

subunits from the model organism *Hydra magnipapillata*. These subunits (HyNaC 1-5) form heterotrimeric channels gated directly by endogenous neuropeptides. Together with FaNaC, a related ion channel from snails, HyNaCs are the only known peptide-gated ion channels. Coinjection of HyNaC 2/3/5 in *Xenopus* oocytes leads to expression of a peptide-gated cation channel with higher permeability for sodium than for potassium.

Here we show that HyNaC 2/3/5 channels have a substantial permeability for calcium, which is in the range of the purinergic P2X₄ receptor. In *Xenopus* oocytes, this calcium influx through HyNaC 2/3/5 strongly activates endogenous calcium-activated chloride channels (CaCCs), leading to a biphasic current. Blocking of the CaCCs reveals a step-like current without apparent desensitization. Moreover, we found that calcium permeability of HyNaCs is largely dependent on a single acidic amino acid at the outer entry of the pore region. Substitution of this acidic residue significantly reduces calcium permeability. The homologous position has been previously reported to be involved in calcium modulation of the related DEG/ENaC channel ASIC 1a (Paukert 2004). Collectively, these results suggest that HyNaCs might play an important role in calcium signalling *in vivo*.

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A Natural Point Mutation Reveals Target Promiscuity of Toxins Isolated from the Sea Anemone *Anthopleura elegantissima*

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Sea anemone venom is a known source of interesting bioactive compounds, including peptide toxins which are invaluable tools for studying structure and function of voltage-gated potassium channels. APETx3 is a novel peptide isolated from the sea anemone *Anthopleura elegantissima*, containing 42 amino acids cross-linked by 3 disulfide bridges. Sequence alignment reveals that APETx3 is a natural occurring mutant from APETx1, only differing in 1 amino acid at position 3. APETx1 is believed to be a selective modulator of human *ether-á-go-go related* (hERG) potassium channels. In this study, APETx1, 2 and 3 have been subjected to an electrophysiological screening on a wide range of 21 ion channels expressed in *Xenopus laevis* oocytes: 10 cloned voltage-gated sodium channels (Na_v1.2-Na_v1.8, the insect channels DmNa_v1, BgNa_v1-1a and the arachnid channel VdNa_v1) and 11 cloned voltage-gated potassium channels (K_v2.1, K_v3.1, K_v4.2, K_v4.3, K_v7.1, K_v7.2, K_v7.3, K_v7.4, K_v7.5, hERG, the insect channel *Shaker* IR). Surprisingly, the Thr3Pro substitution results in a complete abolishment of APETx3 modulation on hERG channels. However, the same substitution provides this toxin the ability to become a potent modulator of voltage-gated sodium channels (Na_vs). APETx3 slows down the inactivation of mammalian and insect channels similar to site 3 toxins such as α -scorpion toxins and sea anemone Na_vs toxins. Our screening reveals that the homologous toxins APETx1 and APETx2 display promiscuous properties as they are also capable of recognizing Na_v channels, causing an inhibition of the sodium conductance.

All together, these data provide new insights in key residues which allow these toxins to recognize distinct ion channels with similar potency but with different modulatory effects. Furthermore, we describe for the first time the target promiscuity of a family of sea anemone toxins believed to be highly selective.

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Positive Charges of Discrepin are Responsible for the Block of K⁺ Channels of Cerebellum Granular Cells

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The peptide Discrepin, isolated from the venom of the buthid scorpion *Tityus discrepans*, blocks preferentially the IA currents of the voltage-dependent K⁺ channel of rat cerebellum granular cells. There are only six scorpion toxins known in the sub-family α -KTx15 and Discrepin is the one with the least identity (approx. 50%) when compared to the others. Furthermore it is the only one whose action on the blockade of the IA current is irreversible. In order to find out which residues are important for the blocking effects of discrepin, two mutants were chemically synthesized ([V6K,D20K] and K13deleted), correctly folded and their physiological properties were examined. Electrophysiological experiments performed with the double mutant V6K-D20K show that increas-

ing the number of positive charges augments the blocking activity: the affinity for the voltage-dependent K⁺ channels increases 10 folds when compared to the native peptide. Viceversa, the deletion of the lysine residue at position 13 removes the interaction of toxin with the channels, confirming the essential role of positive charges played in channel blockade. Additionally, comparative analysis of 3D-structure models of the mutants V6K, D20K and V6K-D20K suggests that probably the N-terminal segment of Discrepin might be the surface implicated in the recognition of the ion-channel.

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Crotamine Toxicity Revisited: Novel Insights Based on K_v Channel Inhibition

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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 IC₅₀ of ~300 nM and the key amino acids responsible for this molecular interaction are highlighted. 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 such as cell penetrating, antitumoral activity and K_v channel inhibiting properties. The potent and selective K_v channel inhibiting properties, as demonstrated in this work, can be an advantage for the use of crotamine or its derivatives as anti-tumor drug. This new property of crotamine might explain some experimental observations and opens new perspectives of pharmacological uses.

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Anti-Obesity Effect of Shk-186, a K⁺ Channel Blocker

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Obesity is a global health problem and there is a pressing need for safe therapeutics. Here, we describe the anti-obesity effects of ShK-186, a K_v1.3-selective peptide inhibitor with picomolar potency and an excellent safety profile in rodents and monkeys. C57BL/J mice fed a diet containing high fat (21%) diet and fructose solution (60%) were administered ShK-186 (20, 100 or 500 µg/kg) or vehicle on alternate days by subcutaneous injection. ShK-186-treatment caused a dose-dependent reduction in weight gain over the 45-day trial, the maximal weight difference being 30%. ShK-186-treatment reduced plasma levels of triglyceride and free fatty acids without affecting cholesterol, HDL, LDL or glucose. CT scan revealed significant reduction in volume of white adipose tissue (WAT) in ShK-186-treated mice compared to vehicle-controls. However, histology revealed no difference in cell size and fat content of white fat cells. In metabolic cage experiments, ShK-186-treatment increased cumulative intake of food and fluid compared to vehicle-treated mice, especially during the light-cycle. Furthermore, their respiratory exchange ratio and energy expenditure were also higher. In PET experiments, ShK-186 treatment increased uptake of 18-fluoro-deoxyglucose (¹⁸FDG) by brown adipose tissue (BAT) and skeletal muscle compared to vehicle-controls; pre-administration of the beta-adrenoceptor blocker propranolol (5mg/kg) inhibited ¹⁸FDG uptake by BAT but not skeletal muscle. ShK-186's anti-obesity effect appears to be mediated by activation of BAT and/or skeletal muscle, two important thermogenic tissues in the body.