Differential action of ω-conotoxins CVID and CVIB on voltage-gated calcium channels in rat sensory neurons

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Selective antagonists of voltage-gated calcium channels (VGCCs) are of considerable interest both as research tools and potential therapeutic agents. The selectivity of VGCC antagonists is essential for dissecting the various Ca^{2+} channel types underlying the whole-cell Ca^{2+} current whereas potency and reversibility play an important role in the use of a VGCC antagonist as a pharmaceutical agent. ω-Conotoxins GVIA, MVIIA and MVIIC have been used routinely as selective blockers of N- and P/Q-types of VGCCs in excitable cells. However, the newly discovered ω-conotoxins from *Conus catus*, CVID has been shown to have the highest selectivity for N-type over P/Q-type VGCCs among the other N-type selective VGCC antagonists (Lewis *et al.*, 2000). The present study investigated the selectivity, potency and reversibility of action of ω-conotoxins CVID and CVIB in isolated sensory neurons dissociated from rat dorsal root ganglia (DRG) and on recombinant VGCCs expressed in *Xenopus* oocytes. Bath application of either CVID or CVIB inhibited depolarization-activated whole cell Ba^{2+} currents in DRG neurons with pIC_{50} values of -8.12 ± 0.05 and -7.64 ± 0.08, respectively. The block of Ba^{2+} currents in DRG neurons by CVID appeared to be irreversible after >30 min washout whereas Ba^{2+} currents exhibited rapid recovery from block by CVIB (>80% within 3 min). ω-Conotoxin CVIB inhibited more of the whole-cell Ba^{2+} current in DRG neurons than CVID and the recoverable component of the Ba^{2+} current inhibited by CVIB was mediated by the N-type VGCC. The potency of CVID and CVIB block of N- and P/Q-type VGCCs was compared with the ω-conotoxins, GVIA, MVIIA and MVIIC. ω-Conotoxins GVIA and MVIIA inhibited Ba^{2+} currents in DRG neurons to a similar degree as CVID. The residual current amplitude obtained in the presence of maximally effective concentrations of the ω-conotoxins was: GVIA, 54 ± 0.2%; MVIIA, 41 ± 0.04% and CVID, 34 ± 1%. The residual current after block by CVIB was 3 ± 5% of control level reflecting non-selective N- and P/Q- action of the toxin. ω-Conotoxin CVIB reversibly inhibited Ba^{2+} currents mediated by N- ($Ca_v2.2$) and P/Q- ($Ca_v2.1$) type VGCCs expressed in *Xenopus* oocytes. The $\alpha_2\delta_1$ auxiliary subunit coexpressed with $Ca_v2.2$ and $Ca_v2.1$ reduced the potency of CVIB as reported previously for CVID at recombinant N-type VGCCs (Mould et al., 2004). The present study demonstrates that ω-conotoxins CVID and CVIB can be successfully used for pharmacological isolation of N- and P/Q- components of the Ca^{2+} conductance in rat DRG neurons. CVID selectively and irreversibly blocked the N-type component of the whole cell Ba^{2+} current in DRG neurons but blocked reversibly the recombinant N-type ($Ca_v2.2$) VGCC. In contrast, CVIB blocked reversibly the N-type component and blocked irreversibly P/Q- component of the whole cell Ba^{2+} current in DRG neurons. ω-Conotoxins CVID and CVIB may be useful as antagonists of N- and P/Q-type VGCCs in sensory neurons involved in processing primary nociceptive information.

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