UDC 577.29 DOI: 10.25808/08697698.2018.202.6S.011

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First two-chain peptide toxin from sea anemone *Heteractis crispa*

Key words: sea anemone, type II toxins, electrophysiology.

Sea anemone venoms contain diverse toxins most of which target voltage-gated sodium channels (Na_V). More than fifty toxins of four distinct structural types have been isolated and characterized from different sea anemone species. All of the sea anemone toxins are expected to bind with site 3 of the Na_V inhibiting channel inactivation alike to scorpion α-toxins and spider δ-toxins do regardless of their different folds. Despite many toxins have been isolated, a limited number of toxins were investigated in terms of ability to discriminate between closely related Na_V subtypes.

In this work, two known type II toxins, RTX-III and δ-SHTX-Hcr1f (= RpII), as well as new, RTX-VI, were isolated from 20% aqueous-ethanol fraction of *Heteractis crispa*. Toxin RTX-VI turned out to be an unusual natural analog of RTX-III consisting of two, not one, disulfide-linked peptide chains and was devoid of Arg13 which is important for the selectivity and affinity of such peptides to Na_V channels. At the same time, CD spectra of three peptides substantially overlapped indicating an identical conformation of these toxins. Electrophysiological screening of *Heteractis* toxins on nine Na_V channel isoforms revealed their different selectivity. Obtained data demonstrate that δ-SHTX-Hcr1f and RTX-VI toxins modulate insect ($BgNa_V1$, $VdNa_V1$) and CNS ($Na_V1.1$-1.3, $Na_V1.6$) Na_V but not skeletal muscle ($Na_V1.4$) or PNS ($Na_V1.8$) channels. Among three investigated toxins, only RTX-III affected cardiac $Na_V1.5$. The absence of Arg13 in RTX-VI structure abolish its effect on the $Na_V1.5$ and $Na_V1.3$ currents, however, this does not prevent toxin binding with $Na_V1.6$ regardless of efficacy decrease. Moreover, distinct from RTX-III, RTX-VI was shown to modulate $Na_V1.2$ of CNS. Thus, *H. crispa* type II toxins could be used as molecular tools for studying of some subtypes of mammalian or insect Nav channels rather than as pharmacological agents.

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This work was partially supported by RFBR according to the research project № 18-04-00631 and the Presidium of Far Eastern Branch of the Russian Academy of Sciences (grant number RUS_ST2017-228).