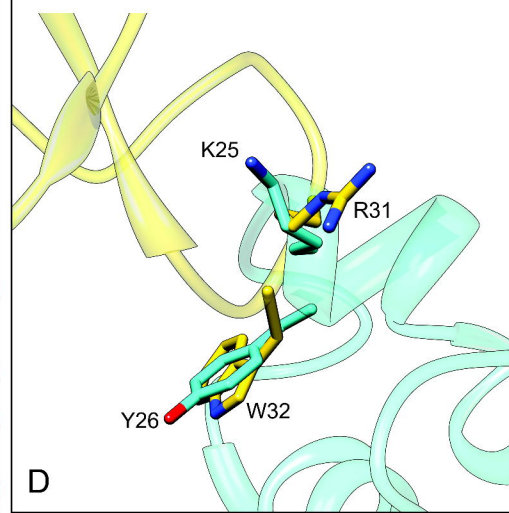
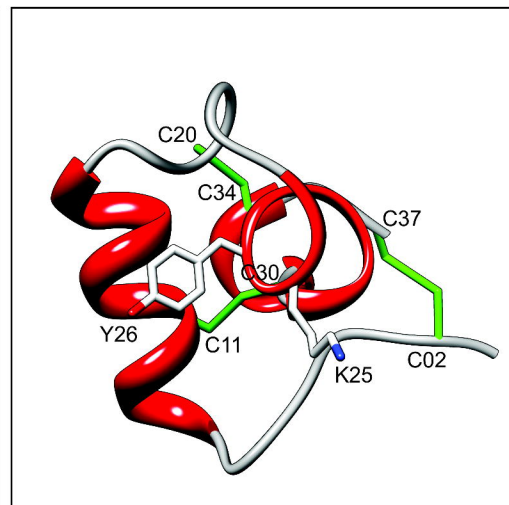
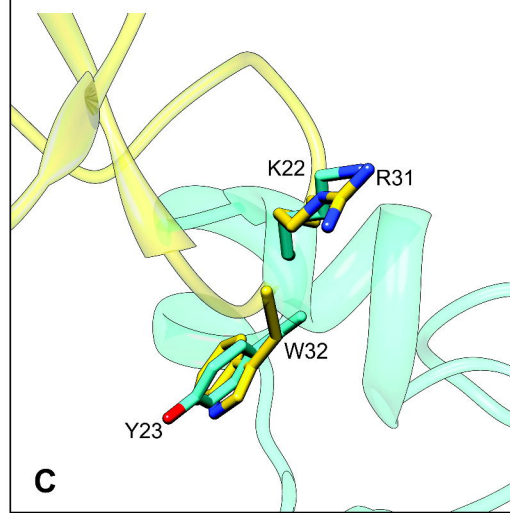
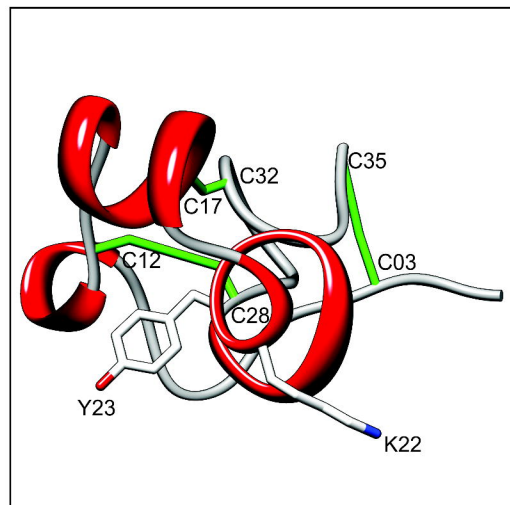
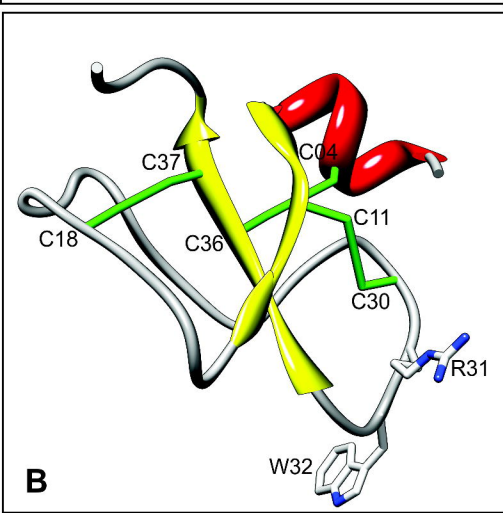
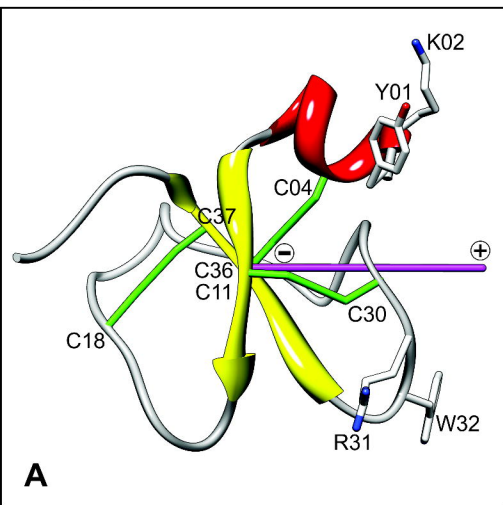


Figure 4

