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A Role for Calcitonin Gene-Related Peptide in the Monosodium Iodoacetate model of Osteoarthritis in the Mouse

Andrea Ogbonna, Anna Clark, Clive Gentry, Marzia Malcangio. *King's College London, London, UK*

Osteoarthritis (OA) is a highly prevalent, age-related, pain condition that poses a significant clinical problem. Here, in the monosodium iodoacetate (MIA) model of OA, we have characterised pain behaviours and associated changes at the first pain synapse in the dorsal horn of the spinal cord. As MIA injection in the rat can cause changes in Calcitonin Gene-Related Peptide (CGRP) content within the dorsal root ganglia (DRG; Ferreira-Gomes *et al.*, 2010) a potential role for CGRP in MIA-induced OA is investigated.

Male C57Bl/6 mice (20-30g) received intra-articular injections of 0.5, 0.75 and 1mg MIA and mechanical paw withdrawal thresholds were monitored for up to 28 days. An intrathecal injection of the peptide antagonist $CGRP_{8-37}$ (5nmol/5µl/mouse) was given 21 days post MIA and paw withdrawal thresholds measured 1 and 3 hours after administration. Following behavioural studies immunohistochemical (IHC) analysis of spinal cord and DRG was carried out. In addition, activity-evoked CGRP release was measured from isolated lumbar dorsal horn slices with attached dorsal roots. Twenty-one days following intra-articular MIA or vehicle the L3-L5 dorsal roots of spinal cord slices were stimulated at C fibre strength (20V, 0.5ms, 10Hz) and evoked release of CGRP was quantified using ELISA.

All doses of MIA induced significant mechanical hypersensitivity in the ipsilateral hindpaw which lasted for up to 28 days and was maximal at the highest dose, which was then used throughout the study (AUC contralateral-ipsilateral: saline control 0.7±1.2; 0.5mg MIA 8.5±1.3; 0.75mg MIA 9.9±0.7; 1mg MIA 14.3±1.7; n=7-14, all doses ***$p<0.001$ compared to saline and 1mg MIA $^{\#}p<0.05$ compared to 0,75mg MIA, One Way ANOVA). No evidence of sensory neuron damage was observed in L3 and L5 dorsal root ganglia 7 days after MIA injections. However, both dorsal horn neuron activation and microglial response (c-Fos and Iba-1 immunostaining respectively), but not reactive astrocytes (GFAP immunostaining), were observed in the ipsilateral dorsal horn 28 days after intra-articular MIA. Evoked CGRP release was greater from dorsal horn slices of MIA treated mice (Basal release 31.8±4.2 pg/8ml fraction, evoked release 122.2±30.7pg/8ml fraction) compared to control (Basal release 22.2±3.3pg/8ml fraction, evoked release 67.1±10.8pg/8ml fraction, n=5-6, *$p<0.05$, One Way ANOVA). Furthermore, the peptide antagonist $CGRP_{8-37}$ attenuated established MIA-induced mechanical hypersensitivity (50% PWT 1h post i.t.: MIA/Vehicle 0.43±0.13g; MIA/$CGRP_{8-37}$ 0.77±0.11g, n=12-13, *$p<0.05$, One Way ANOVA).

These data show that MIA induced-knee disruption in the mouse is associated with referred mechanical hypersensitivity and increased release of CGRP from primary afferent fibres in the dorsal horn, where second order neuron activation is associated with a microglial response. Antagonism of CGRP receptor activation provides a therapeutic avenue for the treatment of pain in osteoarthritis.

Reference List:

Ferreira-Gomes, J, Adaes, S, Sarkander, J & Castro-Lopes, JM. (2010). Phenotypic alterations of neurons that innervate osteoarthritic joints in rats. *Arthritis Rheum*, **62**, 3677-3685.