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GW406381, a novel COX-2 inhibitor, attenuates spontaneous ectopic discharge in sural nerves of rats following chronic constriction injury

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Abstract

There are several lines of evidence to suggest that cyclooxygenase-2 (COX-2) plays an important role in the generation and maintenance of neuropathic pain states following peripheral nerve injury. However, COX-2 inhibitors are generally ineffective in reversing mechanical allodynia and hyperalgesia in models of neuropathic hypersensitivity. Here, we have investigated the effects of GW406381, a novel COX-2 inhibitor, on mechanical allodynia, hyperalgesia and generation of spontaneous ectopic discharge in rats following chronic constriction injury (CCI) of the sciatic nerve and compared it with rofecoxib. GW406381 (5 mg/kg, 5 days of treatment) significantly reversed the CCI-induced decrease in paw withdrawal thresholds (PWTs), assessed using both von Frey hair and paw pressure tests, whereas an equi-effective dose of rofecoxib (5 mg/kg, 5 days of treatment) in inflammatory pain models was ineffective. In rats treated with GW406381, the proportion of fibres showing spontaneous activity was significantly lower (15.58%) than that in the vehicle (32.67%)- and rofecoxib (39.66%)-treated rats. Ibuprofen, a non-selective COX inhibitor, at 5 mg/kg, orally dosed three times a day for 5 days did not significantly affect the PWTs in CCI rats. In naïve rats, GW406381 did not significantly change the PWTs. These results illustrate that COX-2 may indeed play an important role in the maintenance of neuropathic pain following nerve injury, but that only certain COX-2 inhibitors, such as GW406381, are effective in this paradigm. Whilst the mechanisms underlying this differential effect of GW406381 are not clear, differences in drug/enzyme kinetic interactions may be a key contributing factor.

Keywords: GW406381; Rofecoxib; Neuropathic pain; Ectopic discharge; Chronic constriction injury (CCI)

1. Introduction

Neuropathic pain is a common syndrome, which may result from peripheral or central nerve injury or as a result of pathological changes induced by metabolic disease, viral infection, traumatic injury or chemotherapeutically induced nerve damage (for review see Zimmermann, 2001). Clinically, neuropathic pain is characterised by mechanical and thermal allodynia, hyperalgesia and spontaneous ongoing pain which are often refractory to current available treatments (Woolf and Mannion, 1999; Collins and Chessell, 2005).

Though the role of COX-2 in inflammatory diseases has been confirmed in both animal models and in humans (Taylor, 1999; Clemett and Goa, 2000; Bianchi and Broggini, 2002; Broom et al., 2004), its role in neuropathic pain is still to be defined. There have been several lines of experimental evidence to suggest that the up-regulation of COX-2 mRNA and protein occurs in injured human and rat nervous system tissues in multiple neuropathic pain models (Ma and Eisenach, 2002, 2003; Durrenberger et al., 2004; Takahashi et al.,

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2004). However, the efficacy of COX-2 inhibitors in preclinical studies of neuropathic hypersensitivity remains equivocal (Lashbrook et al., 1999; Broom et al., 2004; Padi and Kulkarni, 2004), and no randomised, placebo-controlled studies of COX-2 inhibitors in clinical neuropathic pain have been reported. In addition, there appear few reported clinical studies of non-selective COX inhibitors in treating neuropathic pain conditions.

Ectopic discharge is a common electrophysiological phenomenon observed in various models of neuropathic pain (Burchiel et al., 1985; Sheen and Chung, 1993; Liu et al., 2000a,b). With substantial evidence indicating that the spontaneous ectopic discharge originates from injured afferent axons (Wall and Gutnick, 1974; Matzner and Devor, 1994; Devor and Seltzer, 1999; Pan et al., 1999; Chen et al., 2001; Zhang et al., 2003) and/or in dorsal root ganglia (DRG) (Devor et al., 1992b; Kajander et al., 1992; Li et al., 2000; Michaelis et al., 2000), it appears within the same temporal window as mechanical allodynia and hyperalgesia following nerve injury (Abdi et al., 1998; Pan et al., 1999; Han et al., 2000; Liu et al., 2000a; Chen et al., 2001). It is thought that ectopic discharge generated from the neuroma and DRG following nerve injury forms a persistent afferent barrage transmitted into the spinal cord which consequently leads to the sensitisation of peripheral and central nervous systems and chronic pain (Kajander and Bennett, 1992; Sheen and Chung, 1993; Tal and Eliav, 1996; Devor and Seltzer, 1999; Liu et al., 2000a,b).

In this study, we have examined the effect of oral administration of a novel COX-2 inhibitor, GW406381 (see Beswick et al., 2004; Bingham et al., 2005), on paw withdrawal thresholds (von Frey hair test and paw pressure test) in CCI and naïve rats and compared it to ibuprofen, a non-selective COX inhibitor and rofecoxib, a COX-2 inhibitor which shares a similar efficacy profile to traditional COX-2 inhibitors. In addition, the effects of GW406381, rofecoxib and vehicle on the generation of spontaneous ectopic discharge in the sural nerve in the CCI model of neuropathic pain were also evaluated.

2. Materials and methods

Male Sprague–Dawley rats, weighing 150–180 g at the time of surgery, were used for this study. The animals were housed in perspex cages in groups of four in a controlled environment of constant temperature and moisture (temperature: 21 ± 1 °C and light and dark circle of 12–12 h) and fed *ad libitum*. All procedures in this study were approved by UK Home Office in accordance with the Animals (Scientific Procedures) Act 1986.

2.1. Surgery

The chronic constriction injury (CCI) model was prepared following the standard protocols described previously (Bennett and Xie, 1988). Briefly, rats were anaesthetised with a 5% isofluorane/95% oxygen gas mixture for induction followed by an intraperitoneal injection of sodium pentobarbitone (50 mg/kg). The lateral side of the left thigh was shaved and disinfected with 75% ethanol. A small incision of about 1 cm was made parallel to the femur. The muscle was carefully separated to expose the sciatic nerve. Four loose ligatures were placed on the sciatic nerve with 4–0 suture silk thread at 1 mm intervals. The wound was closed in layers with suture silk and the animals were placed in a recovery chamber with the temperature controlled at 30 °C. The animals were placed back to their home cage after complete return of consciousness and free movement. A dose of amoxicillin (0.1 ml, 15 mg) was routinely injected intraperitoneally after surgery to prevent infection.

2.2. Behavioural tests

2.2.1. Mechanical allodynia (von Frey hair test)

The assessment of paw withdrawal threshold (PWT) in response to mechanical stimulation was carried out on the 14th day after surgery and was repeated after 5 days of drug dosing. The PWT was measured using a series of graduated von Frey hairs (Semmes–Weinstein monofilaments, Stoelting). The animals were placed in individual perspex boxes on a raised metal mesh for at least 30 min before the test. Starting from the filament of lowest force, each filament was applied perpendicularly to the centre of the ventral surface of the paw until slightly bending for 6 s. If the animal withdrew or lifted the paw upon stimulation, then a hair with force immediately lower than that tested would be used. If no response was observed, then a hair with force immediately higher would be tested. The lowest amount of force required to induce reliable responses (positive in three out of five trials) was recorded as the value of PWT.

2.2.2. Paw pressure test (Randall–Selitto test)

Paw withdrawal threshold in response to pressure was assessed using an analgesy meter (Ugo-Basile, Italy). The PWT was first tested 14 days after CCI surgery and re-tested after 5 days of drug dosing. The paw of the rat was placed onto a small plastic platform and increasing pressure was applied to an area between the third and fourth metatarsus of the dorsal surface of the paw with a cone-shaped probe (tip diameter 1 mm). The pressure was steadily increased at a rate of 10 g/s and stopped when the rat withdrew the leg. The average value of two consecutive measurements from each side was used for analysis. Only those CCI rats with clear hyperalgesia (PWT difference between CCI side and normal side $\geqslant$30 g) were used for drug dosing.

2.3. Electrophysiology

After behavioural testing, rats were anaesthetised with thiobutabarbital sodium (inactin, Sigma) at 120 mg/kg, i.p. for induction, 20–60 mg/kg i.v. through an intravenous cannula for the maintenance and thereafter throughout the experiment if required. The right carotid artery and jugular vein were cannulated separately for monitoring blood pressure and drug application, respectively. The body temperature was monitored and controlled within a physiological range through a thermo-blanket system. Electrocardiogram was routinely

monitored. The sural nerve was exposed via a dorsal incision on the hind limb and covered with warm mineral oil. The skin was stitched onto a metal O-ring to form an oil pool for recording nerve activity. Using a microscope, the sural nerve, away from the sciatic nerve injured area, was separated carefully from the surrounding connective tissues and sectioned at the level just above the ankle. A small bundle of nerve filaments were teased from the proximal cut end of the sural nerve which was still connected with CNS and sciatic nerve but disconnected with peripheral innervated area, and looped onto a unipolar silver wire recording electrode with an indifference electrode connected to the connective tissues nearby, allowing the spontaneous activity generated from either the ligated area (sensitised axons) or from DRG (somata) to be recorded. The electrical signal was amplified through a Digitimer AC amplifier (NL104) and filtered with low pass set at 50–500 Hz and high cut at 5k to 50 kHz. The signals were then recorded through a CED micro-1401 interface to a PC for recording and off-line analysis. Recording from a fibre with spontaneous activity lasted for at least 1 min and for a silent fibre, at least 30 s.

2.4. Drug administration

GW406381 and rofecoxib (both produced and synthesised by the department of medicinal chemistry, GSK) were dissolved in a vehicle containing 1% DMSO, 66% polyethylene glycol 200 (PEG200, Sigma) and 33% saline to 5 mg/ml and sonicated before oral dosing. The vehicle and drugs were orally administered twice a day between 8:00–9:00 a.m. and 16:00–17:00 p.m. In a separate experiment, normal saline (1 ml/kg) and ibuprofen, dissolved in normal saline to form a suspension, were orally dosed in CCI rats at 5 mg/kg, three times a day for 5 days. Experimenters were blinded to the names and chemical properties of the test compounds. Doses of compounds were chosen from their dose–response profile in models of inflammatory hypersensitivity as described previously (Bingham et al., 2005).

2.5. Statistical analysis

Data for behavioural tests and firing frequencies are presented as means ± SEM. Statistical evaluations are based on paired or unpaired Student's *t*-test, χ^2 test or ANOVA, as appropriate, with Microsoft Excel built-in analysis programs or GraphPad Prism 4.0 software. A significance level was set at $P < 0.05$.

3. Results

3.1. Behavioural tests

3.1.1. Effects of GW406381 and rofecoxib: von Frey hair test

In age-matched control rats, the PWT values obtained from left and right sides were similar and without statistically significant differences. This threshold was relatively stable over time (9.50 ± 0.33, 10.38 ± 0.71, and 9.75 ± 0.25 g over 3 days, $n = 8$). In this study, von Frey hair test data from the healthy leg were used as the control reference value.

Following vehicle treatment for 5 days, the PWT upon von Frey hair stimulation on the ipsilateral side significantly increased from 1.40 ± 0.13 to 3.42 ± 0.51 g ($n = 9$; $P < 0.01$). In rofecoxib-treated rats, the PWT was also significantly increased from 1.11 ± 0.15 to 4.29 ± 1.49 g ($n = 9$; $P < 0.01$). In GW406381-treated rats, the PWT was significantly increased from 1.16 ± 0.18 g before dosing to 7.56 ± 0.56 g after 5 days of dosing ($n = 9$, $P < 0.001$). There was no significant difference in the PWT after the dosing between the vehicle and rofecoxib groups ($P > 0.05$). However, the PWT was significantly higher after dosing in the GW406381-treated rats compared to that in vehicle group ($P < 0.001$) and rofecoxib group ($P < 0.05$; Fig. 1A).

In contrast, in the contralateral side, the PWT was not significantly affected. The PWTs before and after dosing in the vehicle groups were 9.56 ± 0.29 and 9.89 ± 0.72 g, respectively ($P > 0.05$); in the rofecoxib group, the PWTs were 11.11 ± 0.73 and 11.44 ± 0.91 g ($P > 0.05$); in the GW406381-treated group, the PWTs were 10.67 ± 0.87 and 10.67 ± 0.87 g, respectively ($P > 0.05$; Fig. 1B).

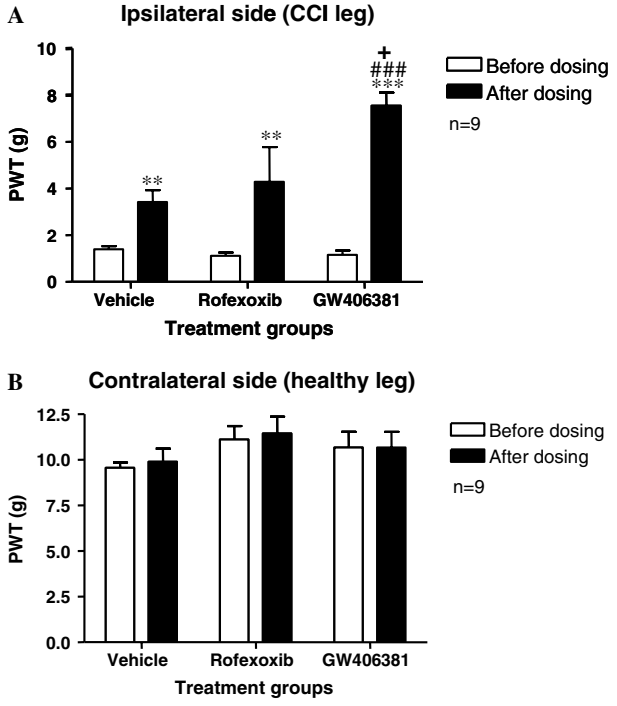

Fig. 1. Effect of vehicle, rofecoxib and GW406381 on the paw withdrawal threshold (PWT) in response to von Frey hair stimulation. (A) PWT in ipsilateral (CCI) side before and after drug dosing. **, ***: compared to the value before drug dosing, $P < 0.01$, 0.001. ###, compared to vehicle group after drug administration, $P < 0.001$; +, compared to rofecoxib group after 5-day drug administration, $P < 0.05$, all Student's *t*-test. (B) Contralateral (healthy) side, PWT was not significantly changed after 5-day vehicle or drug administration.

The effects of GW406381 on PWT in response to von Frey hairs were also examined in eight naïve rats. Before dosing started, the PWT were 10.74 ± 0.58 and 10.88 ± 0.51 g on left and right hind paws, respectively. After 5-day dosing of GW406381, the PWT was not significantly changed. The PWT in the left and right hind paws were 10.74 ± 0.58 and 11.17 ± 0.49 g, respectively. There was no significant change in both sides after drug dosing ($P > 0.05$ for comparison of left and right paws before and after dosing, Fig. 2A).

3.1.2. Effects of GW406381 and rofecoxib: Randall–Selitto test

The results from Randall–Selitto test were more variable between the treatment groups. However, in any group of normal animals, the averaged values of the PWT from left and right sides were always similar and without any statistically significant difference. For example, in a test group of 12 animals, the PWT from left and right sides were 139.33 ± 15.49 and 144.83 ± 15.91 g, respectively Thus there was no significant difference between the two sides ($P > 0.05$). In CCI rats, the degree of difference between ipsilateral and contralateral sides varied between groups of animals. However, the animals chosen for drug-dosing experiments displayed a clear difference in PWT between CCI and healthy legs. The difference in PWT between ipsilateral (injured) and contralateral sides was equal to or larger than 30 g.

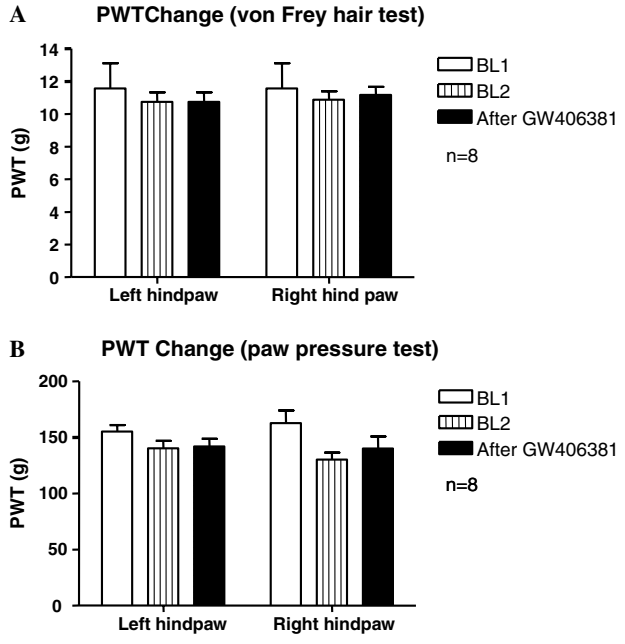

Fig. 2. Effect of GW406381 on paw withdrawal thresholds (PWT) in response to von Frey hairs (A) and paw pressure stimulation (B) in naïve rats. The results shown here in (A) and (B) suggest no significant change in PWT after 5-day treatment with vehicle or GW406381 in both ipsilateral and contralateral sides. $P > 0.05$, one-way ANOVA test. BL1, baseline 1; BL2, baseline 2.

In vehicle-treated CCI rats, ipsilateral PWT was unchanged after 5 days of vehicle dosing (99.90 ± 2.63 g before dosing and 95.20 ± 7.72 g after dosing; $n = 5$, $P > 0.05$). In rofecoxib-treated rats, the PWT was slightly reduced after 5 days of dosing (119.9 ± 15.25 g before dosing and 81.1 ± 8.26 g after dosing, $P > 0.05$, $n = 5$). In contrast, in rats dosed with GW406381 for 5 days, the PWT increased from 100.90 ± 11.97 g before dosing to 146.7 ± 18.73 g after dosing ($P < 0.05$, $n = 5$; Fig. 3A).

On the contralateral side, in the vehicle-treated group, the PWT slightly decreased after 5-day dosing (198.00 ± 22.01 g before dosing to 153.00 ± 21.35 g after dosing, $P > 0.05$). In rofecoxib-treated animals, the PWT on the healthy side fell significantly from 189.10 ± 26.54 g before dosing to 124.20 ± 8.33 g after dosing ($P < 0.05$, $n = 5$). In GW406381-treated rats, in contrast, the PWT was not significantly affected after drug dosing (162.50 ± 12.54 g before dosing to 170.10 ± 10.21 g after dosing, $P > 0.05$, $n = 5$; Fig. 3B).

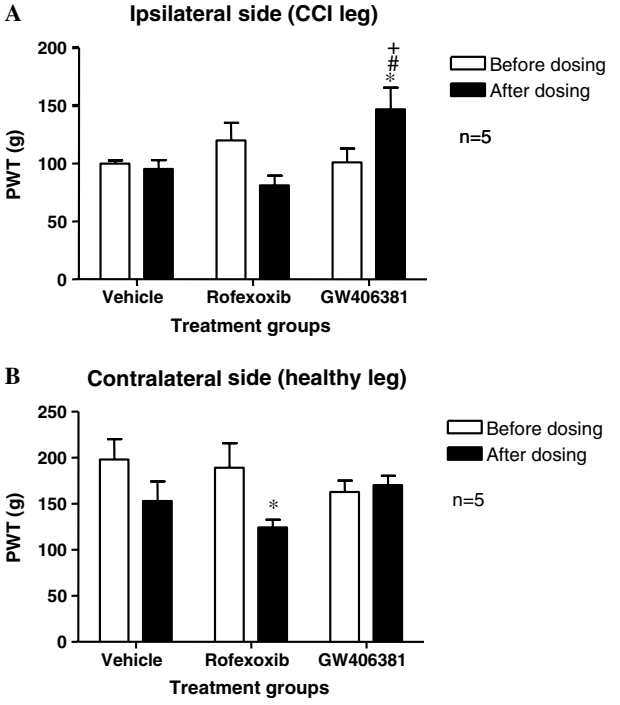

Fig. 3. Changes of paw withdrawal threshold (PWT) in CCI rats in response to paw pressure (Randall–Selitto test) after 5-day oral vehicle or drug administration. (A) In ipsilateral (CCI) side, before vehicle or drug dosing, there was no significant difference in PWT among the vehicle, rofecoxib and GW406381 groups. After 5-day dosing, in vehicle- or rofecoxib-treated groups PWT was not significantly changed, $P > 0.05$. In GW406381-treated rats, however, the PWT was significantly increased after dosing, compared to pre-dosing, $P < 0.05$ and was significantly higher compared to that in vehicle- and rofecoxib-treated rats ($P < 0.05$, # and +). (B) In contralateral (healthy) side, the PWT was not significantly changed after 5-day dosing in vehicle- and GW406381-treated rats but decreased in rofecoxib-treated rats, $P < 0.05$ compared to pre-dosing level, all Student's t-test.

In a group of eight naïve rats, the effects of GW 406381 were also observed. Before dosing, the PWT in the left and right hind paws in response to paw pressure stimulation were 140.31 ± 6.60 and 130.31 ± 6.15 g, respectively. After 5-day dosing, the PWT were 141.88 ± 6.88 and 140.00 ± 10.94 g in the left and right hind paws, respectively. There was no significant difference either between left and right hind paws or before and after dosing ($P > 0.05$, Fig. 2B).

3.1.3. Effects of saline and ibuprofen on paw withdrawal threshold in CCI rats: von Frey hair test

In a group of eight CCI rats, the effect of saline on PWT in response to von Frey hairs was examined. In the ipsilateral side of saline-treated rats, post-surgery, the PWT was significantly lowered to 1.07 ± 0.13 g from baseline before surgery of 12.15 ± 1.49 g ($P < 0.001$). After 5-day dosing of saline, the PWT was not significantly changed (1.07 ± 0.13 g) compared to that before dosing ($P > 0.05$, Fig. 4A). In the contralateral side, the PWT after surgery and after 5-day dosing of saline was not significantly different from that before surgery ($P > 0.05$, Fig. 4B).

Similarly, in eight CCI rats treated with ibuprofen, the PWT in the ipsilateral side was significantly lowered after surgery (from 9.72 ± 0.51 before surgery to 1.21 ± 0.20 g on the14th day after surgery, $P < 0.001$). Five-day treatment with ibuprofen at 5 mg/kg, three times a day, did not significantly change the PWT (1.36 ± 0.16 g), $P > 0.05$, compared to that after surgery (Fig. 4A). In the contralateral side, the PWT after surgery (9.57 ± 0.54 g) and after dosing of ibuprofen (9.87 ± 0.48 g) was not significantly different from that before surgery (10.00 ± 0.58), $P > 0.05$, compared to the PWT before surgery (Fig. 4B).

3.1.4. Effects of saline and ibuprofen on the paw withdrawal threshold in CCI rats: Randall–Selitto test

In the above eight CCI rats, the effect of saline and ibuprofen on the PWT in response to paw pressure stimulation was simultaneously observed as von Frey hair test. In saline group, the PWT in ipsilateral side was 139.69 ± 9.2 g before surgery. After CCI surgery, the PWT was significantly lowered to 94.69 ± 7.84 g ($P < 0.01$, Fig. 5A). After 5-day dosing of ibuprofen, the PWT was not significantly different (93.44 ± 5.36 g) from that before dosing. In the contralateral side, the PWT before, after surgery and after ibuprofen dos-

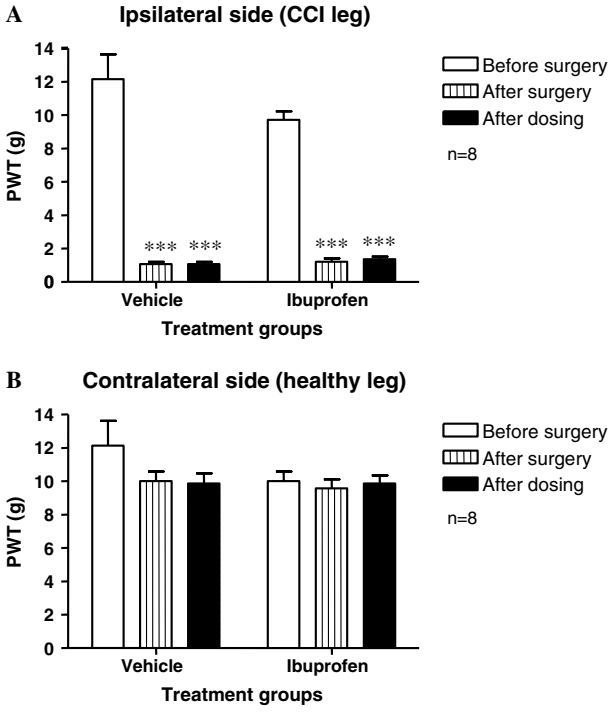

Fig. 4. Effects of ibuprofen on paw withdrawal threshold (PWT) in response to von Frey hair stimulation in CCI rats. (A) In ipsilateral (CCI) side, PWT before, after surgery and after ibuprofen dosing at 5 mg/kg, three times a day for 5 days. After surgery, PWT in ipsilateral side was significantly decreased, compared to pre-surgery level. After 5-day dosing of ibuprofen, there was no significant change in the lowered PWT. (B) On the contralateral side, PWT was not significantly changed either after surgery, or after ibuprofen dosing ($P > 0.05$). ***, compared to pre-surgery level, $P < 0.001$. One-way ANOVA and Student's *t*-test.

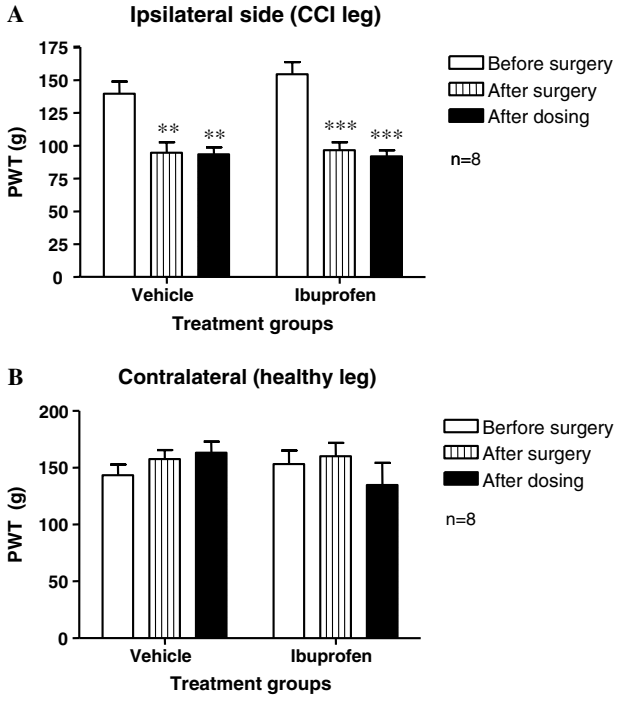

Fig. 5. Effect of ibuprofen on paw withdrawal threshold (PWT) in response to paw pressure in CCI rats. (A) In ipsilateral side (CCI side), PWT was significantly ($P < 0.01$ and 0.001) lower than that before surgery but was not different in the groups treated either with vehicle or with ibuprofen at 5 mg/kg for 5 days, compared to PWT after surgery. (B) PWT was not significantly changed in both groups after surgery and after 5 days of vehicle or ibuprofen dosing. One-way ANOVA and Student's *t*-test.

ing were 143.44 ± 9.26, 157.50 ± 8.16 and 163.13 ± 9.73 g, respectively. No significant difference was observed among the PWTs at different times ($P > 0.05$, Fig. 5B).

3.2. Electrophysiology

3.2.1. Patterns of spontaneous activity recorded from sural nerve fibres in CCI rats

In the present study, the common patterns of spontaneous activity observed in sural nerve fibres included typical burst-like firing (Fig. 6Aa), fast burst-like firing (Fig. 6Ab), paired/triplet firing (Fig. 6B), regular and irregular firing (Figs 6C and D). Burst-like firing was characterised by bursts of firing lasting between 0.5 and 2 s with intervals between the bursts of 1 and 4 s. Each burst contained between 6 and 20 action potentials. However, these characteristics varied largely from fibre to fibre. Two subtypes of burst-like patterns of firing were distinguishable: (a) fast bursting pattern (Fig. 6Ab) containing 4–8 potentials with intervals between bursts of 100 and 200 ms; (b) regular discharges of action potentials with relatively regular intervals between two bursts. Paired/triplet firing, the activity appeared always in doublets or triplets, as in Fig. 6B, where each burst contained two or three action potentials with intervals of around 100 ms. In regular firing fibres, action potentials discharged regularly in a tonic firing pattern (Fig. 6C). Irregular firing fibres discharged action potentials randomly with no obvious pattern and usually at low frequency (from less than 10 impulses per minute to a few hundred impulses per minute; Fig. 6D). The numbers of fibres displaying these various firing patterns and their incidence in the various treatment groups are displayed in Table 1. Although there was a range of spontaneous activity patterns observed in CCI rats, this was rarely, if ever, observed in naïve animals.

3.2.2. Effects of GW406381 and rofecoxib on the proportions of fibres displaying spontaneous activity

The percentage of spontaneously active fibres in all teased fibres (bundles) in vehicle- and drug-treated rats was calculated. Data were grouped from two series of experiments. Each batch contained four rats for each treatment group (total eight rats in each group). In vehicle-treated rats, 756 small bundles were examined. Among them, 247 bundles were 'active' (32.67%). In rofecoxib-treated rats, among 928 bundles studied 368 'active' bundles were detected (39.66%). Finally, in GW406381-treated rats, among 719 bundles recorded, 112 bundles discharged spontaneous activity (15.58%). Statistical analysis revealed that the percentage of active bundles in the rofecoxib-treated group was significantly higher than that in the vehicle group ($P < 0.001$). In contrast, the percentage of active fibres in the GW406381-treated group was significantly lower than that observed in the vehicle and rofecoxib groups ($P < 0.001$, Fig. 7).

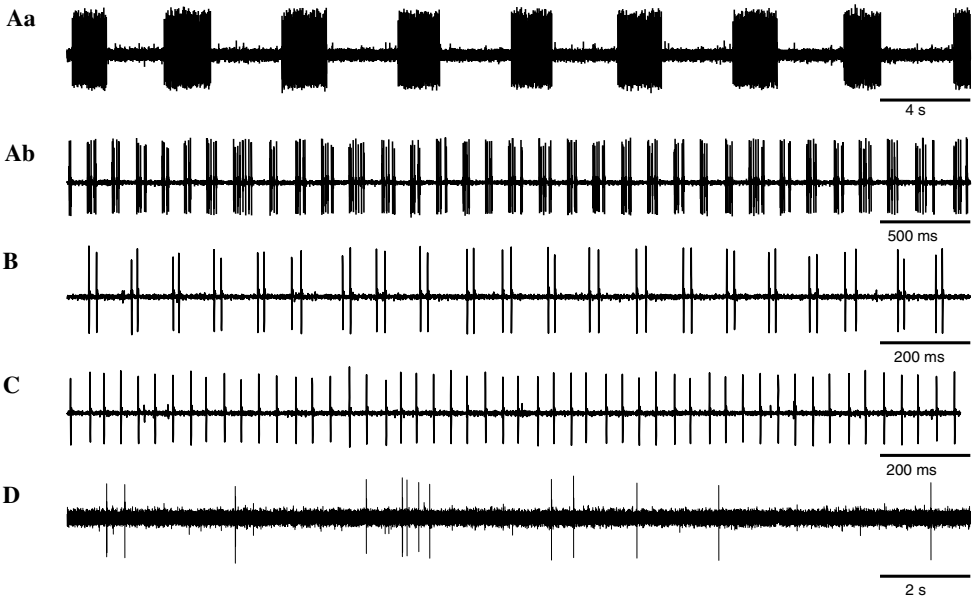

Fig. 6. Firing patterns of spontaneous activity in sural nerve fibres of CCI rats. (Aa) is typical burst-like firing; (Ab) can also be categorised as burst-like, but with shorter intervals between bursts and less spikes in each burst, therefore it can be classified as fast burst-like firing; (B) paired/triplet firing: the firing appears always in pairs (in some cases, in three spikes, e.g. tripled firing, or a mixed pattern of paired/tripled firing, not shown here); and, (C) is an example of regular firing, with very regular intervals between spikes. (D) An example of irregular firing, e.g., atypical spontaneous activity without noticeable pattern, usually with lower frequency. For more details see the text and Table 1.

Table 1
Effects of rofecoxib and GW406381 on spontaneous activity recorded from sural nerve fibres in CCI rats

Pattern	Vehicle	Rofecoxib	GW406381	Total
Burst-like (*n*)	118	190	39	347
Frequency (imp/min)	1391.86 ± 71.99	1371.55 ± 59.45	1017.51 ± 117.28**,##	
Regular (*n*)	21	39	14	74
Frequency (imp/min)	1023.67 ± 189.77	820.44 ± 126.25	632.93 ± 172.39	
Paired/tripled (*n*)	45	58	20	123
Frequency (imp/min)	1208.73 ± 80.50	999.19 ± 57.27**	922.85 ± 63.60**	
Irregular (*n*)	63	81	39	183
Frequency (imp/min)	141.71 ± 16.21	161.02 ± 17.62	93.13 ± 16.59*,#	
Total	247 (756)	368 (928)	112 (719)	727 (2403)

The number of fibres, patterns and frequency of firing associated with spontaneously active fibres in CCI rats treated with vehicle, rofecoxib and GW406381. See Fig. 3 and text for details of the firing patterns. The numbers in brackets are the total number of fibres recorded, including fibres with and without spontaneous activity *, **: compared to vehicle group, $P < 0.05$ and 0.01; #, ##: compared to rofecoxib group, $P < 0.05$ and 0.01.

3.2.3. Effects of GW406381 and rofecoxib on the frequency of spontaneous activity

The average firing frequency of the spontaneously active fibres was calculated for each group. In vehicle-treated rats, the firing frequency of the spontaneous activity ranged from 18 to 3903 impulses/min, and the average frequency of firing in this group of 247 active fibres was 1008.33 ± 52.43 impulses/min. In the rofecoxib-treated group, of 368 active fibres, the frequency of discharges ranged from 4 to 3956 impulses/min, the average frequency being 988.01 ± 42.84 impulses/min. This was not significantly different from that observed in the vehicle group ($P > 0.05$). In contrast, in 112 active fibres in GW406381-treated rats, the fibres fired action potentials at a frequency ranging from 9 to 2543 impulses/min with an average frequency of 630.65 ± 61.19 impulses/min. The frequency of spontaneous activity in the GW406381-treated group was significantly lower than that in the vehicle and rofecoxib groups ($P < 0.001$, compared to vehicle and rofecoxib groups, respectively; Fig. 8A and B).

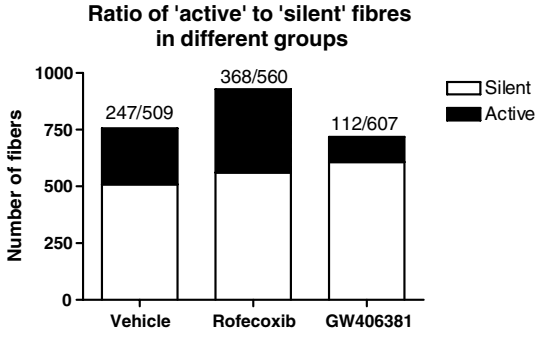

Fig. 7. Ratio of positive (active) to negative (silent) fibres in vehicle-, rofecoxib- and GW406381-treated CCI rats ($n = 8$ in each group). The numbers over each column represent the numbers of 'active' and 'silent' units from different groups. The percentage of active fibres is significantly lower in GW406381-treated rats, $P < 0.001$ compared to the vehicle- and rofecoxib-treated rats, χ^2 test.

The data on the firing patterns and frequencies in the various treatment groups are summarized in Table 1.

4. Discussion

4.1. COX-2 and neuropathic pain

Inflammation and nerve degeneration, seen as a direct effect following nerve injury, may play an impor-

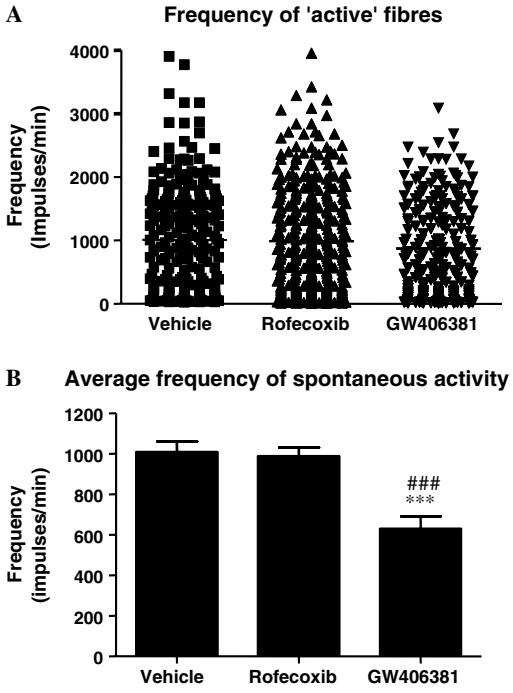

Fig. 8. Frequency of spontaneous activity in active fibres recorded from vehicle-, rofecoxib- and GW406381-treated CCI rats. (A) Frequency of spontaneous activity in fibres of different treatment groups. (B) Averaged frequency of activity in vehicle-, rofecoxib- and GW406381-treated CCI rats (8 rats in each group, 247, 368 and 112 units in vehicle, rofecoxib and GW406381groups, respectively). ***, compared to the vehicle group, $P < 0.001$; ###, compared to rofecoxib group, $P < 0.001$, Student's *t*-test.

tant role in the early stages of the development of neuropathic pain states (Wu et al., 2001, 2002; Ma and Eisenach, 2002; Ma et al., 2002; Schäfers et al., 2004). COX-1 and COX-2 take part in the synthesis of multiple inflammatory mediators, including prostaglandins, which are potent algesic factors in sensitising neuronal responses. Between them COX-1 is largely constitutively expressed, while COX-2 is induced following insult. Non-selective COX inhibitors (e.g. NSAIDs), though effective in treating inflammatory diseases, are poorly effective or ineffective in the systemic treatment of both preclinical and clinical neuropathic hypersensitivity (Lashbrook et al., 1999), and are associated with adverse gastrointestinal side effects, which limit their long-term use. Our results from this study that ibuprofen, one of clinically used non-selective COX inhibitors, had no significant efficacy in relieving allodynia and hyperalgesia following CCI are in line with these findings. COX-2-selective inhibitors have an improved GI side-effect liability, and have a good therapeutic effect in treating inflammatory diseases (Hinz and Brune, 2004), but again their effects in treating neuropathic pain states clinically are anecdotally poor, although no randomised controlled studies have been formally undertaken. Similarly, traditional COX-2 inhibitors are systemically poorly effective or ineffective in preclinical models of mechanical neuropathic hypersensitivity. However, two reports suggest that chronic oral dosing of COX-2-selective inhibitors, etodolac and celecoxib, can alleviate heat-evoked hyperalgesia in CCI rats (Schäfers et al., 2004; Suyama et al., 2004), but mechanical hypersensitivity appears to be unresponsive to COX-2 inhibitors (Bingham et al., 2005). Similarly, chronic dosing of rofecoxib (i.p.) in the spared nerve injury model failed to prevent the development of the mechanical, thermal allodynia and hyperalgesia or inhibit existing allodynia and hyperalgesia (Broom et al., 2004).

There is emerging evidence however, that prostaglandins, which are considered amongst the most potent inflammatory mediators, are also associated with the generation or modulation of ectopic discharges. In several neuropathic pain models, PGE2, or its analogs, applied to the neuroma, evoked or potentiated ectopic discharges (Devor et al., 1992a; Michaelis et al., 1998; Omana-Zapata and Bley, 2001). In another study, induction of inflammation around the saphenous nerve trunk with carrageenan and CFA, which increased the synthesis of COX-2 and prostaglandins, also resulted in spontaneous activity in axons as well as mechanical hyperalgesia behaviourally (Eliav et al., 2001). These spontaneous activities are believed to be associated with the maintenance of central hypersensitivity and may directly mediate persistent, ongoing pain (Campbell et al., 1992; Gracely et al., 1992), which is often defined as an uncomfortable burning sensation and is difficult to treat.

4.2. Efficacy of GW406381

In this study, we have examined the efficacy of orally dosed rofecoxib and a novel COX-2 inhibitor, GW406381, on the mechanically evoked paw withdrawal thresholds in normal and CCI rats and the generation of spontaneous activity in sural nerve axons of CCI rats. We have found that chronic dosing with GW406381 in normal rats did not significantly change both PWTs measured with von Frey hairs and paw pressure. However, in CCI rats, GW406381, not only significantly raised the PWTs, both in von Frey hair and Randall–Selitto tests, but also reduced the number of fibres showing spontaneous activity as well as the frequency of firing in these fibres. In contrast, rofecoxib had no significant effect in reversing CCI-induced decreases in PWT measured with von Frey hairs, and in the Randall–Selitto test. Moreover, rofecoxib failed to reduce the proportion of active fibres and firing rates in electrophysiological experiments. These studies support results from other laboratories where rofecoxib did not reverse allodynia in neuropathic animals (Lashbrook et al., 1999; Broom et al., 2004). Rofecoxib has attenuated allodynia in one study, but only at a very high dose (50 mg/kg, i.p., De Vry et al., 2004). Doses of both GW406381 and rofecoxib in this study were based on pharmacodynamic and pharmacokinetic parameters described by Bingham et al. (2005).

GW406381, similar to rofecoxib, is a potent and highly selective COX-2 inhibitor (Beswick et al., 2004; Bingham et al., 2005). GW406381 differs from rofecoxib in two key respects (Bingham et al., 2005): (1) GW406381 has approximately twice the ability to penetrate the CNS as rofecoxib (steady-state brain–blood ratios of 1.53 and 0.8, respectively), and (2) GW406381 has a slow dissociation following binding to the COX-2 enzyme ($0.0041\ min^{-1}$ vs $0.698\ min^{-1}$, GW406381 and rofecoxib, respectively). As the primary site of ectopic discharge generation is in the periphery, it seems unlikely that the former reason accounts for this differentiation in this study. However, further investigation is required to fully elucidate the mechanism underlying the differences observed between these two COX-2 inhibitors. As GW406381 is highly selective for COX-2, with no known off target activity (Bingham et al., 2005), interaction with another molecular target also seems unlikely. We have also partly ruled out the possibility that the non-selective inhibition of COX-2 may lead to the increased PWTs, since ibuprofen did not show a significant efficacy in reversing lowered PWT in CCI rats.

We have observed that the vehicle we used in this study had some effect in reversing the PWT in response to von Frey hairs. This is possibly due to polyethylene glycol (PEG 200), since some authors claim that it can help CNS tissue repair and function re-establishment

(Laverty et al., 2004; Luo et al., 2004). However, in this study we see clear drug differences with GW406381 compared to vehicle group ruling out that reversal in this case is coming from vehicle alone.

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In summary, the present study examined the effects of orally dosed GW406381, a novel COX-2 inhibitor, on mechanical allodynia, hyperalgesia and generation of spontaneous ectopic discharge in the sural nerve of CCI rats, and compared it with rofecoxib, a known COX-2 inhibitor. Only GW406381 significantly reversed the decease in paw withdrawal thresholds assessed using von Frey hairs and paw pressure tests (Randall–Selitto test). Moreover, in rats treated with GW406381 the proportion of fibres with spontaneous activity was significantly lower than that in the vehicle- and rofecoxib-treated rats. These results illustrate that COX-2 may indeed play an important role in the maintenance of neuropathic pain following nerve injury, but that only certain COX-2 inhibitors, such as GW406381, are effective in this paradigm.

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