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OSTEOARTHRITIS-DEPENDENT CHANGES IN ANTINOCICEPTIVE ACTION OF $NA_V1.7$ AND $NA_V1.8$ SODIUM CHANNEL BLOCKERS: AN *IN VIVO* ELECTROPHYSIOLOGICAL STUDY IN THE RAT

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Abstract—Voltage-gated sodium channel blockers are not traditionally recommended for osteoarthritis (OA) pain therapy, but given the large peripheral drive that follows OA development there is a rationale for their use. Using a rat model of monosodium iodoacetate (MIA)-induced OA we used *in vivo* electrophysiology to assess the effects of the $Na_V1.7$- and $Na_V1.8$-selective antagonists, ProTxII and A-803467 respectively, on the evoked activity of spinal dorsal horn neurons in response to electrical, mechanical and thermal stimuli applied to the peripheral receptive field. These studies allow examination of the roles of these channels in suprathreshold stimuli, not amenable to behavioral threshold measures. Spinal administration of ProTxII significantly reduced neuronal responses evoked by mechanical punctate (von Frey (vF) 8–60 g) and noxious thermal (45 and 48 °C) stimuli in MIA rats only. A-803467 significantly inhibited neuronal responses evoked by vF 8–60 g and 48 °C heat after spinal administration; significantly inhibited responses evoked by brush, vFs 26–60 g and 40–48 °C stimuli after systemic administration; significantly inhibited the electrically evoked Aδ-, C-fiber, post-discharge, Input and wind-up responses and the brush, vFs 8–60 g and 45–48 °C evoked neuronal responses after intra plantar injection in the MIA group. In comparison A-803467 effects in the sham group were minimal and included a reduction of the neuronal response evoked by vF 60 g and 45 °C heat stimulation after spinal administration, no effect after systemic administration and an inhibition of the evoked response to 45 °C heat after intra plantar injection only. The observed selective inhibitory effect of ProTxII and A-803467 for the MIA-treated group suggests an increased role of $Na_V1.7$ and 1.8 within nociceptive pathways in the arthritic condition, located at peripheral and central sites. These findings demonstrate the importance of, and add to, the mechanistic understanding of these channels in osteoarthritic pain.

Key words: osteoarthritis, pain, dorsal horn, sodium channels, electrophysiology.

INTRODUCTION

Osteoarthritis (OA) constitutes one of the largest cost burdens to healthcare in the western world with pain being the dominant symptom and reason for clinical presentation (Hiligsmann et al., 2013; Neogi, 2013). Non-steroidal anti-inflammatory drugs (NSAIDs) are first-line treatments, often in combination with paracetamol or opioids, but analgesic efficacy is largely modest at best at tolerable doses, or is hampered by significant adverse effects with dose escalation (Harvey and Hunter, 2010; Zhang et al., 2010a). For these reasons, many patients resort to total joint replacement to relieve their pain, yet chronic pain remains for a significant proportion (about 20–40%) of patients (Kirwan et al., 1994; Creamer et al., 1996; Ethgen et al., 2004). This highlights the complexity of OA pain and the significant unmet clinical need.

OA is characterized by inflammation (episodic and chronic) and swelling of joints and also significant pain in the area surrounding the joint and often in areas distant to the affected joint (referred pain), thus suggesting that both peripheral and central nociceptive mechanisms are at play (Farrell et al., 2000; Malfait and Schnitzer, 2013; Zhang et al., 2013). The transmission of pain from the peripheral site of injury, beyond the peripheral transducers, requires activation of voltage-gated sodium channels (VGSCs) located on peripheral nociceptors. Abundant data exist showing that maladaptive changes in VGSCs are critical for mediating variety of chronic pain conditions in both animals and humans (Eijkelkamp et al., 2012; Dib-Hajj et al., 2013) thus modulating their activity is a rational strategy for chronic pain therapy.

Sodium channel blockers for the treatment of OA pain are not currently recommended, yet they may have a key role in controlling OA pain since there is strong evidence for abnormal firing in peripheral and central neurons in the arthritic condition, which must involve alterations in VGSCs (Schuelert and McDougall, 2006, 2008, 2009;

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Abbreviations: C-LTMs, C-low-threshold mechanoreceptors; DRG, dorsal root ganglia; KO, knockout; MIA, monosodium iodoacetate; NSAIDs, non-steroidal anti-inflammatory drugs; OA, osteoarthritis; PWF, paw withdrawal frequency; PWT, paw withdrawal threshold; RM ANOVA, repeated-measures analysis of variance; SEM, standard error of the mean; vF, von Frey; VGSCs, voltage-gated sodium channels; WDR, wide dynamic range.

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McDougall et al., 2009; Rahman et al., 2009; Sagar et al., 2010; Kelly et al., 2012, 2015; Bullock et al., 2014) and a genetic mutation in the encoding gene for the 1.7 sodium channel sub-type has been correlated with increased pain sensitivity in OA patients (Reimann et al., 2010) but see (Valdes et al., 2011). Furthermore, analgesic efficacy of the lidocaine patch and intravenous and intra-articular injection of non-selective VGSC blockers has been observed in osteoarthritic patients (Creamer et al., 1996; Burch et al., 2004; Gammaitoni et al., 2004; Kivitz et al., 2008; Dworkin et al., 2011; Duarte et al., 2014).

There is a rationale for sodium channel blockers for OA pain therapy, based on heightened peripheral drive, which could be present in both early inflammatory and later non-inflammatory stages. In addition there may be neuropathic components to the pain in sub-groups of patients (Duarte et al., 2014; Thakur et al., 2014). Our aim was to further characterize the role of $Na_v1.7$ and 1.8 channels in a rat model of monosodium iodoacetate (MIA) (2 mg)-induced OA of the knee joint; a well-established model for the mechanistic study of osteoarthritic pain that has also been pharmacologically validated with respect to established analgesics including NSAIDs (Vonsy et al., 2008; Zhang et al., 2013). This dose of MIA (2 mg) has been shown to produce an up-regulation of the neuronal damage marker, cAMP-dependent transcription factor (ATF-3), in peripheral nerves that innervate the knee joint, a reduction in intra-epidermal nerve fiber density and alterations in spinal cord neuroimmune cells (Ivanavicius et al., 2007; Im et al., 2010; Thakur et al., 2012, 2014) features that are consistent with neuropathy. Therefore this model would be useful for assessing the analgesic potential of drugs for OA patients with neuropathic traits (Hochman et al., 2011; Duarte et al., 2014). Using *in vivo* electrophysiology, we have investigated, for the first time, the effects of ProTxII, a tarantula toxin that potently inhibits $Na_v1.7$ channels with about fifteen to a hundred fold selectivity over other VGSCs (Middleton et al., 2002; Schmalhofer et al., 2008; Xiao et al., 2010), and A-803467, a selective $Na_v1.8$ VGSC blocker (Jarvis et al., 2007), on the evoked activity of wide dynamic range (WDR) dorsal horn neurons in response to stimulation of the peripheral receptive field in this model of OA. The effects of ProTxII were examined after topical spinal application only because it was previously shown that ProTx-II only inhibited C-fiber action potential propagation in desheathed but not in intact nerve preparations, suggesting that the toxin could not penetrate the blood nerve barrier (Schmalhofer et al., 2008). For this reason we did not extend our ProTxII study to intraplantar and systemic routes as we did not expect that the toxin would be able to reach the channel. The effects of the selective $Na_v1.8$ channel blocker A-803467 given via three different routes of administration (topical spinal, systemic and intraplantar injection) were assessed in order to shed light on the sites of action of the drug. *In vivo* electrophysiology allows for spinal nociceptive processing and central sensitization to be studied experimentally and provides information on suprathreshold responses, which are likely to equate to high levels of pain transmission as reported by patients, therefore adding to behavioral data where the analgesic effect of drugs on threshold responses are generally measured.

EXPERIMENTAL PROCEDURES

Sprague–Dawley rats (Central Biological Services, University College London, UK) weighing 130–140 g at time of injection and 240–270 g at time of *in vivo* electrophysiology were employed for this study. All experimental procedures were approved by the UK Home Office and followed the guidelines under the International Association for the Study of Pain (Zimmermann, 1983).

Induction of OA

On day 0 isoflurane anesthetized Sprague–Dawley rats received an intra-articular injection of 2–mg MIA in 25 μl of 0.9% saline through the infrapatellar ligament of the knee. Sham animals were injected with sterile 0.9% saline only. Following injection animals were allowed to recover and then re-housed in cages under a 12-h alternating light/dark cycle with *ad libitum* access to food and water.

Assessment of pain related behavior

Development of mechanical and cooling hypersensitivity. Behavioral responses to stimulation of the ipsilateral hind paw were recorded once the animals had acclimatized to the testing area (Perspex cages with a wire mesh floor) for at least 30 min. Tactile hypersensitivity was tested by touching the plantar surface of the hindpaw with von Frey (vF) filaments (Touch-test TM, North Coast Medical Inc., San Jose, CA, USA) using the "up-down method" (Chaplan et al., 1994), starting with 2.0 g then ranging from 0.4 g to 15 g. Positive withdrawals were counted as biting, licking and withdrawal during or immediately following the stimulus. The strength of the vF filament was increased or decreased following a negative or positive response respectively. This up-down procedure was applied 4 times following the first change in response. Data are presented as 50% paw withdrawal threshold (PWT) for each group ± standard error of the mean (SEM). Sensitivity to cooling stimulation was assessed as the number of withdrawals out of a trial of five applications of a drop of acetone to the plantar surface of the ipsilateral hind paw. Paw withdrawal frequency (PWF) was quantified and presented as a percentage of the maximal response i.e. (number of foot withdrawals/five trials) × 100.

Hind-limb weight bearing. Changes in hind paw weight bearing was measured using an incapacitance tester (Linton instruments, Norfolk, UK). Animals were placed in a perspex chamber designed so that the animal is upstanding and the hindpaws rest on a separate small electronic balance so that the weight distributed on the right and left hind paw could be measured. Once the animal was settled three consecutive readings (each measured over 3 s) were recorded. The average of a