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Research Report

Alpha-conotoxin Vc1.1 alleviates neuropathic pain and accelerates functional recovery of injured neurones

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Abstract

This paper demonstrates the capacity of the neuronal nicotinic acetylcholine receptor (nAChR) antagonist α-conotoxin Vc1.1 to inhibit pain responses in vivo. Vc1.1 suppressed pain behaviors when tested in two models of peripheral neuropathy of the rat sciatic nerve, the chronic constriction injury (CCI) and partial nerve ligation (PNL) models. Mechanical hyperalgesia was assessed using an Ugo Basile Analgesymeter. Vc1.1 was administered by intramuscular bolus injection near the site of injury at doses of 0.036 μg, 0.36 μg and 3.6 μg in CCI rats and at a dose of 0.36 μg in PNL rats. Vc1.1 was also administered contralaterally in CCI rats at doses of 0.36 μg and 3.6 μg. Treatment started after the development of hyperalgesia and continued for 7 days. Vc1.1 significantly attenuated mechanical hyperalgesia in both CCI and PNL rats for up to a week following cessation of treatment. Vc1.1 also accelerated functional recovery of injured neurones. A blister was raised over the footpad innervated by the peripheral terminals of the injured nerve. The ability of these terminals to mount an inflammatory vascular response upon perfusion of the blister base with substance P provided a measure of functional recovery. This study shows that α-conotoxin Vc1.1, a neuronal nAChR antagonist, suppressed mechanical pain responses associated with peripheral neuropathy in rats in vivo and accelerated functional recovery of the injured neurones. A role for neuronal nAChRs in the analgesic activity of Vc1.1 is proposed.

Theme: Sensory systems
Topic: Pain modulation: pharmacology

Keywords: α-conotoxin Vc1.1; Neuronal nicotinic acetylcholine receptor; Mechanical hyperalgesia; Functional recovery

1. Introduction

Conotoxins are small peptides of 12 to 19 amino acids, that act as highly selective antagonists of ion channels in animal cells. A subgroup of these, the 4, 7 loop class of α-conotoxins, interacts selectively with neuronal-type nAChRs [8] and the degree of selectivity for different nAChR subtypes is remarkable [23]. This renders α-conotoxins attractive as prospective drug leads. We recently identified and characterized α-conotoxin Vc1.1 from a tropical marine snail, *Conus victoriae*, found in waters off the coast near Broome, Western Australia. A molecular approach has been used to identify α-conotoxin Vc1.1, whose amino acid sequence was deduced by sequencing a cDNA library obtained by RT-PCR from the mRNA isolated from a total RNA extract from the venom ducts of a *Conus victoriae* [30]. The coding sequence was 201 base pairs, which translates into 66 amino acids. The predicted pre-region of the protein precursor was 21 amino acids, the pro-region 29 amino acids, and the mature peptide 16 amino acids in length. The peptide Vc1.1, as synthesized by solid-phase techniques, had 16 amino acids with a C-terminal amide and with two disulfide bonds connected in a 4, 7 loop structure. Purification by RP-HPLC gave a single major peak (>70% pure) whose mass was confirmed by ESI-MS as 1806.6. The

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peptide was soluble in water and stable in solution when tested after 8 weeks. The characteristics of Vc1.1 are those of a specific antagonist of neuronal nAChRs [30].

We showed that Vc1.1 can specifically antagonize neuronal but not muscle-type nAChRs [30]. This conotoxin was also shown to suppress the vascular response to selective stimulation of sensory nerves in rats [30] suggesting that antagonists of nAChRs had the potential to suppress pain transmission in these animals. The analgesic effect of strong nicotinic agonists, such as epibatidine, nicotine and analogues has been well documented and supports the involvement of nicotinic transmission in pain perception [7,34]. There is, however, no precedent for the use of nicotinic antagonists to suppress pain transmission in animals in vivo.

Previous studies using N-type calcium channel blockers suggest that voltage-dependent calcium channels (VDCCs) mediate pain [35]. In particular, the N-type VDCC has been implicated in pain-related behaviors and calcium channel blockers such as the ω-conotoxins have attracted attention as antinociceptives [4]. Specific antagonists for neuronal N-type calcium channels have been shown to reduce heat hyperalgesia and mechanical allodynia in the CCI model when administered directly to the site of nerve injury [39], and subcutaneous administration of an N-type, but not P- or Q-type, Ca^{2+} channel antagonist attenuated mechanical hyperalgesia in the PNL model of pain [38].

In this study, we nevertheless demonstrate the ability of the neuronal nAChR antagonist Vc1.1 to modulate neuropathic pain in rats. We have used two commonly used models of neuropathic pain, the chronic constriction injury (CCI) model [3] and the partial nerve ligation (PNL) model [33] of the rat sciatic nerve. These models reflect pain behaviors exhibited by different populations of human neuropathic pain in patients [18].

We have also assessed the ability of Vc1.1 to modulate functional recovery of the injured neurones. As an indicator of the functional activity of C-fibres, we used an established blister model combined with laser Doppler flowmetry to assess the ability of the injured nerves to mount a peripheral inflammatory response upon perfusion of the blister base with substance P [2,15,16].

2. Materials and methods

2.1. Animals

Outbred, young (3 to 4 months old), male Sprague–Dawley rats with an average weight of 250–350 g were housed in groups of 4, in a constant temperature room (21 ± 0.5 °C), under a 12/12-h light/dark cycle and had free access to food and water ad libitum. Anesthesia was induced with sodium pentobarbitone (Nembutal 60 mg/kg i.p.) and additional doses of pentobarbitone 15 mg/kg were given throughout the experiment. Body temperature was maintained at 37 °C. Animals were killed by anesthetic overdose at the end of the experiment. All animal experiments were performed with the approval of the Royal Melbourne Hospital Research Foundation Animal Ethics Committee and all experimental procedures adhered to the National Health and Medical Research Council and IASP guidelines.

2.2. Surgery

2.2.1. Chronic constriction injury model

Under anesthesia and aseptic conditions the production of chronic neuropathy was achieved using a modified version [15,16] of the CCI model of Bennett and Xie [3]. The right sciatic nerve in the mid thigh region of the rat was exposed by blunt dissection through the *biceps femoris* and was separated from surrounding connective tissues. Four ligatures (4-0 chromic gut) were loosely tied around the sciatic nerve so that they touched, but barely constricted the nerve. In all rats, contralateral sides were not disturbed. Animal behavior was observed after surgery.

2.2.2. Partial nerve ligation model

Under anesthesia and aseptic conditions the production of chronic neuropathy was achieved using a modified version of the PNL model of Seltzer et al. [33]. The right sciatic nerve in the mid thigh region of the rat was exposed by blunt dissection through the *biceps femoris* and was separated from surrounding connective tissues. For the PNL group, the dorsal 1/3 to 1/2 of the right sciatic nerve was tightly ligated with 6-0 silk sutures.

2.3. Measurement of mechanical paw withdrawal threshold

Mechanical paw withdrawal thresholds were assessed with a slightly modified version of the Randall–Selitto method [26] using an Ugo Basile Analgesymeter (Varese, Italy). This instrument exerts a force that increases at a constant rate. The force was applied to the hind paw of the rat, which was placed on a small plinth under a cone-shaped pusher with a rounded tip (1.5 mm in diameter). The rat was held upright with the head and limb to be tested free, but most of the rest of the body cradled in the hands of the experimenter. The paw was then put under the pusher until the rat withdrew the hind paw or 300 g was reached on the linear scale, which ever occurred sooner. Each hind paw was tested twice, with a 15-min interval between the measurements and mechanical paw withdrawal thresholds were calculated as the average of two consecutive measurements.

2.4. Treatment protocol in injured animals

A total of nine groups of animals with nerve injury received either saline or Vc1.1. Seven groups of CCI animals received either saline or Vc1.1 ipsilaterally (targeted therapy) close to the injury site at mid thigh region at 0.036 μg, 0.36 μg or 3.6 μg or contralaterally at 0.36 μg or 3.6 μg.