PW211

Vc1.1, an α-conotoxin, reverses mechanical allodynia and inhibits ectopic discharge in neuropathic pain models.

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Aim of Investigation

It has been suggested that the α-conotoxin Vc1.1 exerts its analgesic effect via an action at nicotinic acetylcholine receptors (nAChR) and/or via $GABA_B$ receptors coupled to N-type voltage-gated calcium channels (VGCC). To further explore the utility of Vc1.1 for various pain conditions and the site and mechanisms of action of this compound, here we have examined the effects of Vc1.1 on mechanical allodynia and ectopic discharge (ED) of peripheral nerves in rat models of neuropathic pain (chronic constriction injury, CCI and Chung models) and have examined the cellular/ionic mechanisms of action of Vc1.1 in spinal cord slice preparations and acutely isolated dorsal root ganglion (DRG) neurones prepared from neuropathic pain models.

Methods

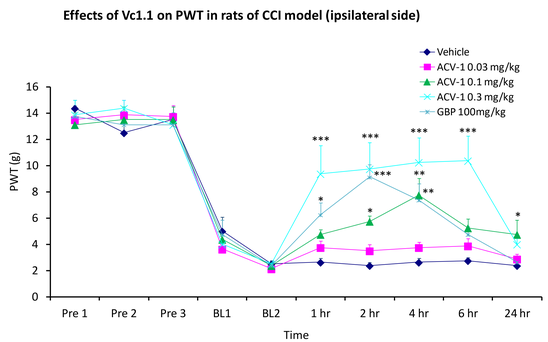
Rat CCI models were prepared by placing 4 loose ligations on the left sciatic nerve. Chung models were prepared by tightly ligating the L5 spinal nerve. Two weeks following surgery, the status of the neuropathic pain condition was confirmed by monitoring mechanical allodynia using von Frey hairs prior to behavioural and electrophysiology experiments. For behavioural studies, model rats were placed in individual plastic boxes on a metal mesh leaving the plantar surface exposed to von Frey hair testing. Vc1.1 was administered intramuscularly (i.m.) following measurement of baseline paw withdrawal threshold (PWT) using von Frey hairs and changes in PWT monitored up to 24 hours.

For *in vivo* electrophysiology, rats were anaesthetised with urethane, and ectopic discharge originating from the neuroma and DRG were recorded from small bundles of sural nerve or dorsal root filaments. Windup of dorsal horn neurones of spinal cord in Chung models was induced by a train of stimulus composed of 16 square pulses in 5 sec at an intensity of 2×Threshold every 5 min. The dose of Vc1.1 was 0.1mg/kg, iv for all *in vivo* electrophysiological studies.

Whole-cell patch clamp recordings were obtained from lamina I/II dorsal horn neurones in thick (450-500 µm) longitudinal spinal cord slices, with dorsal roots maintained intact, prepared from Chung and CCI models. For isolated DRG neurone recording whole-cell patch clamp recordings were obtained from acutely dissociated small, medium and large diameter neurones prepared from Chung model animals.

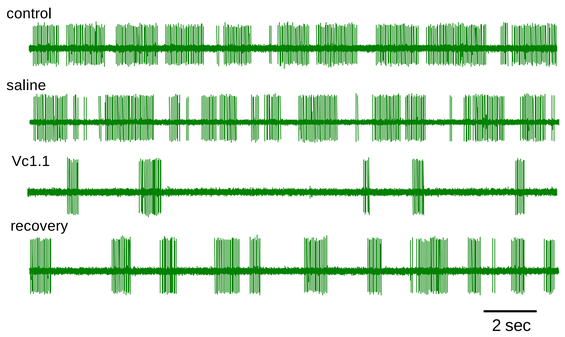
Results

❶ In rat CCI models, intramuscular injection of Vc1.1 dose-dependently reversed the mechanical allodynia.

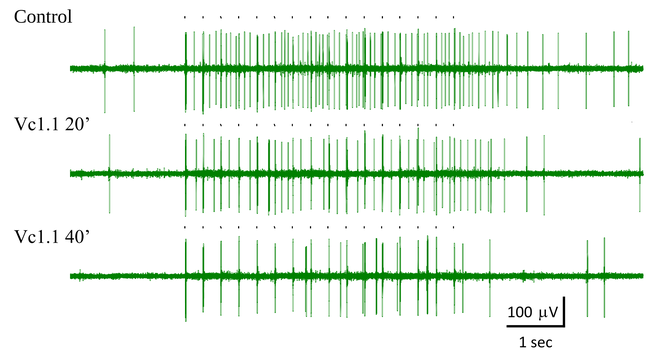


*, **, ***: $P < 0.05$, 0.01 and 0.001, compared to vehicle group, one-way ANOVA, n=8 for each group.

❷ *In vivo* electrophysiology revealed that following i.v. Vc1.1 dosing at 0.1 mg/kg in CCI rats, ED of neuroma and DRG-origin was reduced to 39% and 37% of control level, respectively ($P < 0.05$ and 0.01, compared to the saline group, n=4, DRG data not shown).

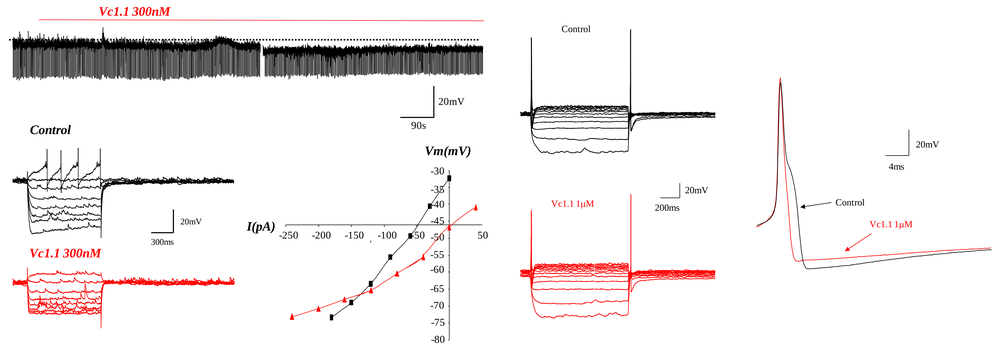


❸ At spinal cord dorsal horn level, Vc1.1 significantly reduced electrically evoked windup of wide dynamic range (WDR) neurones (n=3, $P < 0.03$ compared to vehicle group) in Chung model animals.



❹ (Left) In lamina I/II dorsal horn neurons, Vc1.1 (300 nM) induced a membrane hyperpolarisation of -6.4 ± 1.1 mV (n=5) associated with a reduction in neuronal input resistance. Voltage-current relations indicated membrane hyperpolarisation induced by Vc1.1 was associated with activation of an inwardly rectifying potassium conductance.

❺ (Right) Whole-cell patch clamp recordings from DRG neurones revealed no obvious change in passive membrane and subthreshold active conductances in these neurones in the presence of Vc1.1. However, a distinct reduction in the action potential duration and repolarisation phase of action potentials was observed in a subset of DRG neurones.



Conclusions

Vc1.1 significantly reverses mechanical allodynia in neuropathic rats and reduces ectopic discharge in peripheral nerves. This effect is partly mediated via a reduction in ectopic discharge associated with injured peripheral nerve spinal cord sensitisation. The analgesic mechanism of action of this compound involves activation of receptor-signal transduction pathways consistent with activation of an inwardly rectifying potassium channel at the level of the dorsal horn and inhibition of a calcium conductance contributing to action potential firing at the level of the DRG.