



Original experimental

Systemic administration of WY-14643, a selective synthetic agonist of peroxisome proliferator activator receptor- α , alters spinal neuronal firing in a rodent model of neuropathic pain

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HIGHLIGHTS

- Peroxisome proliferator activated receptor alpha (PPAR α) is a nuclear hormone transcription factor.
- PPAR α is widely distributed in the peripheral and central nervous systems.
- PPAR α agonists (e.g. WY-14643) have antinociceptive effects in rodent models of neuropathic pain.

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ABSTRACT

Background and aims: The clinical management of chronic neuropathic pain remains a global health challenge. Current treatments are either ineffective, or associated with unwanted side-effects. The development of effective, safe therapies requires the identification of novel therapeutic targets using clinically relevant animal models of neuropathic pain.

Peroxisome proliferator activated receptor alpha (PPAR α), is a member of the nuclear hormone family of transcription factors, which is widely distributed in the peripheral and central nervous systems. Pharmacological studies report antinociceptive effects of PPAR α agonists following systemic administration in rodent models of neuropathic pain, however the neuronal mechanisms and sites of action mediating these effects are unclear.

The aim of this study was to investigate the effects of systemic administration of the synthetic PPAR α agonist, WY-14643 on mechanically-evoked responses of spinal cord dorsal horn wide dynamic range (WDR) neurones in the spinal nerve ligated (SNL) model of neuropathic pain in rats. In addition, comparative molecular analysis of mRNA coding for PPAR α and PPAR α protein expression in the spinal cord of sham-operated and neuropathic rats was performed.

Methods: Lumbar L5–L6 spinal nerve ligation was performed in male Sprague–Dawley rats (110–130 g) under isoflurane anaesthesia. Sham controls underwent similar surgical conditions, but without ligation of the L5–L6 spinal nerves. Hindpaw withdrawal thresholds were measured on the day of surgery -day 0, and on days -2, 4, 7, 10 and 14 post-surgery. At day 14 extracellular single-unit recordings of spinal (WDR) dorsal horn neurones were performed in both sham and SNL neuropathic rats under anaesthesia. The effects of intraperitoneal (i.p.) administration of WY-14643 (15 and 30 mg/kg) or vehicle on evoked responses of WDR neurones to punctate mechanical stimulation of the peripheral receptive field of varying bending force (8–60 g) were recorded. In a separate cohort of SNL and sham neuropathic rats, the expression of mRNA coding for PPAR α and protein expression in the ipsilateral and contralateral spinal cord was determined using quantitative real time polymerase chain reaction (qRT-PCR) and western blotting techniques respectively.

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Results: WY-14643 (15 and 30 mg/kg i.p.) rapidly attenuated mechanically evoked (8, 10 and 15 g) responses of spinal WDR neurones in SNL, but not sham-operated rats. Molecular analysis revealed significantly increased PPAR α protein, but not mRNA, expression in the ipsilateral spinal cord of SNL, compared to the contralateral side in SNL rats. There were no changes in PPAR α mRNA or protein expression in the sham controls.

Conclusion: The observation that levels of PPAR α protein were increased in ipsilateral spinal cord of neuropathic rats supports a contribution of spinal sites of action mediating the effects of systemic WY-14643. Our data suggests that the inhibitory effects of a PPAR α agonist on spinal neuronal responses may account, at least in part, for their analgesic effects of in neuropathic pain.

Implication: Selective activation of PPAR α in the spinal cord may be therapeutically relevant for the treatment of neuropathic pain.

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1. Introduction

Lesions of the somatosensory nervous system results in the development of neuropathic pain, often characterized by tactile allodynia and/or hyperalgesia [22,26,27]. The underlying mechanisms of neuropathic pain are complex and not well elucidated. Treatments are hampered by limited efficacy [1,32] and/or unwanted side-effects, for review see [12]. Thus, identification of new therapeutic targets may offer the opportunity for the development of more effective and safe therapies for the treatment of neuropathic pain.

Animal models of neuropathic pain are essential tools for elucidating the mechanisms underlying this type of chronic pain. Commonly used rodent models of neuropathic pain such as chronic constriction injury [4] and spinal nerve ligation [21] mimic peripheral nerve injury which results in lowered threshold and/or increased sensitivity to mechanical and thermal stimuli, mediated by central sensitization mechanisms within the spinal cord [17].

The peroxisome proliferator activated receptor alpha (PPAR)- α , is a member of the nuclear hormone family of transcription factors, for review see [6], and is widely distributed within the peripheral and central nervous systems [29].

A growing body of evidence from animal studies support a role for PPAR- α agonists as important modulators of pain processing. Systemic administration of synthetic, or endogenous PPAR α agonists attenuates pain behaviour in rodent models of neuropathic pain [16,24]. PPAR α is expressed at key sites associated with physiological pain processing including the spinal cord, a major site of somatosensory processing [2,15,31]. Rapid long lasting PPAR α activation in the spinal cord in the chronic Freund Adjuvant model of inflammatory pain [2] supports a role for PPAR α signalling in the modulation of spinal nociceptive processing in models of chronic pain. It is well established that increased sensitization of spinal neurones, and activation of resident macrophage or glia cells, within the spinal cord are key mechanisms that contribute to aberrant pain responses in animal models of neuropathic pain [17,19]. The importance of these mechanisms is consolidated by the demonstration of antinociceptive effects of inhibitors of activated glia in rodent models of neuropathic pain [11,28,33]. Despite the immunohistochemical evidence that PPAR α is widely distributed in both neuronal and non-neuronal cells in the spinal cord [3,29], the effects of PPAR α activation on spinal neuronal responses in models of neuropathic pain is unknown.

The aim of the present study was to investigate the effects of systemic administration of the synthetic PPAR α agonist, WY-14643 on spinal nociceptive processing in the spinal nerve ligated (SNL) model of neuropathic pain in rats. Specifically, the effects of WY-14643 on innocuous and noxious mechanically-evoked neuronal responses of spinal cord dorsal horn wide dynamic range (WDR) neurones were determined. In addition, comparative molecular

analysis of mRNA coding for PPAR α and PPAR α protein expression in the spinal cord of sham-operated and neuropathic rats was performed.

2. Methods

All animal experiments were performed in accordance with the UK Home Office Animals (Scientific Procedures) Act of 1986 and international association for the study of pain (IASP) guidelines. Experiments were performed on adult male Sprague–Dawley rats (240–300 g) obtained from Charles River, UK and group-housed in a light-controlled room (12 h light/dark cycle) with ad libitum access to food and water.

2.1. Spinal nerve ligation

SNL model of neuropathic pain was performed according to the method of [21] as previously described [18]. Briefly, male Sprague–Dawley rats (110–130 g) were anesthetized using isoflurane (induction, 3%; maintenance, 1–1.5%; in 33% O₂/67% N₂O) and placed in a prone position. A midline incision was made at the L3–S2 level, left paraspinal muscles at L4–S2 level were separated from spinal processes. Part of the L6 transverse process was removed with fine rongeurs, and the L4–L6 nerves were identified. The L5–L6 spinal nerves were isolated and tightly ligated distal to the dorsal root ganglia and proximal to the sciatic nerve formation with 6-0 silk. The wound was closed in two layers using absorbable sutures and wound clips, after complete hemostasis. This process was repeated in sham-operated rats but without ligation of the L5–L6 spinal nerves. Rats were group-housed post-surgery. The posture and behaviour of rats were monitored over a 48 h period.

2.2. Pain behaviour

Hindpaw withdrawal thresholds were measured on the day of surgery –day 0, and on days- 2, 4, 7, 10 and 14 post-surgery, using published methods. Briefly, sham or SNL rats were placed in a transparent glass cubicle with a wire mesh grid floor. A series of eight Von Frey hair (VFH) monofilaments (Semmes-Weinstein Monofilaments; North Coast Medical Inc., USA, via Linton Instrumentation, Norfolk, UK) with logarithmically incremental stiffness (1.0 g, 1.4 g, 2.0 g, 4.0 g, 6.0 g, 8.0 g, 10 g, and 15 g) were used to determine the hindpaw withdrawal threshold using the up and down method [9].

2.3. Electrophysiology

At two weeks following sham or SNL surgery, rats were prepared for in vivo extracellular recordings of spinal neurones according to methods described previously [18]. Briefly, rats were anesthetized with isoflurane inhalation anaesthetic (induction, 3%; surgery,

Table 1

Electrophysiological characteristics of spinal wide dynamic range (WDR) neurones. Data are mean $\pm$ s.e.m. ($n = 5$ neurones in 5 sham rats and $n = 17$ neurones in 17 SNL rats).

	Sham ($n = 5$)	SNL ($n = 17$)
Depth (μm)	590 $\pm$ 55.68	710 $\pm$ 30.13
C-fibre latency (ms)	158 $\pm$ 30.75	174 $\pm$ 14.34
C-fibre threshold (mA)	0.95 $\pm$ 0.08	0.98 $\pm$ 0.06

2%; maintenance, 1–1.5%; in 33% O₂/67% N₂O), a tracheal cannula was inserted and rats were placed in a stereotaxic frame. Core body temperature was maintained at 36.5–37.5 °C throughout the experiment by means of a heating blanket connected to a rectal temperature probe. A laminectomy was performed to expose segments L4–L5 of the spinal cord. The spinal cord was held rigid by clamps rostral and caudal to the exposed section of spinal cord (L4/5). Extracellular single-unit recordings of WDR dorsal horn neurones (Table 1) were made with glass-coated tungsten microelectrodes. Electrodes were lowered vertically through the cord with a SCAT-01 microdrive (Digitimer, Welwyn Garden City, UK), and depths of recorded neurones from the spinal cord surface noted. Receptive fields of neurones covering one or two toes were identified using pinch. Single-unit activity was amplified and filtered (Digitimer). Signals were digitized and analyzed using a Cambridge Electronic Design micro1401 interface and Spike 2 data acquisition software (Cambridge Electronic Design, Cambridge, UK). Responses of neurones to a train of 16 transcutaneous electrical stimuli (0.5 Hz; 2 ms pulse width) applied to the centre of the receptive field were recorded. All neurones selected were WDR, exhibiting a short-latency A β -fibre-evoked response (0–20 ms after stimulus) and A δ -fibre-evoked response (20–90 ms after stimulus). These neurones also exhibited longer-latency C-fibre-evoked responses (90–300 ms after stimulus) and post discharge responses (300–800 ms after stimulus). Responses of neurones to punctate mechanical stimulation of the peripheral receptive field of varying bending force of 8 and 10 g (innocuous) and 15, 26, and 60 g (noxious) were characterized. Individual von Frey hairs were applied to the centre of the receptive field for 10 s in ascending order with a time period of 10 min maintained between stimulations. Pharmacological studies were performed once stable control responses (<10% variation) were obtained.

2.4. Drug preparation and administration

All treatments were given as intraperitoneal (i.p.) injections once stable baseline-evoked responses of WDR neurones were recorded. Rats were randomly assigned treatment groups and blinded to the experimenter. In the first series of experiments, a single systemic dose of the synthetic PPAR α agonist, WY-14643, (Enzo life sciences, Exeter, UK), 15 mg/kg ($n = 5$ neurones in 5 rats), or vehicle made up of [(0.3% Tween 80/0.9% NaCl and 50% ethanol) combined in a ratio of 1:1, $n = 5$ neurones in 5 rats], was administered and the time course profile of WY-14643, on evoked responses of spinal neurones determined. The ethanol concentration in the vehicle was the minimum required to ensure complete solubility of WY-14643 solution.

In a second cohort of rats, we compared the effects of two doses of WY-14643 (15 mg/kg and 30 mg/kg) on evoked responses of spinal neurones in sham ($n = 5$ neurones in 5 rats) and SNL ($n = 7$ neurones in 7 rats) vs. the effects of vehicle in SNL rats ($n = 5$ neurones in 5 rats). Based on the first series of experiments, the two doses of WY-14643 were administered 60 min apart and effects were recorded for 60 min. Doses of WY-14643 were based on the effects of a synthetic PPAR agonists on pain behaviour in the mouse sciatic nerve ligation model of neuropathic pain [24].

Table 2

Rat primer and probe sequences for PPAR α and β -actin.

PPAR α forward	tggagtcacgcatgtgaag
PPAR α reverse	tggtccggttcttttctgaatct
PPAR α probe	cttcttccggcgaactattcggctaaagc
β -actin forward	gccatgtacgtagccatcca
β -actin reverse	tctccgagtcacatcaaatg
β -actin probe	gttcctgtatgcctctggtcgaccac

2.5. Measurement of PPAR α mRNA and protein expression in the spinal cord

In order to perform a comparative anatomical study of PPAR α mRNA and protein expression in the spinal cord, a separate cohort of sham and SNL neuropathic rats were specifically generated for this purpose. The development of mechanical allodynia was monitored in these cohort and at day 14 post-surgery, rats were sacrificed and ipsilateral and contralateral spinal cord tissues harvested for the measurement of PPAR α mRNA using quantitative real time polymerase chain reaction (qRT-PCR) and protein expression by Western blotting analysis according to methods described previously [30].

2.5.1. RNA extraction and cDNA synthesis

Approximately 50 mg of frozen tissue was homogenized in 2 ml of ice cold Tri reagent (Sigma–Aldrich) and total RNA extracted according to methods described previously [30]. Total RNA clean up and on-column DNase digestion were performed using RNeasy purification columns (Qiagen). RNA concentration, integrity and purity were determined using a Nanodrop spectrophotometer. For cDNA synthesis, 1 μg of total RNA was reverse-transcribed using superscript III reverse transcriptase (Invitrogen, UK) in a total reaction volume of 20 μl for 1 h at 50 °C and the reaction terminated at 70 °C for 15 min. The final concentration of cDNA was 50 ng/ μl .

2.5.2. Taqman qRT-PCR

The relative standard curve method based on Taqman qRT-PCR was used for quantifying gene expression. Samples were prepared in a total reaction volume of 25 μl [13 μl Taqman 2 $\times$ reagent, 0.75 μl forward primer (10 μM), 0.75 μl reverse primer (10 μM), 0.5 μl Probe (10 μM), 5 μl water, and 5 μl cDNA]. qRT-PCR was performed using the Step One Plus sequence detection system (Applied Biosciences, UK). Gene expression was determined relative to β -actin. Primers and probes for all genes (Table 2) were designed using Primer 3 software and synthesized by MWG Biotech (Germany).

2.5.3. Western blotting

Briefly, approximately 50 mg of spinal cord tissue was homogenized in 1 ml of RIPA lysis buffer [150 mM NaCl, 25 mM Tris–HCl, pH 7.6, 0.5% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulphate, 1 mM Na₃VO₄ 10 mM NaF, 1 $\times$ Complete Protease inhibitor (Sigma–Aldrich, UK, Cat#11697498001)] and centrifuged at 15,000 $\times$ g for 20 min. Protein content was assayed in supernatant fraction following centrifugation using a Pierce kit assay (Thermo Fischer, UK, Cat#23225) according to the manufacturer's instructions. 50 μg of protein was separated on a 10% sodium dodecyl sulphate polyacrylamide gel and transferred onto a nitrocellulose membrane followed by overnight incubation at 4 °C with rabbit polyclonal primary antibody to PPAR α (Abcam, UK Cat# AB8934, 1:200 dilution) and mouse monoclonal primary antibody to β -actin (Sigma–Aldrich, UK, Cat#A2228, 1:5000). Preliminary control experiments were performed to validate the specificity of the antibody using rat liver and muscle protein, both of which highly express PPAR α , as positive controls and adipose tissue as a negative control for PPAR α expression [8], Supplementary Fig. 1.

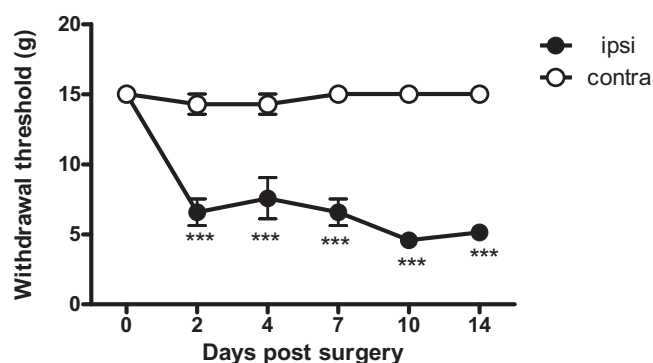


Fig. 1. Development of mechanical allodynia in the hindpaw of SNL rats. Ipsilateral hindpaw withdrawal thresholds progressively decreased up to 14 days post-surgery. Data are mean $\pm$ s.e.m. *** $p < 0.0001$ vs. contralateral hindpaw ($n = 17$ SNL rats).

Blots were washed in TBS/0.1% Tween (TBST) buffer and incubated with IRDye® conjugated goat polyclonal anti-rabbit or anti-mouse IgG (LI-COR® Biosciences, UK, 1:10,000 dilution) as appropriate. Scanning and densitometric analysis of blots were performed using a LI-COR® ODYSSEY infrared imaging system.

Supplementary Fig. S1 related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.sjpain.2015.06.004>

2.6. Statistical analysis

Data are expressed as mean $\pm$ s.e.m. and were analyzed using PRISM statistical software (Graphpad PRISM®). Hindpaw withdrawal threshold data was analyzed by 2-way repeated measures ANOVA with time (post-surgery) and side of surgery (ipsilateral or contralateral), considered as two factors. Mean-maximal effects of WY-14643 on evoked responses of WDR neurones were analyzed by unpaired *t*-test (experiment 1 data), or by 1-way ANOVA (experiment 2 data). Bonferroni post hoc tests were performed where appropriate and $p < 0.05$ was considered statistically significant. Unpaired *t*-test was used to analyse PPAR α mRNA and protein expression data.

3. Results

3.1. The development of mechanical allodynia in the ipsilateral hindpaw

The development of allodynia was monitored in sham and SNL-operated rats by testing hindpaw sensitivity to mechanical stimuli from days 2 to day 14 post-surgery. Analysis of hindpaw mechanical withdrawal thresholds in SNL-operated rats revealed significant time (post-surgery) $F_{(5,60)} = 22.53$, $p < 0.0001$, side $F_{(1,60)} = 141.26$, $p < 0.0001$ and time $\times$ side interaction $F_{(5,60)} = 21.66$, $p < 0.0001$ effects. Mechanical withdrawal thresholds of the ipsilateral hindpaw were significantly reduced at day 2 post-surgery, compared with the contralateral hindpaw (Fig. 1), and progressively decreased until day 14 post-SNL surgery when rats were used for electrophysiological studies. By contrast, mechanical withdrawal thresholds of the ipsilateral hindpaw of sham-operated rats did not alter over the duration of the study (data not shown).

3.2. Effects of systemic administration of WY-14643 on evoked responses of WDR neurones

3.2.1. Time-course profile of evoked responses in WDR neurones following systemic WY-14643 administration

In the first series of experiments, the time-course of a single systemic dose of WY-14643 (15 mg/kg) on mechanical-evoked

responses of WDR neurones was studied for 2 h in a cohort of SNL-operated rats. There was a significant rapid reduction in innocuous (8 g and 10 g) and noxious (15 g) evoked firing of WDR neurones within 30 min of WY-14643 administration, in comparison with pre-drug baseline levels (Fig. 2A). Inhibitory effects of WY-14643 were maintained for 20 min and then responses returned to baseline values at 60 min post WY-14643 administration.

3.2.2. Comparison of mean maximal effects of WY-14643 on neuronal responses in Sham and SNL rats

In the next series of experiments, the effects of two doses of WY-14643 (15 and 30 mg/kg) on mechanically-evoked responses of spinal WDR neurones were studied in sham ($n = 5$) and SNL ($n = 7$) rats. Both doses of WY-14643 significantly ($p < 0.05$), attenuated 8 g, 10 g and 15 g evoked responses of WDR neurones in SNL-operated rats, compared with the effects of vehicle ($n = 5$ rats) (Fig. 2B). Importantly, the effects of WY-14643 on low weight (8 g)-evoked responses of WDR neurones were significantly different to the lack of effect of this intervention on 8 g-evoked responses in sham operated rats (Fig. 2B). There was a similar trend for 10 g- and 15 g-evoked responses of WDR neurones in SNL rats, compared to sham operated controls (Fig. 2B). Responses of WDR neurones to supra-threshold mechanical stimuli were not altered by WY-14643 in either group of rats (Fig. 2B).

3.2.3. Expression of PPAR α in the spinal cord of sham and SNL rats

Analysis of mRNA coding for PPAR α did not reveal any significant differences between ipsilateral and contralateral sides of the spinal cord in sham or SNL rats (Fig. 3A, B). In contrast, western blotting analysis revealed a significant ($p < 0.05$) increase in PPAR α protein expression in the ipsilateral side of the spinal cord of SNL rats, compared with the contralateral side. These changes in PPAR α expression were not observed in the spinal cord of sham-operated rats (Fig. 3C–E).

4. Discussion

Intra-peritoneal administration of the synthetic PPAR α WY-14643 rapidly attenuated innocuous and noxious evoked responses of WDR neurones in SNL, but not sham-operated, rats. Molecular analysis of post-mortem tissue revealed a significantly increased PPAR α protein, but not mRNA expression, in the ipsilateral spinal cord of SNL rats, in comparison with the contralateral side. The demonstration of rapid dose-dependent reductions in evoked responses of spinal neurones by systemic WY-14643 in SNL, but not sham-operated, rats is consistent with evidence from behavioural studies of PPAR α agonists in a rodent model of neuropathic pain [24]. The doses of WY-14643 used herein did not alter suprathreshold noxious evoked responses of spinal neurones in SNL rats. We interpret these observations as a reflection of the dose-limiting effects of WY-14643 administered, which may also account for the short duration of action.

The demonstration that systemic administration of a PPAR α agonist modulates spinal nociceptive processing in a rodent model of neuropathic pain is consistent with findings from previous studies [5,13,25]. Repeated treatment with palmitoylethanolamide (PEA), an endogenous agonist of PPAR α [23] reduced pain behaviour in rodent models of neuropathic pain, in part by delaying the recruitment and degranulation of mast cells associated with activated microglia in the spinal cord [5]. Electrophysiological studies demonstrated that repeated PEA treatment attenuated the duration, frequency and evoked activity of spinal neurones in a formalin-induced model of neuropathic pain in rats [25]. One important difference between these previous studies and the data reported herein is the choice of a synthetic PPAR α agonist

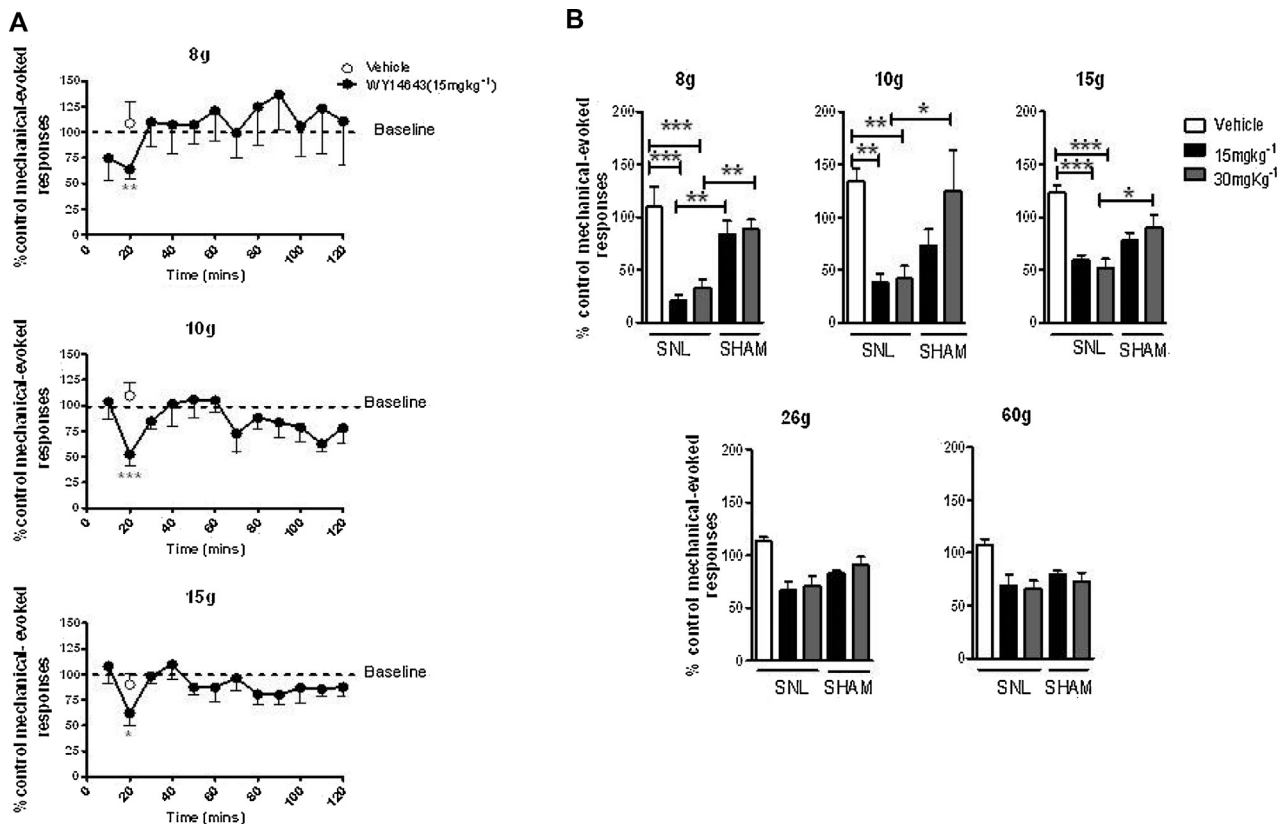


Fig. 2. Effects of WY-14643 and vehicle on mechanically-evoked responses of spinal WDR neurones. (A) Time-course of the effects of WY-14643 (15 mg/kg) on 8g-, 10g- and 15g-evoked responses of spinal WDR neurones in SNL rats. (B) Mean maximal effects of systemic WY-14643 (15 and 30 mg/kg) on 8–60g mechanically-evoked responses of spinal WDR neurones in sham and SNL-operated rats. Data are mean $\pm$ s.e.m. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$ ($n = 5$ neurons from 5 rats for each group for time course data), and ($n = 5$ neurons from 5 sham rats, $n = 7$ neurons from 7 SNL rats and $n = 5$ neurons from 5 rats for vehicle-treated rats).

(WY-14643) rather than endogenous compound (PEA). WY-14643 has advantages from a mechanistic point of view since it allows for the evaluation of the contribution of selective PPAR α activation to spinal nociceptive processing in a model of neuropathic pain. Previous studies have demonstrated the contribution of multiple receptor systems, including the cannabinoid (CB $_1$), transient vanilloid receptor (TRPV $_1$) and PPAR gamma (PPAR γ) receptors in mediating the analgesic effects of PEA in a mouse model of CCI [13]. Pharmacologically, this level of complexity in the mechanisms of action of PEA makes it very attractive, but less so from the mechanistic perspective of understanding the specific contributions of PPAR α activation to spinal nociceptive processing in neuropathic pain.

The expression of PPAR α in the spinal cord has previously been described [3,29]. Importantly, these previous studies localized PPAR α expression to both neuronal and glia cells, although a pre-dominant expression in glia relative to neurons was reported [29]. In keeping with these previous findings, we have demonstrated the presence of both protein and mRNA coding for PPAR α expression in the spinal cord of SNL and sham-operated rats. In the present study, we observed a significant increase in PPAR α protein but not mRNA expression in the ipsilateral side of the spinal cord of SNL rats. Evidence of rapid changes in PPAR α protein expression and activation in a model of inflammatory pain in rats has also been reported, suggesting a functional role in spinal nociceptive processing [2]. The attenuation of evoked responses of spinal neurones by WY-14643 in SNL rats in this study suggests that the increase in PPAR α protein expression may be functionally relevant to spinal nociceptive processing in a neuropathic pain state. This view is also consistent with our observations of a lack of effect of WY-14643 on evoked

responses of spinal neurones, and no change in PPAR α protein expression in the ipsilateral spinal cord in sham-operated rats.

Paradoxically, the expression of mRNA in the ipsilateral spinal cord of SNL rats was not increased in parallel with the increased protein expression at day 14 post SNL. This mismatch between mRNA and protein expression of PPAR α suggests that the increase in PPAR α protein expression arises as a result of ligand-dependent stabilization [6] by endogenous ligands or post-translational modifications [7], which increases the half-life of PPAR α protein in the ipsilateral spinal cord of neuropathic rats. The association of PPAR α with glia cells in the spinal cord, [3,29], and the role of the latter in the development of central sensitization [19,34] may also account for the increase in ipsilateral PPAR α protein expression in the spinal cord of SNL rats.

The facilitation of cross-talk between glia and neurons in the spinal cord is an important mechanism of central sensitization [35] and mediated at least in part by the sustained release of pro-inflammatory cytokines associated with activated glia [20]. Given the well-documented anti-inflammatory properties of PPAR α activation [10,14], it is feasible that the inhibition of pro-inflammatory signalling in the spinal cord of SNL rats may represent a mechanism by which PPAR α activation regulates glia activity. This is supported by the demonstration that PPAR α agonists inhibit secretion of proinflammatory cytokines including TNF α , IL-1 β , and IL-6 cytokine associated with glia activation [36,37].

A limitation in the present study was our inability to carry out a dose-response study in order to determine the optimal dose range of WY-14643 required to modulate evoked responses of spinal neurones in neuropathic pain states. Nonetheless, our studies provide further evidence for the functional role of PPAR α agonists in

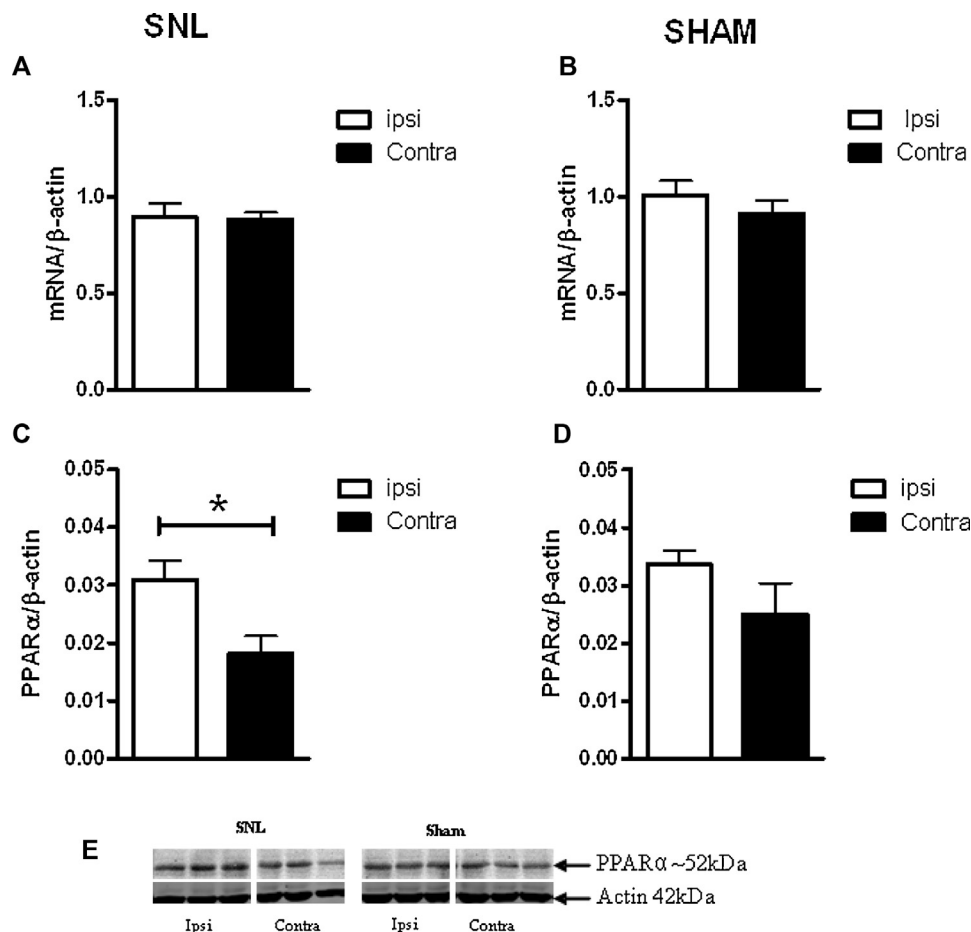


Fig. 3. PPARα expression in the spinal cord of sham and SNL-operated rats. (A–B) mRNA coding for PPARα and (C–E) PPARα protein in the ipsi- and contralateral spinal cord of sham and SNL-operated rats. Data are expressed as mean $\pm$ s.e.m., * p < 0.05 (n = 4 rats per group for mRNA data and n = 3 rats per group for protein data).

modulating neuropathic pain, and support the further investigation of how activation of spinal PPARα attenuates evoked responses of spinal neurones specifically in neuropathic rats. In conclusion, our data suggest that the spinal cord may be a key site of action for the analgesic effects of PPARα agonists in models of neuropathic pain.

Conflict of interest

None.

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