$GABA_B$ receptor gene knockdown impairs inhibition of N-type calcium channels in rat sensory neurons by α-conotoxin Vc1.1

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G protein coupled $GABA_B$ receptors are receptors for γ-aminobutyric acid (GABA) and are widely expressed in the central and peripheral nervous system. They regulate synaptic transmission and signal propagation by modulating the activity of voltage-gated calcium channels and inwardly rectifying potassium channels. Functional $GABA_B$ receptors are heterodimers consisting of $GABA_{B1}$ and $GABA_{B2}$ subunits. The analgesic α-conotoxin Vc1.1, a peptide from the venom of the marine cone snail *Conus victoriae*, inhibits N-type calcium channels in sensory neurons *via* activation of G protein coupled $GABA_B$ receptor (Callaghan *et al.*, 2008). Although there is unambiguous pharmacological evidence demonstrating that Vc1.1 does not interact directly with N-type calcium channels but inhibits them *via* a voltage-independent mechanism involving the $GABA_B$ receptor, to date, there are no molecular studies confirming the interplay between Vc1.1 and the $GABA_B$ receptor. The aim of the present study was to examine the effect of the $GABA_B$ agonist baclofen and Vc1.1 on N-type calcium channel currents recorded in sensory neurons following transient knock-down of the $GABA_B$ receptor using RNA interference (RNAi). Dorsal root ganglion (DRG) neurons isolated from 3-12 days old rats were transfected with small interfering RNAs (siRNAs) targeting both $GABA_B$ subunits. One day after transfection a reduction of over 50% in mRNA levels for $GABA_B$ subunits was observed compared to control cells (mock cells transfected without siRNA and scrambled non-targeting siRNA transfected cells), as demonstrated in quantitative real-time PCR analysis of a minimum of 4 independent experiments. Moreover, suppression of $GABA_B$ protein expression was evaluated immunocytochemically using a high-content imaging system and confocal laser scanning microscopy 2-3 days after transfection. Quantitative analysis of immunofluorescence-labeled $GABA_B$ proteins in DRG neurons revealed significantly reduced $GABA_B$ expression in cells transfected with $GABA_B$-targeting siRNAs compared to control cells. Whole-cell patch-clamp studies conducted 1-3 days after transfection demonstrated that knock-down of functional $GABA_B$ receptor expression significantly reduced the inhibition of N-type calcium channels in response to both baclofen (30 μM) and Vc1.1 (100 nM) in isolated DRG neurons. This is in contrast to neurons transfected with a non-targeting siRNA which were not distinguishable from untransfected neurons. Taken together, these results confirm that α-conotoxin Vc1.1 modulates N-type calcium channels *via* activation of the $GABA_B$ receptor in DRG neurons.

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