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Evidence of a role for descending serotonergic facilitation in a rat model of cancer-induced bone pain

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Abstract

Descending modulation of spinal processing plays an important role in chronic pain states. Monoamine pathways comprise a major component of descending controls from the brainstem to the spinal cord. Recent emphasis has been on facilitatory actions mediated by the 5-HT_3 receptor. We investigated the effects of spinally administered ondansetron, a selective 5-HT_3 receptor antagonist, on electrical- and natural-evoked dorsal horn (DH) neuronal responses in a rat model of cancer-induced bone pain (CIBP). Injection of MRMT-1 cells into the tibiae of Sprague–Dawley rats was used to model CIBP, whilst sham-operated rats were injected with the cell medium alone. Behavioural testing at regular intervals monitored the development of mechanical allodynia, cold allodynia, and ambulatory-evoked pain. In vivo electrophysiology experiments were carried out 15–17 days after surgery, when there were significant behavioural and neuronal alterations in the cancer animals. Spinally administered ondansetron (10, 50, and 100 μg) had no effect on electrical-evoked neuronal responses, but significantly reduced mechanical- and thermal-evoked responses in both the groups of animals. Furthermore, the effects of ondansetron were significantly greater in cancer animals compared to shams. These results therefore suggest a role for descending serotonergic facilitation in CIBP.

Keywords: Cancer pain; Descending facilitation; Electrophysiology; 5-HT_3 receptor; Ondansetron; Spinal cord

Cancer-induced bone pain (CIBP) is a common clinical pain state that has proven difficult to treat effectively as doses of analgesics required to combat the severe episodes of incident pain result in unacceptable side effects [11]. Recent developments in rodent models of CIBP have enhanced our understanding of the mechanisms involved in generating and maintaining this type of pain [11,17,21]. These studies have shown neurochemical reorganisation in the dorsal horn (DH) of areas of spinal cord receiving afferent input from the affected bone [17] and altered spinal neuronal responses unique to CIBP [21], the development of which parallels the behavioural signs of allodynia [5]. The hyperexcitability of DH neurons demonstrated in a rat model of CIBP [5,21] is consistent with the establishment of a state of central spinal sensitization.

Changes in excitability of spinal neurons contributing to central sensitization are dependent on spinal circuits but spinal events can also be controlled by higher centres [20]. Monoamine pathways comprise one of the major components of descending controls running from the brainstem to the spinal cord. Electrophysiological, anatomical, and pharmacological studies with regard to 5-HT, have highlighted areas of the medulla, particularly the rostroventral medulla (RVM) and reticular formation (including the nucleus reticularis magnocellularis—NRMC), as the major output for serotonergic descending influences, with recent studies emphasizing facilitations emanating from these areas [7].

Pharmacological studies have revealed a pro-nociceptive role for 5-HT_3 receptors in the spinal cord. These excitatory ionotropic receptors are located on the terminals of a subset of small diameter primary afferents [22], and their activation through the activity in descending pathways enhances neurotransmitter release in the DH of the spinal cord [6]. Selective blockade of 5-HT_3 receptors results in a reduction of nociceptive neuronal and behavioural responses [1]. This excitatory serotonergic system has been shown to be enhanced following formalin-induced inflammation [8] and peripheral nerve injury [19], but not following carrageenan-induced inflammation

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[8,16] suggesting that, in some pain states, activation of descending facilitatory pathways may make major contributions to spinal excitability. Thus, targeting these systems might offer analgesia that is complementary to the more traditional method of activating the descending inhibitory pathways [13].

Descending 5-HT_3-mediated facilitations are driven by a population of lamina I DH neurons, expressing the NK1 receptor, that form the origins of this spinal–supraspinal circuit relaying in the RVM and acting via a facilitatory action at spinal 5-HT_3 receptors. This loop enhances nociceptive transmission and is necessary for the full coding of mechanical, chemical, and thermal stimuli by deep DH neurons [18]. The circuit receives input from centres, such as the parabrachial area and amygdala, providing a pathway, whereby areas involved in the affective and autonomic aspects of pain could amplify and prolong the sensation to a painful stimulus. Thus fear, anxiety, and vigilance can alter the perception of pain [20], highly relevant in the case of pain from cancer.

We have shown that neuronal and behavioural alterations in the rat model of CIBP are driven by changes in populations of superficial DH neurons [5,21]. As some of these superficial cells will be NK_1-expressing projection neurones, it is likely that there is increased and altered transmission of signals to the brain areas involved in the affective component of pain including a CIBP-induced access of lower threshold inputs. This fits with the clinical condition of CIBP correlating with increased anxiety and depression [12], and the low threshold access may be important in terms of the mechanical allodynia seen behaviourally in the animal models.

Here, we investigate the effects of blocking the final spinal target of these descending facilitations, with spinally administered ondansetron (a selective 5-HT_3 receptor antagonist), on electrical- and natural-evoked DH neuronal responses in CIBP in order to assess the role of the descending serotonergic facilitatory pathways in this pain state.

Experiments were carried out on male Sprague–Dawley rats (University College animal house) weighing approximately 170 g at the time of surgery and 300 g at electrophysiology. All procedures were approved by the Home Office (animal procedures section, London, UK) and follow guidelines of the International Association for the Study of Pain (IASP).Syngeneic MRMT-1 rat mammary gland carcinoma cells (Novartis Institute, London, UK) were prepared as described previously [21]. Cells were cultured in RPMI 1640 (Gibco) with 10% foetal bovine serum, 1% L-glutamine and 2% penicillin/streptomycin (Gibco), washed and suspended in Hank's medium to achieve a final injection concentration of 3×10^3 cells in a volume of 10 μl and kept on ice. For osteotomy and injection of cells (21 rats), anaesthesia was maintained via a nose cone with halothane (1.5–2%) in a mixture of N_2O:O_2 (66:33). The surgical procedure was as described previously [21]. A small incision was made over the distal left tibia and a hole bored through the periosteum. Ten microlitres of cells were injected into the intramedullar cavity, the hole plugged with bone restorative material (IRM, Dentsply, USA), and the wound closed with a metal clip. Sham-operated rats ($n = 18$) were injected with Hank's medium alone.

Animals were tested for behavioural signs of mechanical and cold allodynia and ambulatory-evoked pain preoperatively and on postoperative days 2, 4, 7, 9, 11, 14, and 16 [21]. Mechanical allodynia was assessed by the application of von Frey filaments (1, 5, 9, and 15 g) to the plantar surface of the hind paw for 2–3 s per stimulus alternating between ipsilateral and contralateral hind paws, delivering a total of 10 stimuli to each and with at least 5 min separating ascending von Freys. Brisk withdrawal of the hind paw during or immediately after the stimulus was considered a positive response. Withdrawals to a given von Frey were expressed as a percentage of the maximum possible response. Cold allodynia was assessed by application of a drop of acetone via a syringe to the plantar surface of the hind paw, again alternating between ipsilateral and contralateral sides. A total of five applications were made to each hind paw with at least 5 min separating each. Again, the number of withdrawals was expressed as a percentage of maximum response. Ambulatory-evoked pain was assessed on the rotarod (see [9,10,21]). The apparatus was set to accelerate from 0 to 20 revolutions per minute over 60 s. The time in seconds maintained on the beam before the rat fell was recorded with a maximum cut-off at 150 s. Ambulation was also scored as follows: 0, normal; 1, slight limping; 2, marked limping; and 3, avoidance of use of limb. Rats received two training sessions on the rotorod prior to beginning an experiment, and only the animals scoring between 90 and 120 s at each training session were used for subsequent studies.

Electrophysiology experiments were conducted as described previously [21] between postoperative days 15–17 when all MRMT-1-injected animals show neuronal and behavioural alterations. Rats were anaesthetized as above and a cannula was inserted into the trachea. A laminectomy was performed to expose the spinal cord at L1–3 vertebral level. Recordings of superficial DH neurones (<300 μm from the surface of cord) and deep DH neurons (>500 μm from the surface of cord) receiving afferent input from the ipsilateral hind paw were made using a parylene-coated tungsten electrode (A-M systems, WA, USA). Neuronal responses were captured and analysed using a CED 1401 interface coupled to a Pentium PC with Spike 2 software (Cambridge Electronic Design). Superficial DH cells were characterised as wide dynamic range (WDR) or nociceptive-specific (NS) as described below. A train of 16 electrical stimuli (2 ms pulse width, 0.5 Hz) at three times the threshold current for C-fibre activation was applied transcutaneously at the centre of the receptive field. The responses evoked by A-beta (0–20 ms), A-delta (20–90 ms) and C-fibre (90–350 ms) were separated according to latency.

Von Frey filaments (bending forces of 1, 5, 9, 15, 30, and 75 g) for punctate mechanical, a standard artists brush (pro Arte No. 8) for non-noxious dynamic mechanical, constant water jet at 32, 35, 40, 45, 48, and 50 °C for thermal and at 4 °C for cold, were applied to the receptive field for 10 s. Any response to 32 °C was subtracted from subsequent thermal/cold responses to account for the mechanical component of the response to the water jet. NS cells were taken as those responding (with more than 10 action potentials per stimulus) at von Frey 9 g and above and 42 °C plus for heat, whilst WDR cells showed increasing responses throughout the range of stimuli. All deep DH neurons