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# The first peptide ASIC1a channel modulators from sea anemones

*Three new ASICs modulators from the sea anemone Heteractis crispa, Hcr 1b-2, Hcr 1b-3, and Hcr 1b-4, exhibit surprising activity on ASIC1a channel. Homology modeling of peptide spatial structures revealed dispositions of basic amino acid residues different from those in prototypical APETx2 toxin from Anthopleura elegantissima inhibiting ASIC3. These results support the hypothesis that Heteractis crispatoxins dock onto ASIC1a channel in different spatial orientation than their closest homolog, APETx2, do upon ASIC3 inhibition.*

*Keywords: sea anemone, peptides, modulators of ASIC channels.*

Pharmacology of natural peptide toxins and diversity of their biological effects are complicated and intensively studied problems. Currently, many investigations focus at screening of biologically active natural compounds as well as clarifying their interaction mode with pharmacologically perspective molecular targets to design more effective analogs. Acid sensing ion channels (ASICs) were initially discovered and recognized as neuronal proton sensors by Waldmann and coworkers [6]. Now these channels are characterized as primary receptors gating by local (patho)physiological tissue acidosis resulting in pain or neurodegeneration. So inhibitors of ASIC1a and ASIC3 channels, that are the main receptors of central and peripheral nervous system, respectively, are considered as neuroprotective or analgesic compounds [1]. Little more than 12 proteinaceous toxins affecting ASICs channels were described from spider, snake and sea anemone venom so far. All of them are basic (pI 8.5–11), relatively small (29–59aa), and cysteine-rich peptides inhibiting ASICs (except for large heterodimeric complex MitTxα/β). Interestingly, toxins isolated from spider and snake venoms modulate ASIC1a and ASIC1a-contaning channels, in contrast to the sea anemone toxins selective for ASIC3 and ASIC3-contaning channels (except for MitTxα/β activating both ASIC1a and ASIC3) [1].

Three new peptides, π-AnmTX Hcr 1b-2, -3, and -4 (41 aa long), from the tropical sea anemone *H. crispa* were identified and characterized as the first ASIC1a modulators from sea anemone venom [4]. The combination of Edman degradation and tandem mass spectrometry allowed us attribute them to the structural class 1b of sea anemone toxins. This class is also represented by Hcr 1b-1, APETx1–APETx4, BDS-I, BDS-II, and crassicorin-I with various selectivity for ASIC3, voltage-gated sodium and potassium channels as well as poorly studded

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group of five APETx-like toxins from *Bunodosoma granulifera*. Hcr 1b-2, -3, and -4 share 78–95% and 46–49% identity with ASIC3 modulators, Hcr 1b-1 from *H. crispa* and APETx2 from *A. elegantissima*, respectively [4]. APETx2 was originally understood as prototypical and selective ASIC3 inhibitor from the sea anemone [2]. However, been studied in great detail, this toxin was shown to inhibit voltage-gated sodium ($Na_V1.2$, $Na_V1.6$, and $Na_V1.8$) and potassium ($K_V3.4$ and$K_V11.1$) channels [5, 2, 3]likewise to other class 1b members. Anyway so far APETx2 was the single peptide among class 1b that was demonstrated to modulate ASICs channels.

Similarly to APETx2 (PDB ID: 1WXN and 2MUB), Hcr 1b-2, -3, and -4 peptides are composed of compact four-stranded β-sheet stabilized by three disulfide bridges. Virtually all amino acid residues forming a putative interaction surface of APETx2 with ASIC3 [3] are conserved between APETx2 and *H. crispa* toxins. But key region of APETx2 vary from Phe15-Tyr16-Arg17 toPhe15-Met16-Leu17in *Heteractis* toxins. Moreover APETx2is proposed to form a basic/hydrophobic cluster (Thr2, Phe15-Tyr16-Arg17, Phe33, and Leu34) to interact with ASIC3 channel [3]. However homology models of Hcr 1b-2, -3, and -4 toxins demonstrate there are no basic residues spatially near to residues Thr2, Phe15-Met16-Leu17, Phe33, and Leu34 in *H. crispa* toxins (Fig. 1A). So Hcr 1b-2, -3, and -4 molecules presumably interact with ASIC3 via an alternative cluster of residues centered around of Lys5, Lys40, or Lys41 (Hcr 1b-2 or Hcr 1b-3) and Arg19, Lys40, or Arg41 (Hcr 1b-4). It should be noted, that some of these basic residues may directly not contribute substantially to interaction with ASICs, even when localized within basic/hydrophobic cluster. For example, Arg31 residue which was emphasized to be a part of such cluster (Phe15-Tyr16-Arg17, Phe33, and Leu34) is not important for APETx2 activity onASIC3 according to alanine mutagenesis [3]. So any hypothesis considering the interaction between APETx-like peptides and ASIC channels need to be verified both by computational and experimental approaches (scanning mutagenesis).

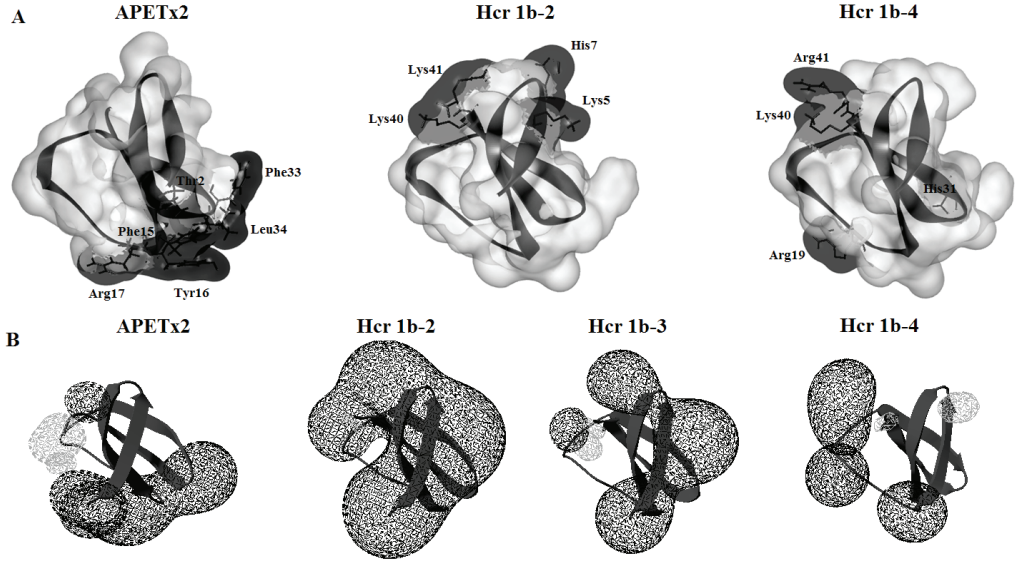


**Figure. 1.** Three dimensional structure of APETx2 (PDB ID: 1WXN) and homology models of Hcr 1b-2, Hcr 1b-3, and Hcr 1b-4. A. Molecular surface representation showing the putative sites of APETx2 interaction with ASIC3 channel, according Jensen and coworkers [3].The spatial location of the basic residues of Hcr 1b-2 (Hcr 1b-3 is not shown since being the same) and Hcr 1b-4 are shown as molecular surface. The basic residues of Hcr 1b-2 and Hcr 1b-4 as well as residues of APETx2 that, when mutated to alanine have a major impact on the peptide's ability to inhibit ASIC3, shown as sticks and colored grey (basic residues) or dark grey (hydrophobic residues of APETx2). B. Equipotential surfaces of APETx2, Hcr 1b-2, Hcr 1b-3, and Hcr 1b-4. Negative and positive potential are colored light grey and black, respectively.

Due to differences in sequence, homology models of Hcr 1b-2, -3, and -4 toxins are different from APETx2 in term of surface charge distribution. Remarkably, distribution of positive electrostatic potential of Hcr 1b-4 is distinct from those of both APETx2 and other *Heteractis* toxins (Fig. 1B). There is every likelihood, based on electrostatic properties, that homologous toxins, APETx2, Hcr 1b-2 or Hcr 1b-3, and Hcr 1b-4 have three unique spatial orientations in their complex with ASIC channels. This discrepancy may also be responsible for Hcr 1b-2, -3, and -4 favoring ASIC1 channel, while APETx2 preferentially target ASIC3.

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