BJA

British Journal of Anaesthesia, ■ (■): 1–12 (2018)

doi: 10.1016/j.bja.2018.01.016
Advance Access Publication Date: xxx
Laboratory Investigation

LABORATORY INVESTIGATION

Adiponectin regulates thermal nociception in a mouse model of neuropathic pain

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Abstract

Background: Adiponectin, a cytokine secreted by adipocytes, plays an important role in regulating glucose and lipid metabolism. However, the role of adiponectin in pain conditions is largely unknown. This study aimed to identify the role and mechanism of adiponectin in nociceptive sensitivity under physiological and pathological states utilising adiponectin knockout (KO) mice.

Methods: Wild type (WT) and adiponectin KO mice were subjected to partial sciatic nerve ligation (pSNL) or sham operation. Pain-like behavioural tests, including thermal allodynia, hyperalgesia, and mechanical allodynia, were performed before and after pSNL from Day 3–21. Dorsal root ganglions (DRGs), lumbar spinal segments at L3-5, and somatosensory cortex were collected for protein measurement via western blotting and immunofluorescence staining.

Results: Compared with WT mice, KO mice had significantly lower (40–50%) paw withdrawal latency to innocuous and noxious stimuli before and after pSNL. In DRG neurones from KO mice, where adiponectin receptor (AdipoR) 2 is located, phosphorylated p38 mitogen-activated protein kinase (p-p38 MAPK) and heat-sensitive transient receptor potential cation channel subfamily V member 1 (TRPV1) were significantly higher (by two- to three-fold) than from WT mice. In spinal microglia and somatosensory cortical neurones, where AdipoR1 is mainly located, p-p38 MAPK and TRPV1 were also higher (by two- to three-fold) in KO compared with WT mice, and altered signalling of these molecules was exacerbated (1.2- to 1.3-fold) by pSNL.

Conclusions: Our results show that adiponectin regulates thermal nociceptive sensitivity by inhibiting activation of DRG neurones, spinal microglia, and somatosensory cortical neurones in physiological and neuropathic pain states. This study has relevance for patients with adiponectin disorders, such as obesity and diabetes.

Keywords: adiponectin; hyperalgesia; neuralgia; neuroinflammation; p38 mitogen-activated protein kinase

Editorial decision: January 20, 2018; **Accepted:** January 20, 2018

Editor's key points

- Adiponectin may have a role in neuropathic pain.
- Neuropathic pain was modelled in adiponectin knockout (KO) and wild type mice.
- KO mice were hypersensitive to thermal stimuli and this was worse after nerve ligation.
- Adiponectin regulates thermal nociception and may be important for patients with conditions where adiponectin is implicated, such as diabetes and obesity.

Adiponectin, initially called adipocyte complement-related protein of 30 kDa, is a cytokine secreted exclusively by adipocytes and it has several isoforms.[1,2] Adiponectin exerts diverse functions in organs/tissues, such as liver, muscle, heart, and brain by virtue of binding to its receptors [adiponectin receptor 1 (AdipoR1) and AdipoR2].[3–6] Adiponectin is present in serum, and can pass the blood-brain barrier and enter cerebrospinal fluid at a 100-fold lower concentration.[7] It acts in the brain to control energy expenditure and body weight,[6,8] and inhibits glial cells in the brain to reduce inflammation.[9] More recently, adiponectin deficiency in aged mice was reported to lead to spatial memory and learning impairments and fear-conditioned memory deficit, and anxiety.[10] Adiponectin is also involved in exercise-induced hippocampal neurogenesis and antidepressant effects in mice.[11] Although the role of adiponectin in the brain has been well documented,[12] its effect in the spinal cord has been rarely studied. A study reported that intrathecal injection of adiponectin attenuated carrageenan-induced inflammatory pain in rats[13] and obese rats with inflammatory hyperalgesia had decreased concentrations of adiponectin in the spinal cord.[14] Adiponectin is also capable of suppressing spinal cord autoinflammation in experimental autoimmune encephalomyelitis.[15] However, the molecular mechanism governing the effect of adiponectin in nociception and inflammation in the spinal cord under physiological and pathological conditions, such as neuropathic pain, is not clear.

Neuropathic pain is evoked by injury in the somatosensory nervous system and characterised as chronic burning or stabbing pain. Neuropathic pain can also manifest as refractory pain from normally innocuous stimuli (i.e. allodynia) and higher than expected pain from noxious stimuli (hyperalgesia). Peripheral nerve injury not only results in dysfunction of sensory neurones *per se* and their central projection pathways, but also leads to neurogenic inflammatory responses.[16] In the periphery, neurogenic inflammation can be induced by heat-sensitive transient receptor potential cation channel subfamily V member 1 (TRPV1) activation or nerve injury, and established by release of neuropeptides, such as calcitonin gene-related peptide (CGRP) and pro-inflammatory cytokines, leading to peripheral sensitisation.[17,18] In the spinal cord, neuroinflammation can be triggered by the increased peripheral neuronal activity and lead to immune responses including the release of inflammatory mediators, such as tumour necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6, contributing to central sensitisation.[19] Moreover, it has been known that activation of p38 mitogen-activated protein kinase (p38 MAPK) after nerve injury promotes long-term changes in synaptic plasticity, resulting in persistent neuropathic pain.[20,21] In a peripheral neuropathic pain model, TRPV1 is expressed in cortical neurones and contributes to synaptic strength.[22] Given the potent effects of adiponectin in regulating neuroinflammation and its involvement in pain processing as described previously, we therefore hypothesised that adiponectin may regulate nociception by inhibition of neuroinflammation in the somatosensory nervous system.

Methods

Animals and experimental design

Adult male adiponectin knockout (KO) mice (6–8 weeks) with a C57BL/6J background, and age-matched wild type (WT) mice weighting 25–30 g were given food and water *ad libitum* and housed five to six per cage, at 22°C, 50% humidity, on a 12 h light:12 h dark schedule.[11] The procedure was approved by the Committee on the Use of Live Animals in Teaching and Research, the University of Hong Kong and relevant aspects of the Animal Research-Reporting In Vivo Experiments guidelines were followed.

The study included a total number of 50 mice (25 WT and 25 KO mice), which were allocated randomly using online software (http://www.randomization.com/) to naïve group (n=5 from each breed), sham group (including two cohorts: Day 7 for western blotting test and Day 21 for behavioural test, n=5 from each breed), and partial sciatic nerve ligation (pSNL) group (including two cohorts: Day 7 and Day 21, n=5 from each breed). The behavioural tester was blinded as to the treatment group. The sample size was determined based on the minimum average relatedness by the method described by Charan and Kantharia.[23]

The naïve group of WT and KO mice without any treatment were perfused for immunofluorescence staining. All the animals used for behavioural test and western blotting assessment were culled by overdose of sodium pentobarbital (Virbac, Milperra, Australia) followed by tissue collection.

Neuropathic pain model

The pSNL model is a well-characterised neuropathic pain model and was induced as previously described.[24] Briefly, anaesthesia was induced with isoflurane 4% in oxygen and maintained with isoflurane 2–2.5% in oxygen. The depth of anaesthesia was indicated by the lack of pedal withdrawal response to a nociceptive stimulus, monitoring of heart rate (300–500 beats min^{-1}), respiration rate (100–200 bpm), saturated pulse oxygenation (i.e. mucous membrane colour, pink or pale pink), and body temperature (36.5–38°C), which were monitored every 15 min during surgery. After hair removal and sterilisation of the area with ethanol 75% and betadine, a small incision was made below the right biceps femoris. Blunt dissection was performed to separate the gluteus superficialis and expose the sciatic nerve. Upon isolation of the nerve from the fascia, a tight ligation approximately one-third to one-half the diameter of the sciatic nerve was made to the nerve with 7-0 silicon-treated silk suture. The wound was then closed with non-absorbable monofilament 5-0 Nylon sutures. Mice were carefully monitored during surgery until recovery from anaesthesia. Sham operation was performed in the same way, except nerve ligation was not carried out. Mice after pSNL consistently developed ipsilateral mechanical allodynia, which peaked by Day 1 post-surgery and remained at a similar level until Day 21, the period in which the study was carried out.