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Spinal nociceptive reflexes are sensitized in the monosodium iodoacetate model of osteoarthritis pain in the rat

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SUMMARY

Objective: Evidence suggests that osteoarthritis (OA) is associated with altered central pain processing. We assessed the effects of experimentally induced OA on the excitability of spinal nociceptive withdrawal reflexes (NWRs), and their supraspinal control in a preclinical OA model.
Design: Experimental OA was induced in rats with knee injection of monosodium iodoacetate (MIA) and pain behaviour was assessed. 14/28 days post-MIA or saline injection, rats were anaesthetised for spinal NWR recording from tibialis anterior (TA) and biceps femoris (BF) hind limb muscles during plantar hind paw stimulation. Thresholds, receptive field sizes and wind up (incremental increase to repetitive stimulation) were measured in intact (d14/28) and spinalised (severed spinal cord; d28) MIA- and saline-injected rats.
Results: MIA reduced BF mechanical thresholds at day 28. Spinalisation of MIA rats did not prevent this hyperexcitability, and failed to produce the reduction in reflex receptive field (RRF) size observed in saline rats. These data indicate that MIA induces a hyperexcitability of BF NWR circuits that is maintained at the spinal level. In contrast, MIA appeared to have no effect on NWRs evoked by mechanical stimuli in the ankle flexor TA in intact rats, however spinalisation revealed hyperexcitability. Thus, 28 days following MIA-treatment, descending supraspinal inhibition normalised TA NWRs and was only overcome following repetitive noxious stimulation during wind up.
Conclusions: We demonstrate that spinal nociceptive reflex pathways are sensitized following the development of OA, suggesting the presence of central sensitization. Further, our data reflect OA-induced alterations in the descending control of reflex responses. Our findings contribute to a mechanism-based understanding of OA pain.

Introduction

The degenerative joint disease osteoarthritis (OA) is associated with chronic pain reducing quality of life[1]. Despite a good understanding of the degeneration that occurs in OA joints, how this drives chronic pain remains largely unknown. Poor association between radiological findings and pain[2], and the fact that some patients experience pain post-surgical joint replacement[3], suggests that alterations in spinal pain processing (central sensitization) make an important contribution. Data from experimental studies in human knee OA support this theory. Reduced pressure pain thresholds (PPTs), increased mechanical spatial and temporal summation at sites distant to the knee[2,4,5] and expanded referred pain areas are observed[6]. The excitability of spinal cord neurons receiving input from joint nociceptors is influenced by descending inhibition and facilitation[7,8]. The potency of heterotopic descending noxious inhibitory control is reduced in human knee OA and is credited with enhancing and driving the spread of pain[2,5]. Since there is a direct relationship between the degree of sensitization at distal sites, and pain[2], it is critical that studies further examine central sensitization mechanisms.

Clinically relevant animal models of OA are required to gain a broader mechanistic understanding of OA pain. Injection of the chondrocyte metabolism inhibitor monosodium iodoacetate (MIA) into the rat knee joint induces pathology and pain responses mirroring key aspects of human knee OA[9]. MIA produces a widespread loss of articular cartilage and subchondral bone remodelling by 28 days[10]. MIA-induced hind paw mechanical allodynia, mirrors pain evoked from non-injured sites in human OA[4,9] and correlates with the presence of activated spinal microglia and facilitated

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responses of spinal neurons[9,11], cellular and electrophysiological indicators of central sensitization. Thus, the MIA model provides an opportunity for the study of central pain mechanisms during OA.

Spinal nociceptive processing and the influence of supraspinal descending modulation can be studied experimentally, by examining nociceptive withdrawal reflexes (NWRs)[12,13]. NWRs are polysynaptic and multisegmental spinal reflexes constituting a protective mechanism, producing a rapid limb withdrawal from damaging stimuli[12] and can be recorded in the form of electromyographic (EMG) activity from limb muscles in conscious humans and anaesthetised animals[12,13]. This neurophysiological approach has been applied to chronic pain patients and animal models of chronic pain, providing a directly translatable approach by which to investigate clinically relevant pain mechanisms[13,14]. The facilitated NWRs seen in patients with knee OA[15,16] are electrophysiological correlates of central sensitization also manifest in rat inflammatory monoarthritis[17]. Whether models of OA are associated with NWR hyperexcitability is unknown, and thus no studies have assessed the translational potential of applying this approach to preclinical OA models.

Our aim was to improve the understanding of how spinal excitability is modulated during OA. We examined MIA effects on NWR excitability in a knee flexor muscle, biceps femoris (BF) and an ankle flexor muscle, tibialis anterior (TA). TA is the prime mover that under non-pathological conditions responds to plantar toe stimulation, lifting the toes by ankle flexion. Such a stimulus would also activate BF resulting in a complete removal of the lower half of the limb from the stimulus via knee flexion. The influence of descending controls on spinal excitability post-MIA was investigated.

Methods

Animals

Procedures were performed in accordance with the Animal (Scientific Procedures) Act 1986, UK Home Office regulations and local ethics approval. Male Wistar rats ($n = 64$; 180–200 g, Harlan Laboratories UK Ltd, Bicester, UK) were housed four per cage, given access to food and water *ad libitum*, and maintained on a 12 h light/dark cycle.

MIA-induced OA

Isoflurane (IsoFlo; Abbott Laboratories, Maidenhead, UK; 3% in O_2) anaesthesia was induced. Once areflexic, the left hind limb was clipped then 1 mg MIA in 50 μl sterile saline was injected through the patellar tendon into the left knee. Control rats were injected with sterile saline (50 μl). Rats were returned to the home cage and monitored.

Behavioural measurements

Pain behaviour was assessed by incapacitance testing (Linton Instrumentation, Norfolk, UK)[18]. Weight borne by ipsilateral (injected) and contralateral (non-injected) hind limbs was assessed (days 3–28 post-injection) and was averaged over 3 s; readings were taken in triplicate and means calculated. Results are ipsilateral as a percentage of contralateral weight bearing (no change ~ 100%).

Surgical procedure for reflex recordings

14 and 28 days post-MIA/saline, rats were deeply anaesthetized using isoflurane (2.2–3.5%) in an oxygen and nitrous oxide mixture (1:2). Body temperature was maintained at 37.5 ± 0.5 °C via a rectal probe coupled to a heating blanket (Harvard Apparatus Ltd., Edenbridge, UK). The trachea, left external jugular vein and left carotid artery were cannulated for airway maintenance, anaesthetic administration and mean arterial pressure (MAP; mm Hg) monitoring, respectively. Throughout MAP was monitored by an arterial pressure transducer (SensoNor 840; SensoNor, Horten, Norway) and recorded using Spike2 software (version 4) (Cambridge Electronic Design (CED) Ltd, Cambridge, UK). Anaesthesia was then maintained by an i.v. infusion of Alfaxan® (alfaxalone, 10 mg/ml; mean rate 41 mg/kg/h) at a level at which animals were moderately responsive to brushing of the cornea. Animals were allowed to stabilize for 1 h minimum before EMG recording. In some day 28 MIA/saline-treated rats ($n = 10/11$) the effect of spinalisation (severing the spinal cord to interrupt descending pathways) on reflex excitability was studied. A laminectomy was performed between T8 and T9 vertebrae and the spinal cord was transected at T9. Reflexes were recorded 2–3.6 h post-transection (median time: MIA rats = 161 min; saline rats = 156 min). Experiments were terminated by Alfaxan overdose, and saturated KCl solution.

Stimulation and recording of reflex responses

Figure 1 represents the hind limb set up. Throughout surgical preparation and reflex recording the experimenter was blind to treatment (MIA vs saline). Reflexes were recorded as compound EMG signals from TA and BF muscles ipsilateral to the injected joint and stimulation site using paired, percutaneous, varnish-insulated copper wire electrodes inserted into the muscles. EMGs were amplified (×5000), filtered (1 and 5 kHz), and fed to a CED 1401 connected to a PC running Signal software (version 2.08) (CED Ltd, Cambridge, UK).

Reflex responses, defined as increases in EMG activity above baseline, were evoked by mechanical (4–60 g von Frey monofilaments, Linton Instrumentation) and electrical ipsilateral hind paw stimulation. Hind paw receptive field mapping was

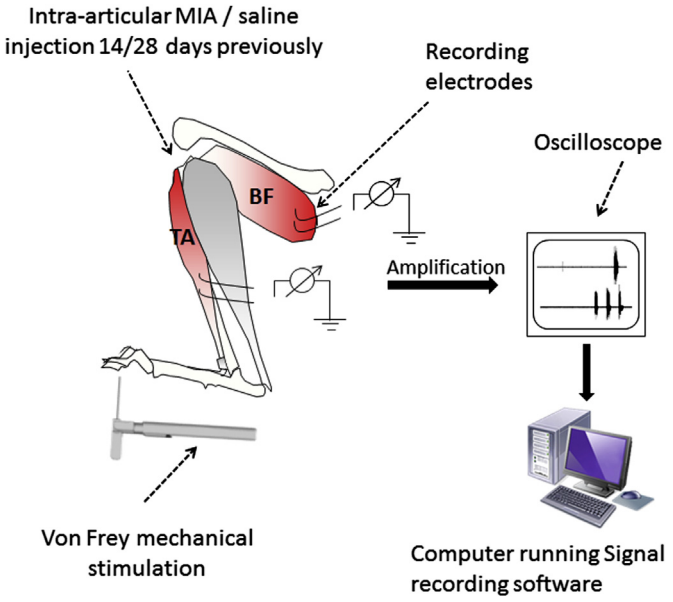

Fig. 1. Schematic representation of the experimental set up used for recording NWRs in the form of EMG activity in the hind limb muscles, BF and TA following hind paw stimulation.