Grading of monosodium iodoacetate-induced osteoarthritis reveals a concentration-dependent sensitization of nociceptors in the knee joint of the rat

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A B S T R A C T

Osteoarthritis (OA) is a degenerative joint disease characterized by joint pain for which there is currently no effective treatment. Previous studies have found that intra-articular injection of monosodium iodoacetate (MIA) caused a dose-dependent destruction of rat knees with concomitant increased pain. In this study, varying degrees of OA were induced by intra-articular injection of 0.1 mg, 0.3 mg and 3 mg MIA. Electrophysiological recordings were made from knee joint primary afferents in response to rotation of the joint and firing frequencies were determined and compared to saline-injected control joints. The analgesic effect of local application of the classic non-steroidal anti-inflammatory drug (NSAID) diclofenac (0.1 mg/0.1 ml bolus) was also determined in each group. Joint afferent firing frequency was significantly enhanced in OA knees compared to saline injected control joints and the magnitude of this sensitization showed a direct relationship with increasing dose of MIA. Diclofenac reduced nociception significantly in the 3 mg MIA treated joint, but had no effect on nerve mechanosensitivity in rats with milder OA. This study shows for the first time that MIA produces a graded sensitization of joint nociceptors making this a useful model for the study of pain mechanisms in joints with progressive OA severity. The anti-nociceptive effect of diclofenac further indicates that the MIA model offers an attractive means of objectively testing potential therapeutic agents.

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Osteoarthritis (OA) is the most common form of joint disease and is widespread in the elderly population [12]. OA primarily affects the weight bearing joints (e.g. knees, hips) and is associated with degeneration of the articular cartilage and subchondral bone. Patients with OA have joint pain that typically worsens with weight bearing and activity. Currently no disease-modifying drugs are available so the objective of pharmacological treatment has been aimed at reducing functional impairment and the associated pain. Studies on new therapeutic drugs for human OA and associated pain have been hampered because of the lack of useful animal models that closely mimic the human disease. The monosodium iodoacetate (MIA) model of OA has been well described in the rat especially in terms of pathological progression of the disease [8], pain behaviour [5,7,18,32] and peripheral nerve sensitization [30]. Injection of MIA into joints inhibits glycoaldehyde-3-phosphate dehydrogenase activity in chondrocytes, resulting in disruption of glycolysis and eventual cell death [5,15,31]. The progressive loss of chondrocytes results in histological and morphological changes to the articular cartilage, closely resembling those seen in human OA [13]. One of the main mechanisms responsible for the generation of joint pain is the activation of nociceptors located on the terminal branches of joint type III (Aδ fibre) and type IV (C fibre) primary afferents [14,25,26]. These afferent nerve fibres show increased activity when a noxious stimulus is applied to the innervated tissue [14,25,26,33]. In animal models of inflammatory joint disease and OA, it has been shown that these joint primary afferent nerves become sensitized, causing enhanced mechanosensation in the affected joint leading to allodynia, hyperalgesia and spontaneous pain [17,27,28,29]. Classical treatment for OA pain includes non-steroidal anti-inflammatory drugs (NSAIDS), opioids and steroids. The limitations of current therapy are such that patients still cite pain as their worst symptom and current analgesic options have significant side effects. It is therefore important to try to elucidate the mechanisms responsible for the induction and maintenance of these pain states to help in the development of more effective analgesics for the treatment of OA. The emphasis of this study concerns the relationship between MIA concentration and the degree of sensitization of afferent nerve fibres. A number of studies have shown that structural damage and pain increase with higher MIA concentration; however, the effect of MIA induced OA on peripheral sensory nerve activity is unknown. In this study we have shown for the first time that there is a direct relationship between MIA
induced severity of OA and the mechanosensitivity of afferent nerve fibres. We were also able to show that local administration of the NSAID diclofenac can reduce nerve sensitization in the most severe form of MIA-induced OA.

Experiments were performed on 44 male Wistar rats (250–450 g) which were housed in cages at room temperature (22 °C) under a 12:12 h light/dark cycle with free access to water and food. The animal handling and surgical procedures outlined in this study all adhered to the Canadian Council for Animal Care guidelines for the care and use of experimental animals. Forty-four rats were deeply anaesthetised with 2% isoflurane in 100% O2 in this study all adhered to the Canadian Council for Animal Care and food. The animal handling and surgical procedures outlined in this study all adhered to the Canadian Council for Animal Care guidelines for the care and use of experimental animals. Forty-four rats were deeply anaesthetised with 2% isoflurane in 100% O2 in this study all adhered to the Canadian Council for Animal Care and food. The animal handling and surgical procedures outlined in this study all adhered to the Canadian Council for Animal Care guidelines for the care and use of experimental animals. Forty-four rats were deeply anaesthetised with 2% isoflurane in 100% O2 in this study all adhered to the Canadian Council for Animal Care and food. The animal handling and surgical procedures outlined in this study all adhered to the Canadian Council for Animal Care guidelines for the care and use of experimental animals. Forty-four rats were deeply anaesthetised with 2% isoflurane in 100% O2 in this study all adhered to the Canadian Council for Animal Care and food. The animal handling and surgical procedures outlined in this study all adhered to the Canadian Council for Animal Care guidelines for the care and use of experimental animals. Forty-four rats were deeply anaesthetised with 2% isoflurane in 100% O2 in this study all adhered to the Canadian Council for Animal Care and food. The animal handling and surgical procedures outlined in this study all adhered to the Canadian Council for Animal Care guidelines for the care and use of experimental animals.

Core body temperature was measured by a rectally inserted thermometer and maintained at 37 °C. The trachea was cannulated and connected to a Harvard rodent respiratory pump to allow artificial ventilation with 100% O2 (stroke volume: 2.5 ml breath frequency: 50 breaths/min). The left carotid artery was then exposed and cannulated with a fine bore catheter (Portex Fine Bore Tubing, 0.5 mm ID, 1.00 mm OD; SIMS Portex Ltd., Kent, England) containing heparinised saline (100 units/ml). The cannula was connected to a pressure transducer to allow continuous blood pressure measurement as recorded by a blood pressure monitor (BP-1, World Precision Instruments, Sarasota, FL, USA). A catheter with heparinised saline was placed in the left jugular vein (Portex Fine Bore Tubing, 0.40 mm ID, 0.80 mm OD; SIMS Portex Ltd., Kent, England) and a single administration of the muscle relaxant gallamine triethiodide (Sigma–Aldrich, Ontario, Canada; 50 mg/kg) was injected to eliminate neural interference arising from the hindlimb musculature. The right saphenous artery was cannulated below the knee joint to permit local close intra-arterial injection of an NSAID to the knee joint. A specialised clamp was fixed to the mid-shaft of the isolated right femur and attached to a stereotaxic frame to prevent the generation of spinally mediated reflexes. The saphe- nous nerve was transected distally to the knee joint. The saphenous nerve was then isolated in the inguinal region and cut centrally to prevent the generation of spinally mediated reflexes. The saphenous nerve stump projecting from the knee was placed on a small, black Perspex stage. Under a dissecting microscope, the perineurium was removed and fine neurofilaments were dissected free from the nerve using fine watchmaker forceps. The neural strands were then placed over a platinum electrode to record single afferent fibre activity. To ensure that recorded fibres originate from the knee joint, the receptive field of the fibres were identified by the elicitation of a response to gentle probing of the knee joint with a glass rod with a 1 mm tip. The mechanical threshold of each recorded joint afferent was determined by a gradual increase of the torque applied to the joint until the fibre started to elicit action potentials. The mechanosensitivity of articular afferents was measured in response to rotational torque which was gradually increased in 10 mNm steps starting from 10 to 40 mNm. Each movement lasted 10 s and the same level of rotation was repeated 3 times every 20 s. In all MIA treated groups, recordings were made before (control) and after local intra-arterial injection of the NSAID diclofenac sodium salt (Tocris Bioscience; Missouri USA). The dose of diclofenac administered was 0.1 mg in 0.1 ml which has previously been shown to be effective in attenuating inflammatory and postsurgical pain [1,6]. Percent changes in nerve firing rate at different torque levels were calculated after NSAID application and compared to the saline injected control group. Neuronal activity was recorded by a data acquisition system (CED1401, Cambridge Electronic Design, Cambridge, UK) and stored on a microcomputer for off-line analysis. The number of action potentials/movement was determined using Spike 2 software (Cambridge Electronic Design, Cambridge, UK).

All data were normally distributed and expressed as mean ± SEM for “n” observations. The effect of different doses of MIA on joint mechanosensitivity was analyzed by two-way ANOVA while the effect of diclofenac was tested by a paired Student t-test. Torque-dependency was tested by one-way ANOVA. All differences were considered statistically significant at a P value less than 0.05. Between 1 and 3 afferent fibres were examined per animal such that a total of 59 units were recorded in this study. The conduction velocities of recorded afferents ranged from 0.47 to 9.29 m/s in the control group (mean 1.9 ± 0.75; n = 26). In the 0.1 mg MIA group the conduction velocities ranged from 0.54 to 4.1 m/s (mean 1.8 ± 0.34; n = 9), in the 0.3 mg MIA group it ranged from 1.0 to 7.3 m/s (mean 3.0 ± 1.1; n = 11) and in the 3 mg MIA group the conduction velocities ranged from 0.98 to 2.84 m/s (mean 2.7 ± 0.94; n = 13). All recorded fibres were found to be slowly adapting. All four animal groups showed an increase in firing rate with increasing levels of torque (10 mNm; 20 mNm; 30 mNm; 40 mNm). This torque-dependent effect was highly significant in all groups (P < 0.0001; two-way ANOVA). The effect of different concentrations of MIA on afferent firing rate at the four applied torque levels was also found to be highly significant among groups (P < 0.001). Specimen recordings showing the concentration-dependent increase in nerve fibre firing rate with MIA is shown in Fig. 1 (40 mNm torque is shown in each trace). In rats treated with 0.1 mg MIA the mean afferent firing rate across all four torque levels was 28 ± 12.6 action potentials/movement cycle. This firing frequency was found to be not significantly different from the saline control group (32 ± 13.2 action potentials/movement cycle). Treatment with 0.3 mg MIA (59 ± 21.4 action potentials/movement cycle) and 3 mg MIA (81 ± 22.0 action potentials/movement cycle) induced a highly significant increase in joint afferent firing rate compared to control (P < 0.0001; Fig. 2). A significant increase in firing rate was also observed comparing the 0.3 mg MIA to the 3 mg MIA group (P < 0.05). The mechanical threshold required to initiate afferent firing in control joints was 15.2 ± 1.5 mNm. In the 0.1 mg MIA group the mechanical threshold was 17.0 ± 2.3 mNm, in the 0.3 mg MIA group it was 14.5 ± 2.4 mNm and in the 3 mg MIA group the mechanical threshold was 12.4 ± 1.5 mNm. Local application of the classic NSAID diclofenac (100 μg) significantly reduced MIA-induced sensitization of nerve fibres in the 3 mg MIA group with noxious joint rotation (P < 0.001) but had no significant effect in rats treated with lower MIA concentrations (Fig. 3). Diclofenac had no effect on mechanosensitivity in control joints (data not shown). All units tested could be activated by local intra-arterial injection of KCl (0.4 mM, 0.1 ml) at the end of the experiment confirming...
that administered reagents reached mechanosensory nerve endings throughout the experiment.

Intra-articular injection of MIA into rat knee joints induces histological changes and pain related behaviours characteristic of human OA [2,15,18]. It is possible to vary the degree of joint pathology by injecting different concentrations of MIA and this has been shown to correlate with pain intensity [2,5]. Most studies have focused on MIA-induced histological changes of the joint and related pain behaviour but not much is known about the concomitant changes in primary afferent discharge properties during the transmission of noxious information from the damaged joint to the central nervous system. Recent studies showed that MIA-induced OA leads to an increased firing rate and reduced activation threshold of afferent nerve fibres [29], which consequently leads to sensitization of spinal neurons in the dorsal horn [10]. The present study revealed for the first time that there is a direct relationship between the concentration of intra-articularly injected MIA and afferent nerve fibre activity. Furthermore, incremental rotational forces applied to the knee resulted in a rise in afferent discharge rate in all animal groups. This torque-dependent increase in firing frequency corroborates an earlier report which described the same phenomenon in normal rat knees [14].

Although some clinical studies have shown a correlation between reported pain and the extent of OA disease [21], many patients with relatively undamaged joints describe intense pain while others with severe OA report little or no pain [9]. Thus, the link between structural tissue damage and joint pain remains unclear highlighting the necessity for robust and relevant animal models to explore further this phenomenon. In the Dunkin Hartley guinea pig model of spontaneous OA, for example, it was found that the degree of knee joint pathology correlates well with increasing age; however, there was no statistical correlation between joint nociception and morphologically determined joint degeneration [16]. Aged Dunkin Hartley guinea pigs, therefore, are a clinically relevant model of OA pain although disease severity cannot be easily controlled for between animals. The MIA model of OA on the other hand offers the ability to control not only disease severity, but as described here, it is also possible to gradate the magnitude of nociception incurred. This model therefore, provides a unique opportunity to evaluate potential analgesics in animals with varying degrees of joint degeneration. One reason why the MIA model correlates well with disease severity while the guinea pig model of...
OA does not may be related to differences in overall joint pathology. Aged Dunkin Hartley guinea pig knees possess areas of meniscal ossification, cartilage apposition, contain osteophytes and have other bony remodelling features not seen in the MIA model. Furthermore, joint degeneration in the MIA model occurs over 14 days whereas spontaneous OA in the guinea pig develops over months which likely differentially impacts the sensory properties of joint nociceptive nerves.

Little is known regarding the exact mechanisms responsible for MIA-induced joint pain but it may be related to early inflammatory reactions in the joint. Initial inflammation can induce a sensitization of peripheral receptors which consequently changes the response characteristics of primary afferent fibres [23] leading to more chronic pain states. Activation of normally non-nociceptive fibres by noxious stimuli may then be perceived as painful (for review see [4,33]). The acute inflammatory response in the MIA model lasts approximately 3 days during which time trophic factors are likely released into the joint that upregulate pro-nociceptive receptor expression leading to a reduction in mechanosensory threshold [19]. A-fibres may start synthesising receptors that are normally only found on nociceptive C-fibres, thus signalling a phenotypic shift with some A-fibres adopting C-fibre characteristics [20]. Inflammation subsequently resolves in the MIA model giving way to the more prolonged degenerative phase of the disease. Although human OA is classically defined as a non-inflammatory disease with limited pain relief by NSAIDs, there are clearly molecular and pathophysiological indices of inflammation in OA that warrant closer inspection [11,22]. Previous investigations into the ability of NSAIDs to alleviate joint pain are controversial. Systemic treatment with diclofenac was found to reduce secondary hyperalgesia during the acute phase of MIA induced OA (day 3 following MIA injection), but failed to have any analgesic effect at later time points [5]. In contrast, other studies observed that systemic application of NSAIDs at days 7 and 14 after MIA injection significantly reduced joint pain [2,3,24]. The present study found that peripheral administration of diclofenac reduced afferent nerve firing rate in animals treated with the highest dose of MIA only. This finding suggests that high dose MIA produces an OA joint with ongoing inflammation that is amenable to local treatment with NSAIDs, whereas the inflammation produced by low dose MIA is transient and therefore insensitive to NSAIDs during the latter stages of OA development. Further studies are necessary, however, to test this assumption that the inflammatory component of MIA-induced OA is concentration-dependent.

In summary, this study shows that increasing rotation of rat knees leads to enhanced firing of joint mechanoreceptors. The magnitude of these discharges is augmented in animals treated with MIA and this effect was found to be concentration-dependent. Thus, the MIA model of OA offers an attractive means of evaluating novel pain therapeutics in animals with various levels of disease severity.

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