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Research paper

Effect of cannabinoids on CGRP release in the isolated rat lumbar spinal cord

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HIGHLIGHTS

- Capsaicin causes CGRP to be released from rat spinal cords.
- This is suppressed by the TRPVR1 antagonist capsazapine.
- Contrary to early reports, cannabinoid CB_2 agonists do not inhibit CGRP release.
- This result is consistent with a lack of functional CB_2 receptors in the healthy spinal cord.

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ABSTRACT

Cannabinoids produce analgesia through a variety of mechanisms. It has been proposed that one mechanism is by modulating the release of CGRP in the spinal cord pain pathways. Previous studies have reported that cannabinoids, particularly CB_2 receptor agonists, can modulate CGRP release in the isolated rat spinal cord. In our experiments, the TRPV1 agonist capsaicin evoked CGRP release and this was supressed by the TRPV1 antagonist capsazepine and by the opioid receptor agonist DAMGO. However, none of the cannabinoid receptor agonists that we tested were able to modulate evoked CGRP release; including WIN 55,212-2, methanandamide, and GW405833. These results question the role of spinal cord cannabinoid receptors in the regulation of CGRP signaling.

1. Introduction

Cannabinoids have analgesic properties and cannabinoid receptors are expressed at many points along the pain pathway [14]. Expression is particularly strong within the superficial spinal cord dorsal horn [6,11]. Transient receptor potential vanilloid 1 (TRPV1) receptors are also expressed in the spinal pain pathways, mostly in small diameter C-fibres [24] and detect noxious heat and inflammatory mediators [8]. Capsaicin can activate these neurons and stimulate the release of calcitonin gene-related peptide (CGRP) [19] in the brain and spinal cord [21] and thereby induce neurogenic inflammation [4]. This sensitises neurons in the sensory pathways, and so the co-localisation of cannabinoid receptor and CGRP densities [11]. Gibson et al. [13] has suggested that the regulation of CGRP release by cannabinoid receptors in TRPV1 expressing afferent terminals in the spinal cord is one mechanism by which cannabinoids can reduce pain.

Ahluwalia and Perretti [1] reported that capsaicin evoked CGRP release from isolated rat spinal cord was inhibited by cannabinoid receptor agonist anandamide. At higher concentrations anandamide stimulated CGRP release *via* TRPV1. Beltramo et al. [3] reported that capsaicin CGRP release was inhibited by the CB_2 receptor agonists, GW405833 and AM1241, an effect inhibited by the CB_2 antagonist SR144528. This result was unexpected, because, although CB_2 receptor immunolabelling in the healthy spinal cord has been reported by Wotherspoon et al. [26], our earlier studies have called into doubt the specificity of the CB_2 antibodies [2,17], a result recently corroborated by Li and Kim [16]. In addition, Zhang et al. [27] reported that CB_2 mRNA was only detectable in spinal cord sections in neuropathic pain models but not in healthy rats. Moreover, we have found that using a GTPγS assay, the CB_2 receptor activity could not be stimulated in the healthy rat spinal cord *ex vivo* [6]. These inconsistencies lead us to attempt to replicate cannabinoid inhibition of CGRP release in isolated spinal cord.

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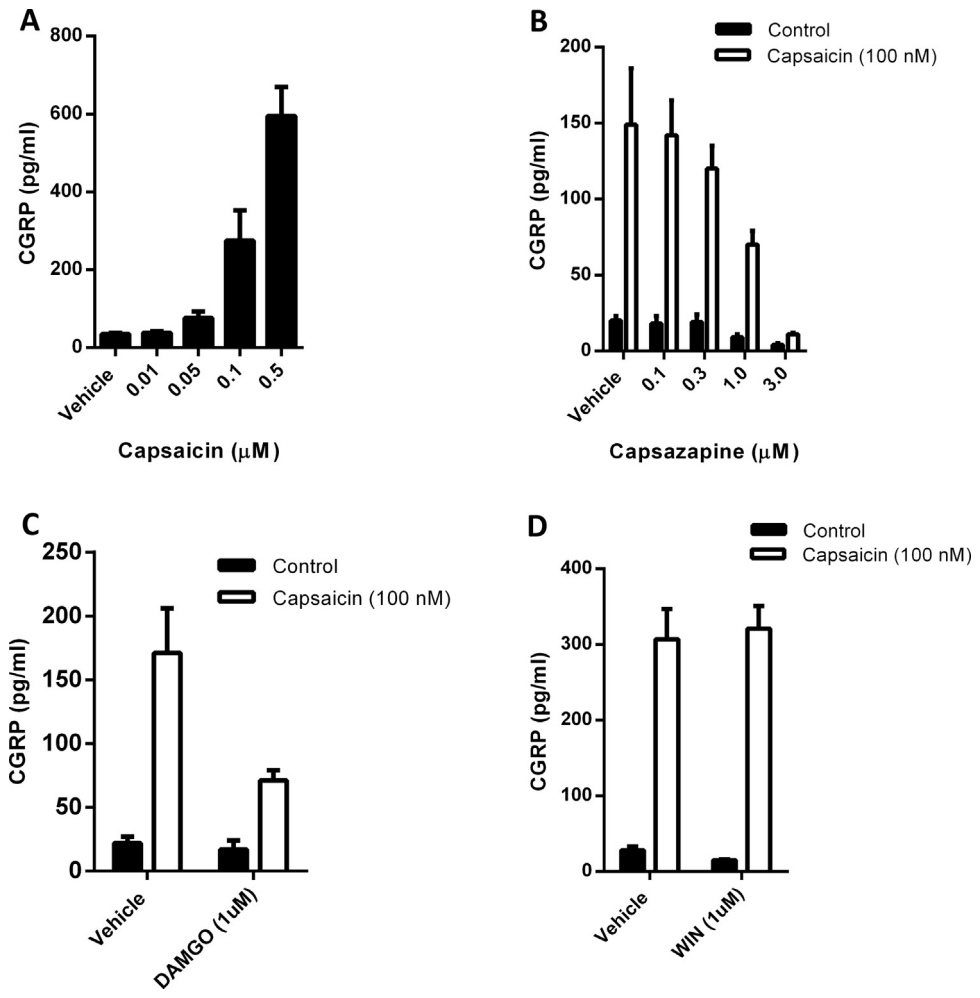

Fig. 1. (A) CGRP release (pg/ml) from rat lumbar spinal cord slices in the presence of various concentrations of capsaicin. (B) Reversal of capsaicin evoked CGRP release by the TRPV1 antagonist capsazapine. (C) Reversal of capsaicin evoked CGRP release by the opioid receptor agonist DAMGO. (D) The potent CB_1/CB_2 agonist WIN55,212-2 does not inhibit CGRP release. Each bar represents mean CGRP release ± SEM in 8 biologically separate experiments performed in technical triplicate. *Significantly different to control ($P < 0.05$).

2. Materials and methods

All experiments conducted at the University of Otago were approved by the Animal Ethics Committee at the University of Otago, under guidelines set down for the ethical and humane use of animals in research under the United Kingdom Animals Act 1986. Male Wister rats of 1–2 months age (250–350 g) were obtained from the Taieri-Hercus Resource Unit (Dunedin, New Zealand). Prior to sacrifice, rats were housed in an enclosure with a 12 h light/dark cycle with *ad libitum* access to food and water.

2.1. Tissue preparation

Lumbar spinal cord tissue was dissected by laminectomy. Rats were sacrificed by decapitation and a sagittal cut was made from the neck of the animal toward the tail to expose the spinous processes of the spinal cord. Two sagittal scalpel incisions were then made on each side of the spinous processes to expose the spinal cord; the spinous processes and spinal lamina and were removed to expose the lumbar spinal cord. A 2.5 cm section was removed and mounted in gelatin [18,22] on a tissue slicer and cut into 500 μM sections, then equilibrated in oxygenated (95% O_2, 5% CO_2) synthetic interstitial fluid (SIF) containing (in mM): 108 NaCl, 3.48 KCl, 3.5 $MgSO_4$, 26 $NaHCO_3$, 1.7 NaH_2PO_4, 1.5 $CaCl_2$, 9.6 sodium gluconate, 5.55 glucose, 7.6 sucrose [5] for 1 h at 32 °C. For dissection of the sciatic nerve, the bicep femoris was exposed and separated to further expose the sciatic nerve. Sections were then prepared as above for the spinal cord.

2.2. Stimulation of CGRP release and measurement with ELISA

Following dissection, spinal cord or sciatic nerve sections were treated with 0.01–0.5 μM capsaicin (Sigma–Aldrich, US) in 1 ml SIF for 15 min at 32 °C before the SIF was removed for assay. In other experiments, sections were exposed to cannabinoids or 0.1–3.0 μM capsazepine (a TRPV1 antagonist, Sigma–Aldrich, US) (Dickenson and Dray, 1991) in SIF for 15 min at 32 °C before incubation with SIF containing both one of these agents and 100 nM capsaicin for a further 15 min. Three cannabinoids were tested in this way on the spinal cord: the potent CB_1/CB_2 agonist WIN55,212-2 (Tocris, US), the CB2 partial agonist GW405833 (Tocris, US) and the CB_1 agonist methanandamide (Sigma–Aldrich, US). WIN55,212-2 was also used in experiments employing sciatic nerve sections. We also tested the effects of an opioid receptor agonist[D-Ala2, N-MePhe4, Gly-ol]-enkephalin (DAMGO) as a positive control [25].

Capsaicin was prepared in SIF starting from a 10 mM stock solution prepared in ethanol. Capsazepine and cannabinoids were diluted in SIF starting from a 10 mM stock solution. GW405833 and methanamdamide were tested at concentrations 0.001–1.0 μM [1,3]. WIN 55,212-2 was tested at 0.01–1 μM.