Selective and voltage dependent inhibition of N-type calcium channels by novel ω-Conotoxins CVIE and CVIF

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N-type Ca^{2+} channel selective ω-conotoxins have recently emerged as potential new drugs for the treatment of severe chronic pain. In this study, two new ω-conotoxins, CVIE and CVIF, were discovered and synthesized following a PCR screen of a *Conus catus* cDNA library. All animal experimentations were performed in accordance to guidelines of the University of Queensland Animal Ethics Committee. Both ω-conotoxins potently displaced ^{125}I-GVIA binding to adult rat brain membrane. In *Xenopus* oocytes, CVIE and CVIF inhibited Ba^{2+} currents through recombinant N-type (α_{1B}, $\alpha_2\delta_1$, and β_3) channels with IC_{50} values of 2.6 ± 0.5 nM (n = 14) and 19.9 ± 3.2 nM (n = 16), respectively. Consistent with our previous study (Mould *et al.,* 2004), both peptides exhibited increased affinity for N-type Ca^{2+} channels in the absence of auxiliary $\alpha_2\delta_1$ subunit. Neither CVIE nor CVIF had any effect on P/Q-, R- or L-type calcium channels. The voltage dependence and the time course of CVIE and CVIF block were analyzed and the current recovered completely from block at -125 mV but only partially at -80 mV, indicating that CVIE and CVIF have a higher affinity to Ca^{2+} channels in the inactivated state. The analogues [R10K]CVIE and [R10K]CVIF did not significantly alter the conserved ω-conopeptide backbone conformation, but increased the rates of onset and offset of ω-conotoxin action and improved recovery from block at -80 mV, compared to the native peptides. In DRG sensory neurons isolated from neonatal rats, CVIE and CVIF potently and selectively inhibited N-type Ca^{2+} channels and the reversibility of block was voltage dependent. The R10K substitution altered the kinetics of block and was associated with an increased rate and recovery from block at -80 mV, compared to the native peptide. In rat spinal cord slices, CVIE and CVIF inhibited reversibly excitatory monosynaptic transmission between primary afferents and dorsal horn superficial lamina neurons. This result suggests the presence of an inactivation-resistant N-type Ca^{2+} channel population in the presynaptic nerve terminals. These N-type channel specific ω-conotoxins are potentially useful neurophysiological tools and inhibitors of nociceptive signaling with therapeutic implications.

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