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Pharmacological characterisation of acid-induced muscle allodynia in rats

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Abstract

Previous studies have shown that repeated injections of acidic saline, given into the lateral gastrocnemius muscle of rats, results in a bilateral reduction in withdrawal threshold to tactile stimulation of the hindpaws. We have now characterised this model of muscoskeletal pain pharmacologically, by evaluating the antinociceptive effects of various analgesics after systemic administration. The μ-opioid receptor agonist morphine (3 and 6 mg/kg) produced a particularly prolonged antiallodynic effect. The glutamate receptor antagonists ([8-methyl-5-(4-(*N*,*N*-dimethylsulfamoyl)phenyl)-6,7,8,9,-tetrahydro-1H-pyrrolo[3,2-*h*]-iso-quinoline-2,3-dione-3-*O*-(4-hydroxybutyric acid-2-yl)oxime] NS1209 and ketamine (6 and 15 mg/kg, respectively), the KCNQ K^+ channel openers retigabine and flupirtine (10 and 20 mg/kg, respectively) and the Na^+ channel blocker mexiletine (37.5 mg/kg) also significantly increased paw withdrawal threshold, although to a lesser degree than morphine. In contrast, the anticonvulsant lamotrigine (30 mg/kg), the cyclooxygenase-2 inhibitor carprofen (15 mg/kg) and the benzodiazepine diazepam (3 mg/kg) were ineffective. All antinociceptive effects were observed at nonataxic doses as determined by the rotarod test. These results suggest that in this model, muscle-mediated pain can be alleviated by various analgesics with differing mechanisms of action, and that once established ongoing inflammation does not appear to contribute to this process.

Keywords: Central sensitisation; Fibromyalgia; GABA (gamma aminobutyric acid); Glutamate receptor antagonist; Muscle pain; Na^+ channel blockers

1. Introduction

Long-term changes in activity and function of nociceptive pain pathways can occur as a result of inflammatory- or neuropathic-mediated damage within both the peripheral and central nervous systems (Woolf and Salter, 2000). A consequence of this can be the expression of accentuated pain-related behaviours, reflecting hyperalgesia and allodynia in response to thermal and mechanical stimulation. Pain associated with muscoskeletal conditions often induce disability and represent a significant financial burden to society in terms of health care costs and lost productivity (Yelin and Callahan, 1995). Chronic pain conditions such as fibromyalgia are characterised by widespread muscle pain and joint tenderness (Wolfe et al., 1990). A recent report of enhanced temporal summation (wind up) in fibromyalgia patients, consistent with central sensitisation (Price et al., 2002), suggests that the aetiological development of chronic musculoskeletal pain in humans may share a number of common underlying mechanisms with other chronic pain conditions including those of neuropathic origin (Zimmermann, 1991). Similar to neuropathic pain in humans, chronic muscoskeletal pain conditions remain somewhat refractory to treatment with currently available analgesics, reinforcing the need to develop appropriate animal models of muscle-associated chronic pain.

Recently, Sluka et al. have developed a model of acid-induced pain in rodents, which has been suggested to have greater face validity to pain of muscoskeletal origin in humans. In this model, two injections of acidic (pH 4) saline separated by 2–5 days, given into the gastrocnemius muscle, have been shown to produce a long-lasting bilateral decrease in hindpaw withdrawal threshold as evaluated by von Frey hair stimulation (Sluka et al., 2001). Morphological analysis of the injected muscle revealed no obvious muscle damage associated with acidic saline injection, whilst injection of lidocaine into the ipsilateral muscle attenuated nociceptive scores of the ipsilateral hindpaw only (Sluka et al., 2001). This latter observation, together with the more recent demonstration that intrathecal injection of

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either glutamate receptor antagonists or opioid receptor agonists attenuates hindpaw hypersensitivity to sensory stimulation, suggests that the maintenance of acid-induced chronic pain in this model is also centrally mediated (Skyba et al., 2002).

In order to provide a thorough pharmacological characterisation of this model of acid-induced chronic pain in the rat, various drugs including morphine, glutamate receptor antagonists, voltage-activated Na^+ channel blockers, KCNQ K^+ channel openers (Rogawski, 2000), carprofen and diazepam, all of which target specific aspects of nociceptive transmission, were administered systemically and the effects on pain-like behaviours observed.

2. Materials and methods

Adult male Sprague–Dawley rats (Möllegaard, Denmark) weighing 200–300 g on the day of the first acidic saline injection were used in this study. The animals were housed on soft bedding, two per cage, with food and water ad libitum. The light–dark cycle was 12:12 h. The experiments were performed according to the Ethical Guidelines of the International Association for the Study of Pain (Zimmermann, 1983) and the Danish Committee for Experiments on animals.

2.1. Injection of the muscle

The method of inducing muscle-mediated chronic pain has been described in detail previously (Sluka et al., 2001). The skin covering the lateral gastrocnemius muscle was shaved, and rats were briefly anesthetized with a gaseous mixture of isoflurane (3–5%) supplemented with oxygen. One lateral gastrocnemius muscle was injected with 100 μl of HCl-adjusted preservative-free physiological saline (9 g NaCl/l; pH 4.0 ± 0.1). Five days later the same gastrocnemius muscle was re-injected using an identical injection protocol. As a control for the injection procedure itself, a separate group of eight animals were injected with saline at pH 7.4.

2.2. Behavioural testing

For testing of mechanical allodynia, mechanical hyperalgesia and cold allodynia, the animals were placed on an elevated metal grid allowing stimulation of the plantar surface of the paw, and the animals were allowed to adapt to their environment for 15 min.

2.2.1. Mechanical allodynia

The presence of mechanical allodynia was assessed using a series of von Frey nylon hairs (0.94–19.4 g) (Stoelting, IL, USA), which were applied in increasing force until the rat withdrew its hindpaw. Each hair was applied five times and the threshold (g) was taken as the lowest force that caused at least three withdrawals out of the five consecutive stimuli (Erichsen and Blackburn-Munro, 2002). Von Frey nylon hairs were calibrated both prior to and throughout the time course of the entire study to ensure that consistent bending forces were routinely applied.

2.2.2. Mechanical hyperalgesia

A pin prick test was used to test for the presence of mechanical hyperalgesia (Blackburn-Munro and Jensen, 2003). The plantar surface of the hindpaw was pressured with the point of a safety pin at an intensity insufficient to penetrate the skin, and the duration of the withdrawal response recorded with a stopwatch.

2.2.3. Cold allodynia

To test for the presence of cold hypersensitivity, ethyl chloride (Perstorps, Sweden) was sprayed onto the plantar surface of the hindpaw and animals were observed for both the intensity of the response and any paw withdrawal duration (Blackburn-Munro and Jensen, 2003). This was then classified according to the following scale: 0—no visible response; 1—startle response without paw withdrawal; 2—clear withdrawal of the paw for (0–1 s); 3—prolonged withdrawal (1–5 s) often combined with flinching and licking of the paw; 4—prolonged repetitive withdrawal (>5 s) and/or vocalization.

2.2.4. Thermal hyperalgesia

Finally, to test for the presence of thermal hyperalgesia, reflex nociceptive pain was assessed using the rat plantar test (Ugo Basile, Comerio, Italy) following a modified method of Hargreaves et al. (1988). Single rats were placed in individual perspex boxes on a glass platform and allowed to habituate for 15 min. A mobile radiant heat source was located under the platform and focussed onto the plantar surface of each hindpaw in turn, enabling paw withdrawal latency values to be recorded. The thermal hyperalgesia response was measured twice before acid injection and then on days 2, 7, 11 and 14 post-injection.

All other behavioural responses to sensory stimulation of the hindpaw described above were assessed before the first intramuscular (i.m.) acid injection and then at 3 and 24 h later. They were then measured again immediately before the second i.m. acid injection and again at 3 and 24 h later. Thereafter, they were measured routinely each week for up to 5 weeks. Only animals with a paw withdrawal threshold to von Frey hair stimulation of 5.41 g or less on the day of the experiment were included for drug testing. At least 7 days after the second injection of acid, either drug or vehicle was injected and behavioural responses measured by an experimenter blinded to treatment.

2.3. Rotarod testing for ataxia

In normal rats changes in motor performance after drug administration were evaluated using the rotarod test (with

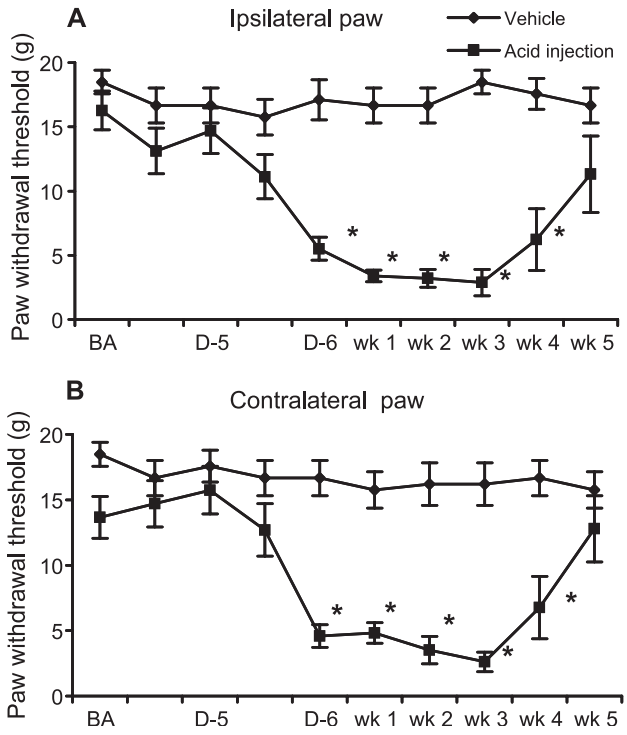

Fig. 1. Sensitivity changes of the hindpaw after repeated injections of acidic saline in the gastrocnemius muscle, in 72 % of the animals injected. Animals that did not develop allodynia were excluded from the study. Withdrawal threshold (g) after stimulation of the plantar surface of the hindpaw was measured with von Frey hairs and the development of mechanical allodynia observed for both the ipsilateral (A) and contralateral (B) hindpaw. The timepoints are: baseline measured before first injection (BA), 3 h after first injection, and immediately before second injection (D-5), 3 h after the second injection, 24 h after the second injection (D-6), and on weeks 1–5 after the second injection (week). A significant increase in mechanical sensitivity occurred for both the ipsilateral and contralateral paws from 24 h after the second injection until 4 weeks (both paws). Data are presented as mean ± S.E.M., $*P<0.05$ vs. baseline.

the exception of morphine, ([8-methyl-5-(4-(*N*,*N*-dimethylsulfamoyl)phenyl)-6,7,8,9,-tetrahydro-1H-pyrrolo[3,2-*h*]-iso-quinoline-2,3-dione-3-*O*-(4-hydroxybutyric acid-2-yl)oxime] NS1209 lamotrigine and mexiletine which have previously been tested using identical doses to those described in the current study (Erichsen and Blackburn-Munro, 2002). The animals were acclimatized to the revolving drum by a training run the day prior to drug testing. They were required to walk on the rotating rod, at 4 rpm for a maximum of 2 min. Only animals that showed no impairment in motor coordination (determined to be present if any rat fell more than two times during the recording period) were included for subsequent testing. On the following day, after baseline responses had been established, animals were administered appropriate drug treatments prior to further testing. Where applicable, the effective dose (ED_{50}) of drug required to induce ataxia (any animal falling more than two times) was calculated as the dose that induced motor impairments in 50% of the animals.

2.4. Drugs

Morphine hydrocloride was obtained from Mecobenzon (Denmark) and mexiletine hydrochloride was purchased from Sigma. NS1209 is a selective, competitive and potent AMPA receptor antagonist (Mathiesen et al., 1998) and was synthesised at NeuroSearch. Retigabine (*N*-(2-amino-4(4-fluorobenzylamino)-phenyl)carbamic acid ethyl ester) and flupirtine (Katadolon) were also synthesised at NeuroSearch A/S. Lamotrigine (Lamictal; 3,5-diamino-6-[2,3-dichlorophenyl]-1,2,4-triazine) was kindly supplied by GlaxoSmithKline (UK). Ketamine (Ketalar, 50 mg/ml), carprofen (Rimidryl Vet., 50 mg/ml) and diazepam were purchased from Unichem, Copenhagen, Denmark. Morphine, NS1209, mexiletine and diazepam were dissolved in physiological saline. Carprofen and ketamine were diluted from stock with physiological saline. Retigabine, flupirtine and lamotrigine were dissolved in Tween 80 and diluted to 10% with physiological saline. Morphine and carprofen were injected subcutaneously (s.c.), and all other drugs were administered intraperitonally (i.p.).

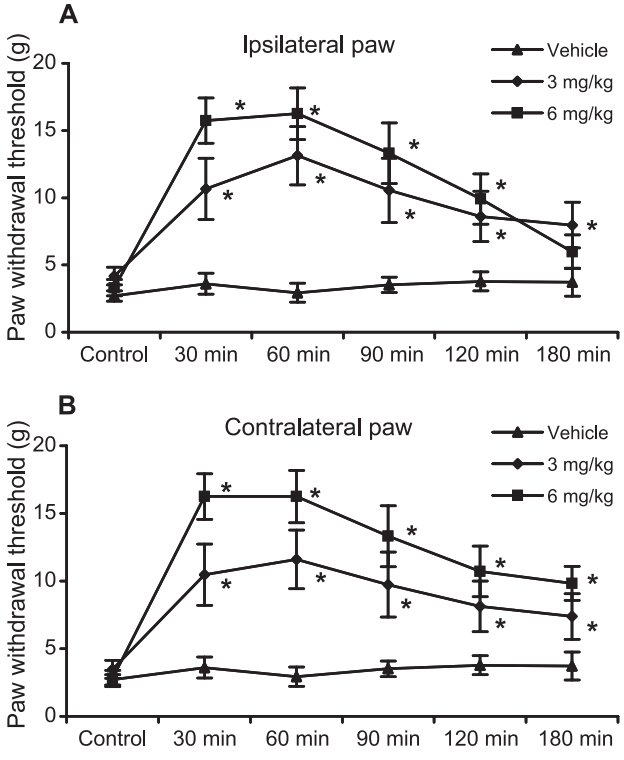

Fig. 2. Effects of morphine on hindpaw withdrawal threshold. Morphine (3 and 6 mg/kg, i.p.) was injected immediately after the baseline (control) response had been obtained. Morphine induced a dose-dependent increase in ipsilateral (A) and contralateral (B) paw withdrawal threshold, which remained significantly different from baseline and vehicle for up to 180 min after injection (ipsilateral paw for 120 min at morphine 6 mg/kg). Data are presented as mean ± S.E.M., $*P<0.05$ vs. corresponding vehicle time points.

2.5. Statistics

Analysis of the data was performed using Statistica (version 6.0). Data for von Frey hair stimulation are presented as mean ± S.E.M. One way repeated analysis of variance (ANOVA) was used to analyse the overall effects of the treatments, followed by posthoc testing with Tukey's test to calculate the difference between groups. In all cases $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. General observations

From a total of 68 animals that received repeated injections of acidic saline, 49 (72%) showed a significant reduction in paw withdrawal threshold in response to von Frey hair stimulation 24 h after the second injection. The remaining 19 animals (28%) displayed reflex responses to innocuous mechanical stimulation that were similar to pre-injection levels and were excluded from the study. Twenty-four hours after the second injection, the withdrawal threshold to mechanical stimulation of the ipsilateral paw decreased from a pre-injection level of 16.2 ± 1.5 to 5.5 ± 0.9 g ($P < 0.001$; Fig. 1). The contralateral paw showed a similar reduction in withdrawal threshold from a pre-injection level of 13.6 ± 1.6 to 4.6 ± 0.9 g ($P < 0.001$). This increased sensitivity remained significant until 4 weeks after the second injection for both hindpaws. There was no significant difference in paw withdrawal threshold between the ipsilateral and contralateral sides. An additional eight animals, injected with saline at pH 7.4, showed no significant change in paw withdrawal threshold up to 5 weeks later (Fig. 1).

To test for the presence of mechanical hyperalgesia and cold allodynia both the ipsilateral and contralateral hindpaws were stimulated with a safety pin or ethyl chloride. No changes in paw withdrawal duration (all < 0.5 s) or cold observation scores (0–1) were observed for either paw when compared with pre-surgery levels (all < 0.5 s and 0–1, respectively), for up to 5 weeks after the second injection of acidic saline. Similarly, when tested for the presence of thermal hyperalgesia, there was no change in paw withdrawal latency to a noxious thermal stimulus for either the ipsilateral or contralateral sides at all time points when compared with pre-injection levels (7.4 ± 0.8 and 7.5 ± 0.4 s, respectively). Finally, in all experiments where drugs were tested, minimal significant differences in drug effects were observed between ipsilateral and contralateral

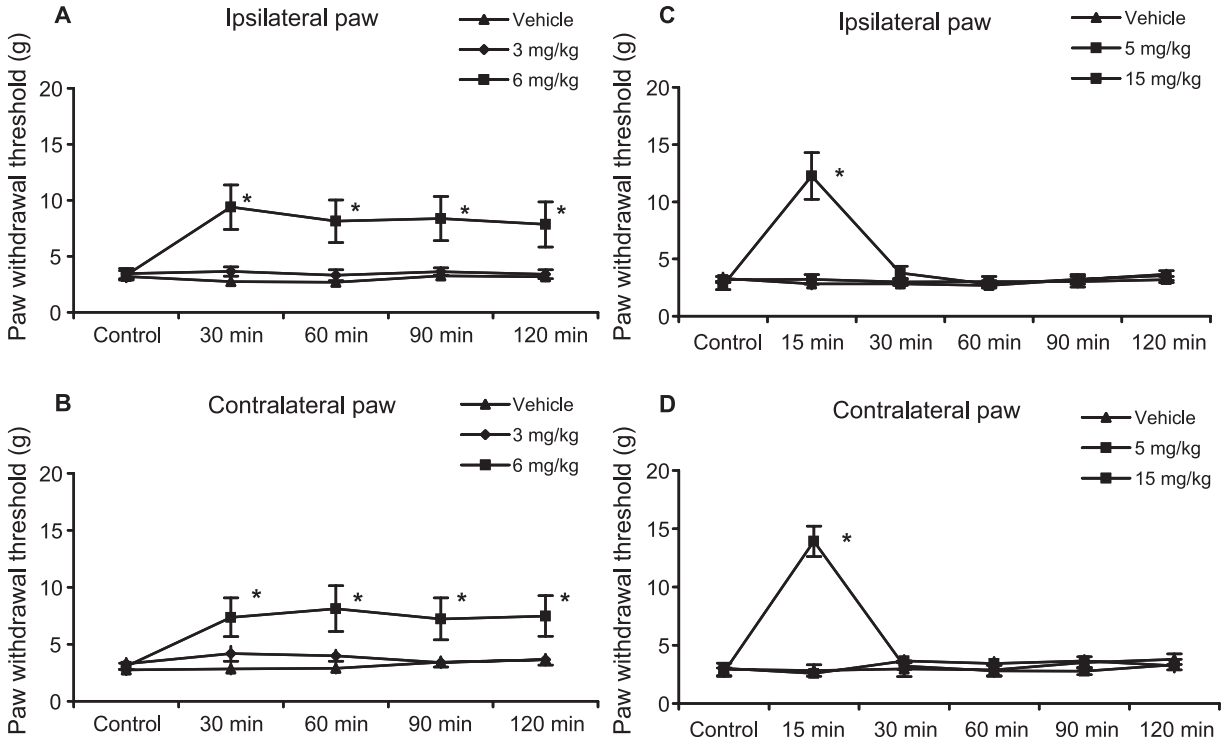

Fig. 3. Effects of glutamate receptor antagonists on hindpaw withdrawal threshold. (A, B) The AMPA receptor antagonist NS1209 (3 and 6 mg/kg, i.p.) was injected immediately after the baseline response (control) had been obtained. At the highest dose tested NS1209 increased paw withdrawal threshold from 30 min after injection and this remained significantly different from vehicle up to 120 min in both ipsilateral and contralateral paws. (C, D) The NMDA receptor antagonist ketamine (5 and 15 mg/kg, i.p.) was injected immediately after the baseline response had been obtained. Although ketamine significantly increased paw withdrawal threshold compared with vehicle (15 mg/kg), this effect was transient, lasting for only 15 min after injection for both hind paws. Data are presented as mean ± S.E.M., *$P < 0.05$ vs. corresponding vehicle time points.

paw responses when compared to vehicle. Thus, in the following sections only values for the ipsilateral paw are given, except in those instances where differences between the two paws were observed.

3.2. Effects of morphine

Systemic administration of morphine produced a dose-dependent increase in paw withdrawal threshold in response to von Frey hair stimulation after repeated application of acidic saline injection (Fig. 2). The lowest dose of morphine (3 mg/kg, s.c.) significantly increased paw withdrawal threshold to 10.7 ± 2.2 ($P < 0.0001$), compared with a threshold level of 3.4 ± 0.7 g, 30 min after injection of vehicle. The increase in paw withdrawal threshold remained significantly different from vehicle 180 min after injection (7.9 ± 1.6 g, $P < 0.05$). At the highest dose of morphine tested (6 mg/kg, s.c.), the increase in paw withdrawal threshold was even greater 30 min post injection (15.7 ± 1.7 g, $P < 0.0001$). This effect remained significantly different from vehicle up to 120 min for the ipsilateral paw (9.9 ± 1.9 g, $P < 0.001$), and up to 180 min (9.8 ± 1.9 g, $P < 0.005$) for the contralateral paw. We have previously shown that morphine has no effect on motor performance in the rotarod test when administered at the doses used in the current study (Erichsen and Blackburn-Munro, 2002).

3.3. Effects of glutamate receptor antagonists

3.3.1. NS1209

When the AMPA receptor antagonist NS1209 was administered i.p. at 3 mg/kg, no change in paw withdrawal threshold was observed when compared with injection of vehicle at any of the timepoints examined (Fig. 3A,B). In contrast, injection of the highest dose of NS1209 (6 mg/kg, i.p.) produced a significant increase in the paw withdrawal threshold to 9.4 ± 2.0 g ($P < 0.001$) 30 min after injection when compared with vehicle-injected animals (2.8 ± 0.4 g). This elevation remained significant until 120 min after administration for the ipsilateral paw (7.8 ± 2.0 g, $P < 0.05$) and for the contralateral paw (7.4 ± 1.7 g, $P < 0.05$). We have previously shown that NS1209 has no effect on motor performance in the rotarod test when administered at the doses used in the current study (Erichsen and Blackburn-Munro, 2002).

3.3.2. Ketamine

Intraperitoneal injection of the NMDA receptor antagonist ketamine at 5 mg/kg, had no effect on paw withdrawal threshold when compared to vehicle for up to 120 min after administration (Fig. 3C,D). In contrast, the highest dose of ketamine (15 mg/kg, i.p.) produced a significant increase in paw withdrawal threshold to 12.2 ± 2 g ($P < 0.0001$) from

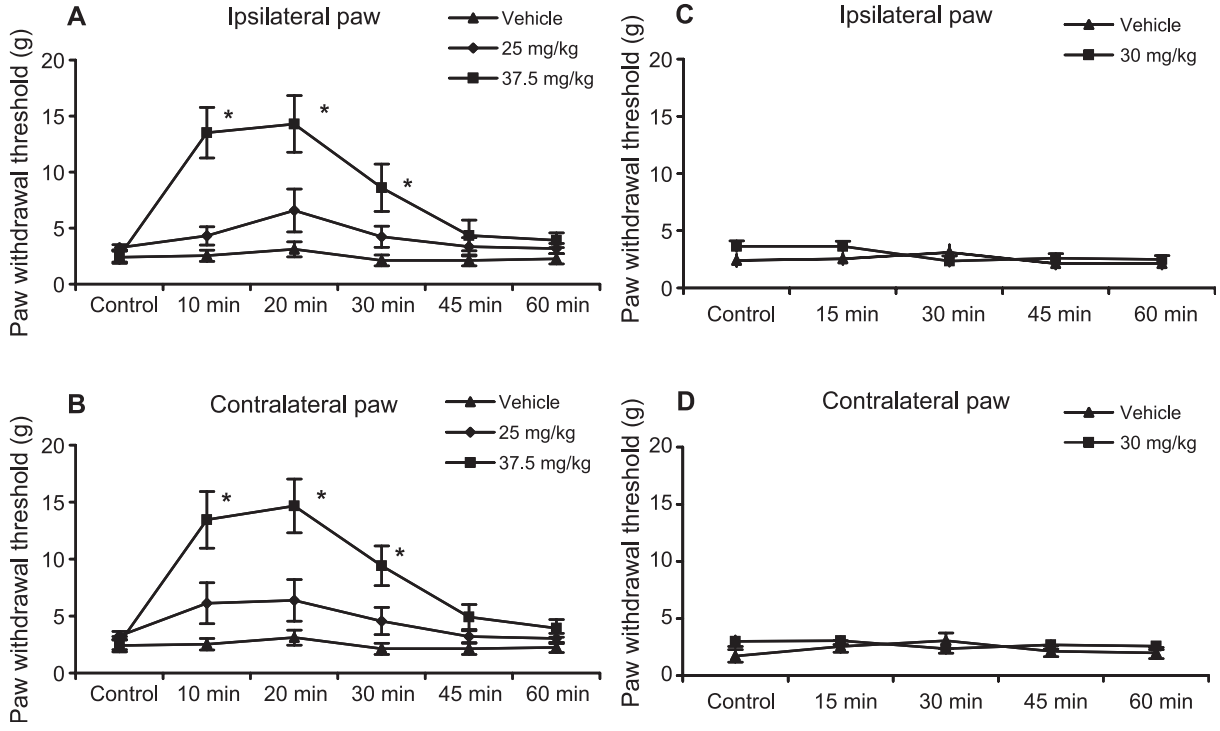

Fig. 4. Effects of voltage-activated Na^+ channel blockers on hindpaw withdrawal threshold. (A, B) The antiarrthymic mexiletine (25 and 37.5 mg/kg, i.p.) was injected immediately after the baseline response (control) had been obtained. At the highest dose tested mexiletine induced a rapid increase in paw withdrawal threshold compared with vehicle. However, this effect was somewhat transient and had disappeared 45 min after injection. (C, D) Injection of the anticonvulsant lamotrigine (30 mg/kg, i.p.) immediately after the baseline response had been obtained had no effect on paw withdrawal threshold. Data are presented as mean ± S.E.M., *$P < 0.05$ vs. corresponding vehicle time points.

15 min after administration compared with injection of vehicle (2.8 ± 1.4 g). However, the increase was transient, and had returned to baseline levels 30 min after injection. At the highest dose of ketamine administered (15 mg/kg, i.p., $n = 6$), no change in motor performance as assessed by the rotarod test was observed when compared with baseline (ED_{50}>15 mg/kg).

3.4. Effects of voltage-activated Na^+ channel blockers

3.4.1. Mexiletine

Administration of the anti-arrthymic mexiletine at the lowest dose of 25 mg/kg (i.p.) had no effect on paw withdrawal threshold when compared with injection of vehicle at any of the timepoints examined (Fig. 4A,B). However, when mexiletine was administered i.p. at a dose of 37.5 mg/kg paw withdrawal threshold was significantly increased to 13.5 ± 2.2 g ($P < 0.0001$) within 10 min, when compared with injection of vehicle. This elevation in paw withdrawal threshold to von Frey hair stimulation remained significantly different from injection of vehicle until 30 min after drug administration (8.6 ± 2.1 g, $P < 0.05$). We have previously shown that mexiletine has no effect on motor performance in the rotarod test when administered at the doses used in the current study (Erichsen and Blackburn-Munro, 2002).

3.4.2. Lamotrigine

Administration of the anticonvulsant lamotrigine (30 mg/kg, i.p.) had no effect on mechanical allodynia when compared with injection of vehicle at any of the timepoints examined (Fig. 4C,D). We have previously shown that lamotrigine has no effect on motor performance when administered at the same dose used in the current study (Blackburn-Munro et al., 2002).

3.5. Effects of KCNQ K^+ channel openers

3.5.1. Retigabine

When the KCNQ K^+ channel opener retigabine was administered at 3 mg/kg, (i.p.), no change in paw withdrawal threshold to von Frey hair stimulation was observed when compared with injection of vehicle at any time point (Fig. 5A,B). In contrast, injection of the highest dose of retigabine (10 mg/kg, i.p.) produced a significant increase ($P < 0.0001$) in paw withdrawal threshold that reached a maximal level of 14.0 ± 1.5 g 30 min after injection when compared to vehicle administration (2.8 ± 0.3 g). This effect remained significantly different from vehicle for a further 30 min (6.6 ± 1.2 g, $P < 0.01$). At the highest dose of retigabine tested, we observed that 50% of animals showed impaired motor performance 15 min after administration as assessed by the rotarod test when compared with baseline (ED_{50} = 10

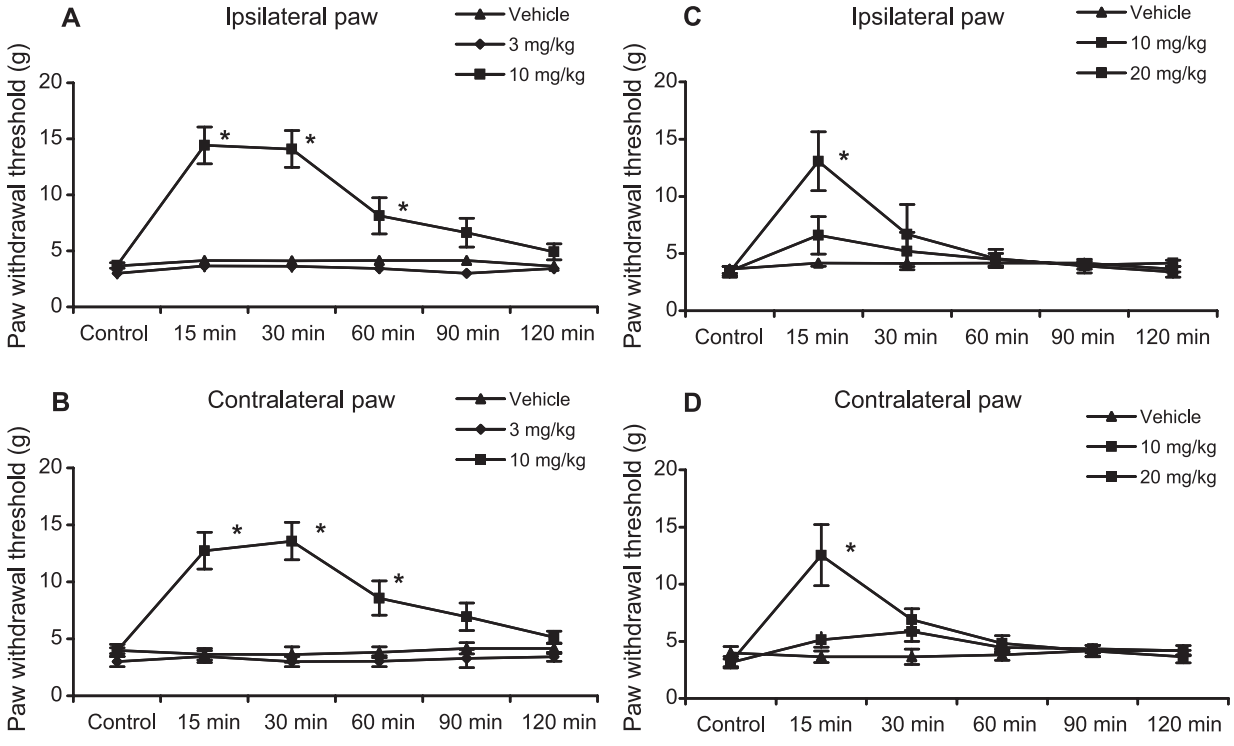

Fig. 5. Effects of KCNQ potassium channel openers on hindpaw withdrawal threshold. (A, B) Retigabine (3 and 10 mg/kg, i.p.) was injected immediately after the baseline response (control) had been obtained. At the highest dose tested retigabine increased paw withdrawal threshold from 15 min after injection and this remained significantly different from vehicle until 60 min after injection for both paws. (C, D) Flupirtine (10 and 20 mg/kg, i.p.) was injected after the baseline response had been obtained. At the highest dose tested a transient increase in paw withdrawal threshold was observed 15 min after administration for both paws. Data are presented as mean ± S.E.M., *$P < 0.05$ vs. corresponding vehicle time points.

mg/kg). However, motor performance had returned to a baseline at after all time points (30, 45 and 60 min) examined after 15 min (ED_{50}>10 mg/kg).

3.5.2. Flupirtine

Intraperitoneal injection of flupirtine at 10 mg/kg, had no effect on paw withdrawal threshold in response to von Frey hair stimulation, when compared with vehicle injection for up to 120 min after administration (Fig. 5C,D). In contrast, 15 min after i.p. injection of 20 mg/kg flupirtine a significant increase in paw withdrawal threshold to 13.0 ± 2.6 g ($P < 0.05$), was observed when compared to injection of vehicle (4.1 ± 0.3 g). However, this elevation in paw withdrawal threshold returned to a baseline level 15 min later, and remained at this level for the remainder of the experiment. At the highest dose of flupirtine administered (20 mg/kg, i.p., $n = 6$), no change in motor performance was observed when compared with baseline (ED_{50}>20 mg/kg).

3.6. Effects of the cycloxygenase-2 inhibitor carprofen

Subcutaneous administration of the selective cycloxygenase-2 inhibitor carprofen at 10 and 15 mg/kg, had no effect on paw withdrawal threshold when compared to injection of vehicle at any of the timepoints examined up to 120 min post-injection (Fig. 6A). At the highest dose of carprofen administered (15 mg/kg, s.c., $n = 6$), no change in motor performance was observed when compared with baseline (ED_{50}>15 mg/kg).

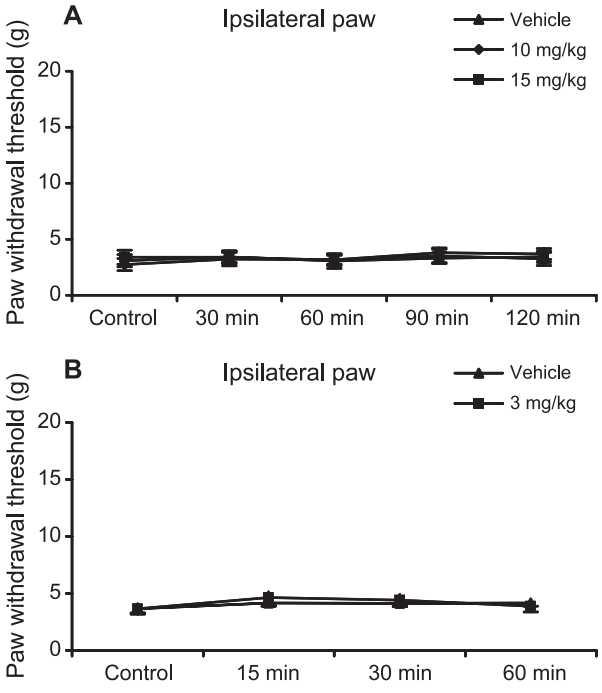

Fig. 6. Effects of carprofen and diazepam on hindpaw withdrawal threshold. (A) The cycloxygenase-2 inhibitor carprofen (10 and 15 mg/kg, i.p.) was injected immediately after the baseline response (control) had been obtained. Carprofen had no effect on paw withdrawal threshold, in the ipsilateral paw, at either dose tested. (B) The GABA modulator diazepam (3 mg/kg, i.p.) was injected immediately after the baseline response had been obtained. Diazepam had no effect on withdrawal threshold in response to von Frey hair stimulation of the ipsilateral hindpaw. Data are presented as mean ± S.E.M.

3.7. Effects of the gamma aminobutyric acid (GABA) modulator diazepam

Injection of the benzodiazepine GABA modulator diazepam at 3 mg/kg (i.p.) had no effect on paw withdrawal threshold when compared with injection of vehicle at any of the timepoints examined up to 60 min after administration (Fig. 6B). When tested subsequently in the rotarod test we observed that 16% of rats ($n = 6$) displayed impaired motor performance 15 min after administration of diazepam (3 mg/kg). On this basis and in the light of the complete lack of antinociceptive effect for the 3 mg/kg dose we decided not to test higher doses in the current study.

4. Discussion

We have shown in the current study that repeated i.m. injection of acidic saline in the rat produces a long lasting bilateral hypersensitivity to innocuous mechanical stimulation of the hindpaw, which is in general agreement with previous observations made for this model (Sluka et al., 2001). We have then gone on to characterize this model pharmacologically by measuring hindpaw reflex nociceptive responses to von Frey stimulation after systemic administration of a range of drugs with proven analgesic efficacy in other animal models of chronic pain. We observed that morphine produced a dose-dependent and particularly prolonged reduction in mechanical hypersensitivity, whilst NS1209, ketamine, mexiletine, retigabine and flupirtine also attenuated mechanical hypersensitivity, although to a lesser extent than morphine. However, lamotrigine, carprofen and diazepam had no apparent effect on mechanical hypersensitivity under the conditions employed in the current study.

In their original description of this model Sluka and colleagues observed that the hindpaw sensitivity of rats ranged from 7 to 60 mN (approximately 0.7–6 g) after two unilateral injections of acidic saline (pH 4) administered 5 days apart; it was not reported if rats failed to develop hindpaw sensitivity within this range, thus suggesting that 100% of animals were ‘responders’ (Sluka et al., 2001). We used an identical injection protocol in the current study to observe an essentially similar decrease in paw withdrawal threshold to levels within the range of 0.94–5.41 g 24 h after the second injection of acidic saline. However, we excluded rats that did not show a post-injection withdrawal threshold below an upper level of 5.41 g (hence 72% of animals were regarded as responders), since we needed to leave an adequate window to test for marginal drug-induced reversal of hindpaw mechanical hypersensitivity.

Although increased sensitivity to mechanical stimuli, indicative of mechanical allodynia and hyperalgesia is well documented in fibromyalgia patients, altered thresholds to heat as well as cold-mediated sensory stimulation have also been documented (Price et al., 2002). Sluka et al. (2001) have previously reported that acid-induced chronic muscle pain in rats may more closely reflect the symptomologial profile of muscle pain in fibromyalgia patients, than other currently available animal models. Surprisingly however, these rats do not appear to show any changes in reflex responses to noxious, thermal stimulation of the hindpaw.

Subcutaneous administration of the selective cyclooxygenase-2 inhibitor carprofen at doses ranging from 5 to 15 mg/kg has previously been reported to attenuate a range of pain related behaviours over a 4–5 h period in a rat model of abdominal pain (Roughan and Flecknell, 2001). However, in the current study, we observed that carprofen had no effect of hindpaw mechanical hypersensitivity after administration, suggesting that ongoing peripheral inflammatory mediated events do not contribute to the pathophysiology of this model. A general lack of histological changes in the injected muscle after repeated i.m. acidic saline injection has been reported, suggesting that central sensitising events contribute to the aetiological development of acid-induced muscle pain (Sluka et al., 2001). This contrasts with carrageenan injection into the muscle, which also produces a long-lasting bilateral increase in hindpaw sensitivity, but is associated with marked inflammatory cell infiltration (Radhakrishnan et al., 2003), which might be expected to contribute to sensitisation of peripherally mediated sensory transmission. A similar lack of detectable peripheral pathology in fibromyalgia patients suggests that central pathophysiological processes also underlie the symptomological profile of the disease. Indeed, recent studies demonstrating that fibromyalgia patient's display enhanced wind-up to repetitive thermal and cold stimuli (Price et al., 2002), in conjunction with imaging studies showing pain-induced activation of several brain structures (Gracely et al., 2002) confirm such suggestions of altered central nociceptive processing. However, we were unable to detect any changes in other measures of pain-like behaviour in this model, which in addition to partially confirming previous observations of Sluka et al. (2001) reinforce the concept of a unique pathophysiology for fibromyalgic pain in humans.

4.1. Effects of morphine

The efficacy of opiate analgesics for the treatment of chronic pain in humans and alleviating pain-like behaviours in animal models, especially those of neuropathic origin, has been variably questioned (Arner and Meyerson, 1988). In spite of these observations we have previously shown that acute administration of morphine at doses comparable to those used in the current study effectively alleviate mechanical hyperalgesia and mechanical/cold allodynia in the spared nerve injury model of neuropathic pain (Erichsen and Blackburn-Munro, 2002). In the present study, administration of morphine produced a dose-dependent reversal in mechanical hypersensitivity in rats after repeated i.m. injection of acidic saline. This supports previous observations where spinal administration of μ-opioid receptor agonists has been shown to attenuate mechanical hypersensitivity in this model (Sluka et al., 2002). A particularly intriguing aspect of our findings pertaining to morphine was the unusually long duration of action of its anti-nociceptive effects which lasted for up to 3 h after administration. This long-lasting effect of morphine suggests that μ-opioid receptor levels located either pre- or postsynaptically to primary fibre input may be increased in this model, although other mechanisms such as persistent activation of downstream kinases (Gutstein et al., 1997) may also explain the prolonged duration of action.

4.2. Effects of glutamate receptor antagonists

AMPA receptor antagonists can prevent the development of hyperalgesia and allodynia observed in response to inflammatory stimuli, nerve and spinal cord injury (Fundytus, 2001; Simmons et al., 1998). We observed in the current study that the selective AMPA receptor antagonist NS1209 produced a robust increase in paw withdrawal threshold, lasting for 90 min in rats with acid-induced chronic pain. This essentially confirms the findings of a previous study where intrathecal administration of the AMPA receptor antagonist NBQX (1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[*f*]quinoxaline-7-sulfonamide disodium) also reduced the bilateral increase in mechanical hypersensitivity, although with a shorter duration of action than NS1209 (Skyba et al., 2002). NMDA receptor antagonists also reduce a wide spectrum of pain-like behaviours in animal models of chronic inflammatory and neuropathic pain (Fundytus, 2001). Intrathecal administration of the NMDA receptor antagonist D-[3H]2-amino-5-phosphonopentanoate (D-[3H]) AP5 has been shown to produce a robust bilateral attenuation of mechanical hypersensitivity in the model used in the current study, but at a dose associated with disturbance of motor function (20 nmol/10 μl) (Skyba et al., 2002); a less robust attenuation of mechanical hypersensitivity was observed at non-ataxic doses. In the present study, intraperitoneal administration of ketamine at a dose presumably devoid of motor impairing effects was associated with a marked, but transient increase in paw withdrawal threshold in response to von Frey hair stimulation. However, the lack of motor impairment observed at this dose is surprising based on the reports from other studies where moderate ataxia has been observed at similar dose levels (Danysz et al., 1994). This finding may relate to the rather low sensitivity of the constant speed rotarod used in this study. Similar results to those described above have been obtained in patients with fibromyalgia where intravenous infusion of ketamine

has been shown to reduce ongoing muscle pain and attenuate temporal summation of preconditioned electrical stimuli (Graven-Nielsen et al., 2000).

4.3. Effects of voltage-activated Na^+ channel blockers

Both mexiletine and lamotrigine have in common the ability to preferentially bind and stabilize inactivated states of the Na^+ channel to prevent Na^+ influx into cells (Clare et al., 2000; Graven-Nielsen et al., 2000). This property endows these drugs with the ability to selectively inhibit Na^+ channels during sustained depolarisation, such as that incurred in the setting of tissue injury. Administration of mexiletine (25–50 mg/kg, i.p.) produces a profound dose-dependent inhibition of second phase nociceptive behaviours in the rat formalin test (Blackburn-Munro et al., 2002). The ability of mexiletine within this dose range, to markedly attenuate hypersensitivity to mechanical and cold stimulation of the ipsilateral hindpaw, has also been recently described in two rat models of neuropathic pain (Erichsen et al., 2003). We also observed that mexiletine reduced hypersensitivity of the hindpaw in response to mechanical stimulation in rats after repeated i.m. injection of acidic saline, albeit with a relatively short duration of action. However, this duration of action is in keeping with the previously described anti-nociceptive effects reported for mexiletine. In contrast, administration of the anticonvulsant drug lamotrigine to rats in the current study had no effect on hindpaw hypersensitivity to mechanical stimulation. This was somewhat surprising given that numerous other studies have reported anti-nociceptive effects for lamotrigine in animal models of persistent and chronic pain (Blackburn-Munro et al., 2002; Erichsen et al., 2003; Nakamura-Craig and Follenfant, 1995). In particular administration of 15 mg/kg lamotrigine has been shown to markedly inhibit second phase pain behaviours in the formalin test (Blackburn-Munro et al., 2002). Furthermore in neuro-injured pain rat's mechanical hyperalgesia in response to pin prick stimulation is also attenuated by 10 mg/kg lamotrigine (Erichsen et al., 2003), while doses as high as 60 mg/kg have been shown to be ineffective in attenuating mechanical allodynia. Thus, we are reasonably confident that the dose of lamotrigine chosen in the present study was large enough to attenuate pain related behaviours. Whether pharmacokinetic differences in the way that mexiletine and lamotrigine bind to the channel (as has been shown for carbamazepine and phenytoin (Kuo et al., 1997) accounts for the disparity in affecting mechanical hypesensitvity in these models remains to be established.

4.4. Effects of KCNQ K^+ channel openers

The M-current is a subthreshold voltage-gated K^+ current that serves to stabilise the membrane potential and control neuronal excitability (Brown and Yu, 2000). Functional studies associate the M-current to homo- or hetero-multimers of KCNQ (2–5) protein subunits (Jentsch, 2000). The anticonvulsant drug retigabine, has been shown to activate KCNQ K^+ channels expressed in mammalian cells (Rundfeldt and Netzer, 2000) and native M-currents in rat sympathetic neurones (Tatulian et al., 2001; Wickenden et al., 2001).

Previously we have shown that oral administration of retigabine (20 mg/kg) attenuates second phase nociceptive behaviours in the rat formalin test (Blackburn-Munro et al., 2002). This attenuating effect of retigabine appeared to be selectively mediated by KCNQ K^+ channels, since it was completely reversed by prior administration of the KCNQ K^+ channel blocker (10,10-bis(4-pyridinylmethyl)-9(10H)-anthracenone) XE-991. In the same study retigabine also reduced mechanical hyperalgesic and cold allodynic responses of the ipsilateral hindpaw in the chronic constriction injury and spared nerve injury models of neuropathic pain. Interestingly, retigabine had no effect on mechanical allodynia as measured by paw withdrawal threshold in response to von Frey hair stimulation. In contrast, we have shown in the current study that i.p. administration of retigabine can reduce mechanical hypersensitivity to von Frey hair stimulation after repeated injection of acidic saline into the gastrocnemius muscle. The simplest explanation for this apparent discrepancy is that retigabine appears to have been administered at a maximally tolerated dose in the current study. We observed that 50% of animals had compromised motor performance as measured by the rotarod test 15 min after injection (although not at 30 and 60 min post-injection), whereas oral administration in the former study had no effect on motor performance at any time point measured. Recently, retigabine has been shown to interact with a site on the $GABA_A$ receptor complex raising the possibility that enhancement of GABA receptor function may contribute to its anticonvulsant properties (Van Rijn and Willems-van Bree, 2003). However, when we tested the $GABA_A$ receptor modulator diazepam in the current study, we saw no effect on hindpaw mechanical sensitivity, suggesting that retigabine does not mediate an anti-nociceptive effect in this model via such a mechanism of action.

Flupirtine is a centrally acting non-opioid analgesic, which is structurally similar to retigabine. Preliminary studies have shown that flurpirtine positively modulates transfected KCNQ K^+ channels with a similar mechanism of action to, but with less potency than retigabine (Ilyin et al., 2002). Other postulated mechanisms of action for the analgesic effects of flupirtine have included activation of inhibitory brainstem monoaminergic pathways which descend to the spinal dorsal horn (Szelenyi and Nickel, 1987; Szelenyi et al., 1989), to indirect inhibition of NMDA receptor activation via stabilization of the resting membrane potential (Kornhuber et al., 1999). Our results show that flupirtine is also less potent than retigabine in vivo, and that it also has a much shorter duration of antinociceptive action. Whilst we cannot definitively say that this action of flupiritine was mediated selectively via modulation of KCNQ K^+

channel function in view of the above observations, taken together with the results obtained for retigabine, it appears that KCNQ K^+ channels contribute to pain behaviour in this model of muscle pain.

In conclusion, we have confirmed previous observations that repeated application of i.m. acidic saline injection produces a long-lasting bilateral mechanical hypersensitivity to innocuous stimulation. This correlates with a number of symptoms presented in human chronic pain conditions, such as that observed in fibromyalgia patients where widespread muscoskeletal pain is regarded as a primary presenting symptom of the disease. However, in contrast to fibromyalgia patients we did not observe any mechanical hyperalgesia or hypersensitivity to thermal or cold stimuli applied to the hindpaw in this model. Overall, the present results suggest that in this model, muscle-mediated pain can be alleviated by various analgesics with differing mechanisms of action, and that once established ongoing inflammation does not appear to contribute to this process.

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