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Contralateral, ipsilateral and bilateral treatments with the κ-opioid receptor agonist U-50,488H in mononeuropathic rats

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Abstract

The effect of repeated contralateral administration of the κ-opioid receptor agonist U-50,488H (*trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide methanesulfonate) on nociceptive behaviour was investigated and compared with ipsilateral and bilateral treatments in a rat model of peripheral unilateral neuropathy (chronic constriction of the common sciatic nerve). Administration of 0.3 mg U-50,488H into the contralateral hindpaw on days 6 and 10 after induction of mononeuropathy increased hindpaw withdrawal latency to mechanical but not to thermal stimulation compared to saline-treated rats. No difference in pain-related behaviour was found between different peripheral (contralateral, ipsilateral and bilateral) treatments with 0.3 mg U-50,488H. Autotomy behaviour was reduced for 6 weeks after sciatic nerve ligation in rats treated contralaterally with the opioid receptor agonist. Antinociceptive effects of contralaterally administered U-50,488H were abolished by the peripherally acting opioid receptor antagonist naloxone methiodide. Our findings indicate that contralateral treatment with U-50,488H attenuates nociceptive behaviour in mononeuropathic rats. These antinociceptive effects are mediated via peripheral opioid receptors.

Keywords: Neuropathic pain; Contralateral treatment; κ-Opioid receptor agonist; U-50,488H; Opioid receptor; (Rat)

1. Introduction

Neuropathic pain, originating after peripheral or central nerve injury, is characterised by allodynia (perceiving pain in response to normally non-noxious stimuli) and hyperalgesia (more intense sensation of pain elicited by noxious stimuli). The precise mechanisms underlying the development and maintenance of allodynia and hyperalgesia are still unknown (Suzuki and Dickenson, 2000; Przewlocki and Przewlocka, 2001). It has been reported that neuropathic pain is due to a sustained activation of peripheral nociceptors which leads to hypersensitivity of primary afferent neurons and central sensitisation of dorsal horn neurons (Woolf, 1993; Woolf and Mannion, 1999).

Management of neuropathic pain remains a major clinical challenge due to the lack of clinically effective treatments (Suzuki and Dickenson, 2000; Przewlocki and Przewlocka, 2001; Machelska et al., 2003). The effectiveness of opioids with respect to neuropathic pain is still controversial (Przewlocki and Przewlocka, 2001). In the past decade, a large body of reports has emerged demonstrating the existence of opioid receptors outside the central nervous system and the generation of opioid analgesia via peripheral mechanisms (Stein, 1995; Stein et al., 2001, 2003). Peripheral antinociception can be achieved by local administration of small, systemically inactive doses of opioids, directly into the injured tissue. These local opioid actions are known to be mediated via μ-, δ- and κ-opioid receptors as they have been shown to be dose-dependent, stereoselective and reversible by antagonists (Stein et al., 1989; Perrot et al., 1999; 2001). Such receptors are expressed on peripheral sensory nerves (Schäfer et al.,

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1995; Stein et al., 1996; Coggeshall et al., 1997). These analgesic effects are particularly prominent in hyperalgesic and inflammatory states and have been demonstrated in both animals and humans (Stein, 1995; Stein et al., 2001, 2003).

Recent evidence indicates that peripheral opioid receptors are also involved in neuropathic pain (Catheline et al., 1998; Walker et al., 1999). In the chronic constriction injury model, it has been demonstrated that peripheral effects of systemically administered morphine are mediated not only by μ- but also by κ-opioid receptors (Catheline et al., 1996). It has been suggested that the decrease in the antinociceptive potency of morphine is due to a reduction in the activity of spinal opioid receptors or opioid signal transduction after nerve injury, although only minor differences in such activity have been reported between damaged and intact nerves (Robertson et al., 1999). Some decrease in the spinal μ-opioid receptor immunoreactivity was demonstrated after axotomy (Zhang et al., 1998).

Several studies have shown that following chronic constriction injury of the sciatic nerve in rats, a time-dependent mechanical and thermal hyperesthesia develops (Attal et al., 1990; Erichsen and Blackburn-Munro, 2002). Even in the case of unilateral constriction of the sciatic nerve, the hyperesthesia appears bilaterally (Yu et al., 1996; Bileviciute-Ljungar and Lundeberg, 2000b). In rats with unilateral nerve injury, this phenomenon was suggested to be caused by exaggerated neuronal activity in wide dynamic range neurons not only on the ipsilateral but also on the contralateral side (Pertovaara et al., 1997; Bileviciute-Ljungar et al., 2001; Suzuki and Dickenson, 2002).

We have reported on the effectiveness in attenuating pain-related behaviour in unilateral inflammation (Bileviciute-Ljungar and Lundeberg, 2000a) and peripheral nerve injury (Bileviciute-Ljungar and Lundeberg, 2000b) after contralateral administration of drugs which reduce nerve activity. A single administration of a local anaesthetic, xylocaine, into the contralateral hindpaw of mononeuropathic rats significantly increased nociceptive thresholds to thermal stimulation for several days (Bileviciute-Ljungar and Lundeberg, 2000b). This antinociceptive action of xylocaine was suggested to be mediated through inhibitory mechanisms in the spinal dorsal horn, as demonstrated by recording the activity in wide range neurons (Bileviciute-Ljungar et al., 2001). In rats with unilateral carrageenan-induced inflammation, the same low dose of xylocaine injected contralaterally increased withdrawal latencies in the inflamed paw (Bileviciute-Ljungar and Lundeberg, 2000a). Similar findings were recently reported for the antinociceptive effect of the κ-opioid receptor agonist U-50,488H (*trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide methanesulfonate) in rats with acute unilateral inflammation (Bileviciute-Ljungar and Spetea, 2001). Contralateral administration of U-50,488H produced a dose-dependent decrease of hyperalgesia induced by carrageenan injection to mechanical stimulation. This action was shown to be mediated by peripheral mechanisms.

In the present study, we investigated the effect of contralateral administration of the κ-opioid receptor agonist U-50,488H on pain-related behaviour in rats with chronic constriction injury of the sciatic nerve. Based on our previous observations in acute carrageenan-induced inflammation, a dose of 0.3 mg U-50,488H, which has been shown not to induce systemic actions (Bileviciute-Ljungar and Spetea, 2001), was tested in mononeuropathic rats. Hindpaw withdrawal latencies to mechanical and thermal stimulation as well as autotomy scores were assessed. Moreover, the efficacy of different peripheral (contralateral, ipsilateral and bilateral) treatments with U-50,488H was compared.

2. Materials and methods

2.1. Chemicals

U-50,488H and naloxone methiodide were purchased from Research Biochemicals (Sigma, MA, USA) and dissolved in sterile 0.9% saline.

2.2. Experimental protocol for animal studies

Experiments were performed on freely moving male albino Sprague–Dawley rats (B&K Universal Lab, Sollentuna, Sweden) weighing 200–250 g, and were approved by the Ethics Committee for Animal Research (North Stockholm, Sweden). Rats were maintained at 21 °C in a 12-h light/dark cycle and had free access to food and water.

Rats were accustomed to testing conditions three times daily for 3 days prior to operation. On the day of operation, the basal values for each animal were obtained. Mononeuropathy on the right side was induced in anaesthetised rats according to Bennett and Xie (1988). Briefly, rats were anaesthetised with intraperitoneal injection of chloralhydrate (350 mg/kg). Sciatic nerve ligation was performed under a microscope. Four ligatures at 1–2 mm intervals were tied loosely around the common sciatic nerve, taking care not to interrupt the epineural circulation. To exclude the effect of surgical intervention, sham-operated rats have also been tested. After operation, animals were allowed 3 days for recovery, and training was performed every day, once daily, before drug treatment. In order to investigate the effects of repeated treatment, identical injections were given on days 6 and 10 post-operation. Injections of either saline or 0.3 mg U-50,488H alone or together with one equimolar dose of naloxone methiodide were given subcutaneously and intraplantarly with a 27-gauge needle in a total volume of 100 μl. Experiments were performed in a blind and randomised order whenever possible.

The antinociceptive effect of contralateral injection of 0.3 mg U-50,488H in comparison to saline was investigated in mononeuropathic rats. Sham-operated rats, injected with either saline or opioid receptor agonist into the contralateral hindpaw, served as additional controls. In

order to evaluate the involvement of peripheral opioid receptors in the antinociceptive action of U-50,488H, the dose of 0.3 mg of the opioid receptor agonist was co-injected into the contralateral paw with an equimolar dose of the peripherally acting opioid receptor antagonist, naloxone methiodide. The efficacy of contralateral vs. ipsilateral and bilateral administration of 0.3 mg U-50,488H was investigated by drug injection into either one or both hindpaws. In the case of bilateral treatment, animals received injections in both the right and left hindpaw of 0.3 mg U-50,488H.

2.3. Experimental procedure for nociceptive behavioural tests

Rats were trained with the experimental equipment as described above, using the Randall-Selitto test and the hot-plate test for half each of the sessions. Hindpaw withdrawal latencies during noxious mechanical and thermal stimulation were tested as previously described (Bileviciute-Ljungar and Spetea, 2001). The order of measurements of the right and left hindpaw was continually alternated and the interval between different stimulations was approximately 5–7 min. Two measurements were carried out for each test and the average value was used to quantify the percentage changes. Hindpaw withdrawal latencies on mechanical and thermal stimulation were measured at 2, 5, 24, 29, 48 and 96 h after the first (day 6 post-operation) and second (day 10 post-operation) injection. Both injections were given at the same time of day.

The Randall-Selitto test (Ugo Basile, Type 7200, Italy) was used to assess withdrawal thresholds to mechanical stimulation. A wedge-shaped, blunt piston with an area of 1×10 mm^2 with a loading rate of 48 g/s was applied to the dorsal surface of the manually held hindpaw and the time required to initiate the struggle response was measured in seconds. A cut-off time of 15 s was applied. The basal values prior to surgical intervention of 59 rats to mechanical stimulation were 5.0 ± 0.2 and 5.1 ± 0.2 s, right and left hindpaw, respectively.

The withdrawal response to noxious heat was determined using the hot-plate test. The entire ventral surface of the rat's hindpaw was placed on a hot-plate which was maintained at a temperature of 50 °C (49.7–50.5 °C). Hindpaw withdrawal latencies were measured in seconds. The cut-off time was 50 s. The basal values prior to surgical intervention of 59 rats to thermal stimulation were 8.1 ± 1.0 and 9.4 ± 1.2 s, right and left hindpaw, respectively.

2.4. Autotomy

Signs of autotomy behaviour, i.e. self mutilation (Bennett and Xie, 1988) were recorded for every digit using the following scale: 0, no signs of self mutilation; 1, a tip of nail removed with signs of old or fresh blood under the nail; 2, a nail totally removed and damage to the distal portion of the digit; 3, damage to the whole digit or the footpad. The graded scale applied was similar to one used by Wiesenfeld and Hallin (1983). Each digit was multiplied by the scale number and a total score for each rat was obtained. Autotomy behaviour was assessed before the first treatment (6 days post-operation, i.e. basal score value) and at 3, 4, 5 and 6 weeks following sciatic nerve ligation.

2.5. Statistical analysis

Statistical analysis was carried out using SPSS software (Statistical Product and Service Solutions, release 10). Each experimental group included 7–12 rats. All results are expressed as the mean ± S.E.M. A P value <0.05 was considered statistically significant.

For the treatment effect, each rat served as its own control and all changes in hindpaw withdrawal latencies are presented as a percentage (%) change from the same pre-injection value obtained on day 6 post-operation, which was calculated according to the formula:

$$\%\ \text{changes} = 100[(P_X - P_0)/P_0]$$

where P is the parameter of nociception, X is the time after drug injection and 0 (zero) is the time before the first injection (the first pre-injection value on day 6 post-operation).

The ANOVA (analysis of variance between groups) test followed by Bonferroni test for significance was used to compare percentage changes in hindpaw withdrawal latencies after contralateral treatment with saline or U-50,488H between the mononeuropathic and sham-operated rats. The same test was used to compare percentage changes in latencies between contralateral, ipsilateral and bilateral treatments with 0.3 mg U-50,488H in mononeuropathic rats.

T-test for paired samples was used to identify differences between the right and left paw withdrawal latency in the same rat. *T*-test for independent samples was used to compare percentage changes in withdrawal latencies after contralateral treatment with U-50,488H alone, and U-50,488H with naloxone methiodide.

In the autotomy studies, the total score for each rat was calculated according to the formula:

$$P = P_X - P_0$$

where P is autotomy score, X is the time after operation and 0 (zero) is the time before the first injection on day 6 post-operation. The autotomy score for each experimental group is presented at 3, 4, 5 and 6 weeks after operation. The non-parametric Mann–Whitney *U*-test was used to compare autotomy behaviour in mononeuropathic rats given different treatments.

3. Results

3.1. Changes in hindpaw withdrawal latencies following contralateral administration of U-50,488H

Administration in the contralateral hindpaw of 0.3 mg U-50,488H significantly increased right hindpaw withdrawal latencies to mechanical but not to thermal stimulation as compared to saline-treated mononeuropathic rats. An increase in the nerve-injured hindpaw in noxious thresholds to mechanical stimulation at 5 and 24 h was measured after the first contralateral injection at day 6 post-operation (Fig. 1A) and also at 2, 5, 24, 29 and 48 h after the second injection at day 10 post-operation (Fig. 1B). No changes have been observed in mononeuropathic rats in withdrawal latencies to thermal stimulation of the injured hindpaw after contralateral drug injection at days 6 or 10 post-operation (Fig. 1C, D). Nociceptive thresholds for mechanical and thermal stimulation of sham-operated rats injected contralaterally in the left hindpaw with 0.3 mg U-50,488H were not affected as compared to animals which received saline injections.

As shown in Fig. 2, concomitant contralateral administration of 0.3 mg U-50,488H with an equimolar dose of the peripherally acting opioid receptor antagonist naloxone methiodide abolished the antinociceptive effects of U-50,488H in the nerve-injured hindpaw. Moreover, hindpaw withdrawal latencies to thermal stimulation were shortened at 48 h ($P<0.05$) and 96 h ($P<0.05$) after the first injection at day 6 post-operation of U-50,488H together with naloxone methiodide as compared to U-50,488H alone, and at 2 h ($P<0.05$) and 24 h ($P<0.01$) after the second injection at day 10 post-operation (data not shown).

The second administration of 0.3 mg U-50,488H at day 10 post-operation did not produce further elevation in withdrawal latencies in the injured side to either mechanical or thermal stimulation as compared to the first drug injection (data not shown). Latencies to mechanical stimulation of the right hindpaw were decreased in mononeuropathic rats treated with vehicle after the second injection at 24 h ($P<0.05$) and 48 h ($P<0.05$) as compared to the first injection (data not shown).

3.2. Differences between the right and left hindpaw in mononeuropathic rats following contralateral administration of U-50,488H

Changes from the basal values (pre-surgery) in the right and left hindpaw withdrawal latencies to mechanical and

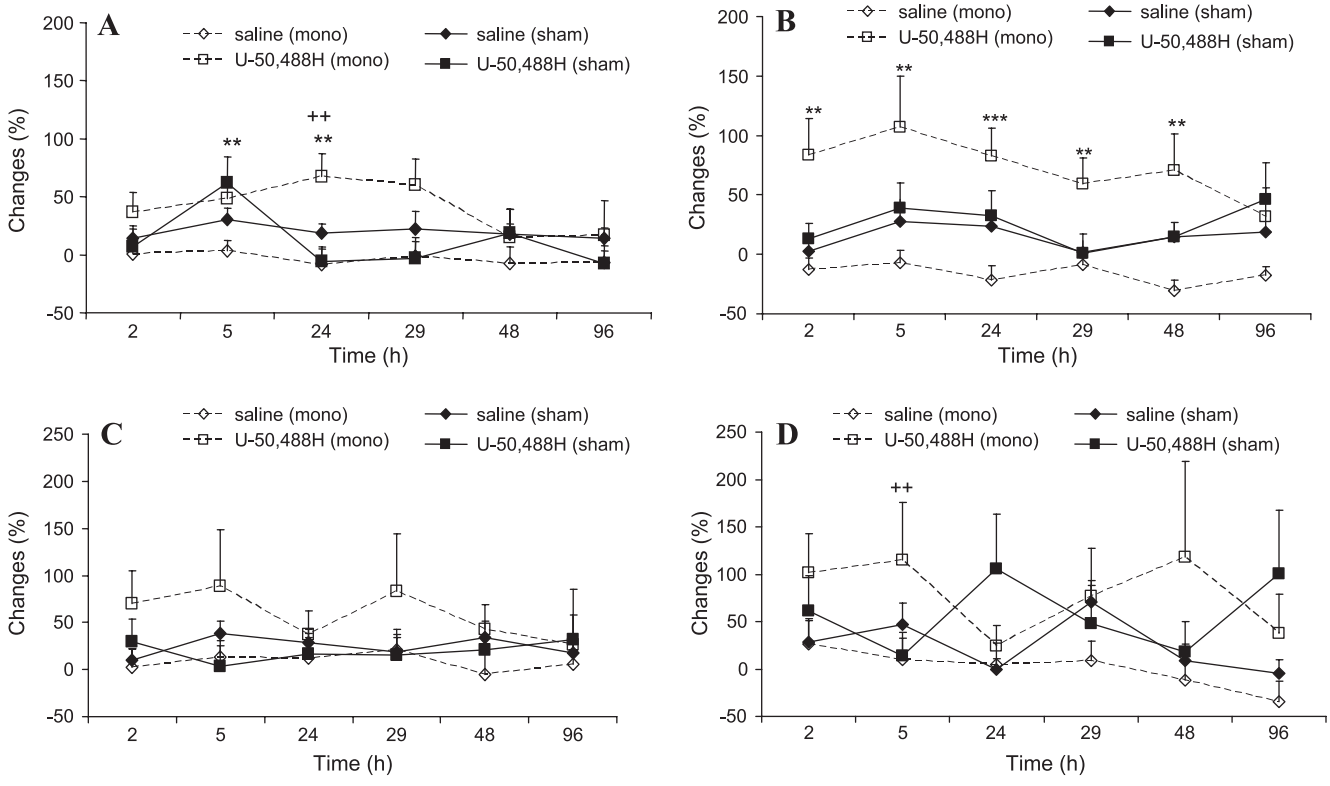

Fig. 1. Changes in hindpaw withdrawal latencies to mechanical (A, B) and thermal (C, D) stimulation of the right hindpaw after contralateral treatment with 0.3 mg U-50,488H. Mononeuropathic (mono) and sham-operated (sham) rats were injected with either saline or 0.3 mg of U-50,488H into the contralateral hindpaw. Results are shown after the first injection on day 6 post-operation (A, C) and after the second injection on day 10 post-operation (B, D). Data are presented as percentage (%) changes from the pre-injection value obtained on day 6 and expressed as mean ± S.E.M. (vertical axis). The horizontal axis indicates hours following injection. *Denotes significant differences between mononeuropathic rats treated with saline or U-50,488H, $n=8-11$. **$P<0.01$, ***$P<0.001$. $^{+}$Denotes significant differences between mononeuropathic and sham-operated rats treated with U-50,488H. $n=8-12$. $^{++}P<0.01$.

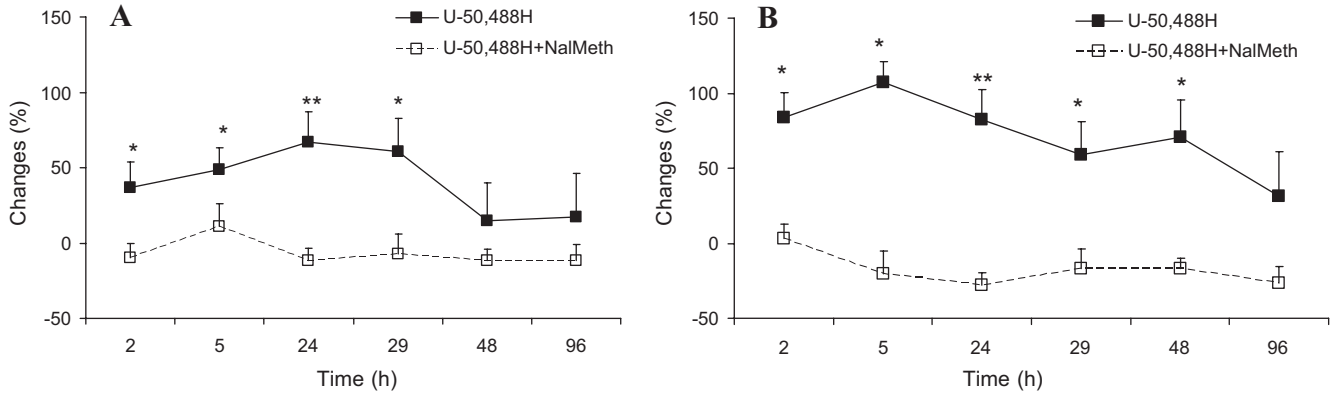

Fig. 2. Changes in hindpaw withdrawal latencies to mechanical stimulation of the right hindpaw in mononeuropathic rats after injection into the contralateral hindpaw of 0.3 mg U-50,488H either alone or together with an equimolar dose naloxone methiodide (NalMeth). Results are shown after the first injection on day 6 post-operation (A) and after the second injection on day 10 post-operation (B). Data are presented as percentage (%) changes from the pre-injection value obtained on day 6 and expressed as mean ± S.E.M. (vertical axis). The horizontal axis indicates hours following injection. *Denotes significant differences between the group contralaterally treated with U-50,488H alone vs. the group treated with U-50,488H and NalMeth. $n = 8$. *$P < 0.05$, **$P < 0.01$.

thermal stimulation were determined on day 6 post-surgery/pre-drug administration in mononeuropathic and sham-operated rats. No significant differences between the changes in latencies of the right and left hindpaw in both sciatic nerve-ligated and sham-operated rats to noxious pressure and heat have been found. In mononeuropathic rats ($n = 40$), the calculated changes in response times to mechanical stimulation were $-20.5 \pm 6.0\%$ and $-16.6 \pm 4.6\%$, right and left paw, respectively, and to thermal stimulation $-21.2 \pm 9.2\%$ and $-27.6 \pm 8.3\%$, in the right and left paw, respectively. In sham-operated rats ($n = 19$), the calculated changes in response times to mechanical stimulation were $-23.6 \pm 7.8\%$ and $-18.4 \pm 5.1\%$, right and left paw, respectively), and to thermal stimulation were $-1.1 \pm 15.6\%$ and $-22.8 \pm 8.4\%$, right and left paw, respectively.

Injection of either saline or 0.3 mg of U-50,488H into the contralateral hindpaw of mononeuropathic rats affected withdrawal latencies on both right (operated) and left (injected) sides. This observation is based on the fact that no differences were detected between the right and left side, except for an increase in thresholds to mechanical stimulation on the right side at 24 h after the second drug administration ($P < 0.05$) and an increase in withdrawal latencies to thermal stimulation on the right side at 96 h after the first injection of U-50,488H ($P < 0.05$) (data not shown).

3.3. Comparison of contralateral, ipsilateral and bilateral treatments with U-50,488H

Generally, no differences were detected in hindpaw withdrawal latencies of the injured hindpaw to both mechanical (Fig. 3) and thermal stimulation (data not shown) when comparing contralateral injection of 0.3 mg U-50,488H with ipsilateral and bilateral treatments. An exception was noted at 5 h after the first treatment with U-50,488H, where contralateral administration was more effective in increasing

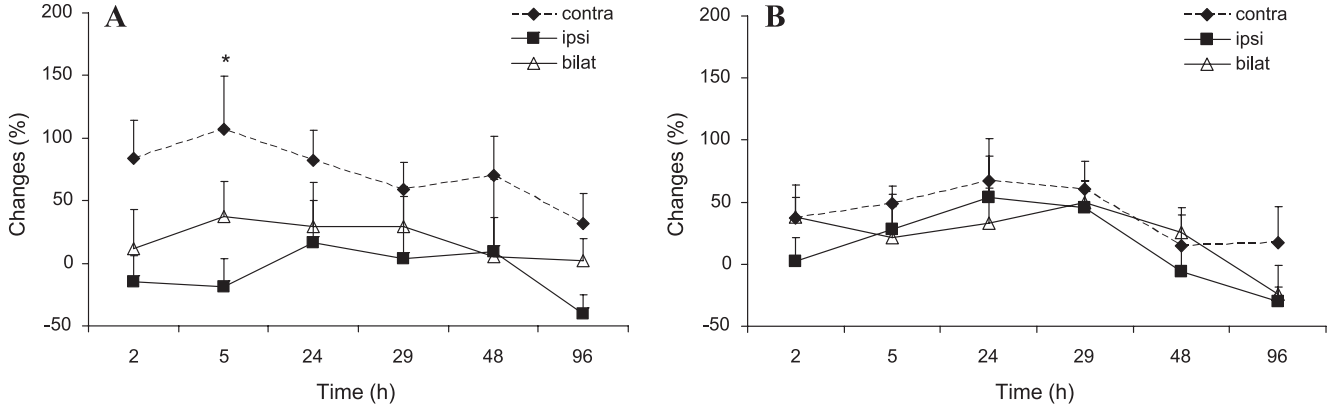

Fig. 3. Changes in hindpaw withdrawal latencies to mechanical stimulation of the right hindpaw in mononeuropathic rats injected into the contralateral (contra), ipsilateral (ipsi) or bilateral (bilat) hindpaws with 0.3 mg of U-50,488H. Results are shown after the first injection on day 6 post-operation (A) and after the second injection on day 10 post-operation (B). Data are presented as percentage (%) changes from the pre-injection value obtained on day 6 and expressed as mean ± S.E.M. (vertical axis). The horizontal axis indicates hours following injection. *Denotes significant differences between contralateral vs. ipsilateral injection with 0.3 mg U-50,488H. $n = 8$. *$P < 0.05$.

Table 1
Autotomy behaviour at week 3, 4, 5 and 6 after operation in mononeuropathic rats treated with 0.3 mg U-50,488H

Treatment	*n*	Week 3	Week 4	Week 5	Week 6
Saline, contralateral	11	2.0 (0.7–4.6)	3.0 (1.5–6.0)	4.0 (2.1–6.7)	7.0 (3.2–7.2)
U-50,488H, contralateral	8	0.0 (−0.3–0.8)[a,d]	0.3 (−0.5–1.3)[a,c]	0.0 (−0.3–0.8)[b,c]	0.0 (−0.9–2.1)[b,c]
U-50,488H, ipsilateral	6	0.5 (−0.9–4.2)	0.0 (−1.2–3.9)	0.5 (−1.1–3.8)	0.5 (−1.4–4.4)
U-50,488H, bilateral	6	−4.0 (−6.3–3.9)	−1.0 (−3.5–1.9)	0.0 (−4.0–5.2)	2.0 (−3.3–5.7)
U-50,488H + NalMeth, contralateral	7	3.0 (0.7–4.5)	4.0 (0.6–5.2)	5.0 (0.4–6.4)	5.0 (0.7–7.0)

Data are given as mean ± S.E.M. Autotomy score was calculated as a total score and subtracted from the score before drug treatment (day 6 post-operation). The autotomy score for each experimental group is presented as the median with 95% confidence interval. Non-parametric Mann–Whitney *U* test was used for statistical analysis.

[a] $P < 0.05$ when comparing mononeuropathic rats injected contralaterally with U-50,488H vs. saline.

[b] $P < 0.01$ when comparing mononeuropathic rats injected contralaterally with U-50,488H vs. saline.

[c] $P < 0.05$ when comparing mononeuropathic rats injected contralaterally with U-50,488H vs. U-50,488H + naloxone methiodide (NalMeth).

[d] $P < 0.01$ when comparing mononeuropathic rats injected contralaterally with U-50,488H vs. U-50,488H+naloxone methiodide (NalMeth).

thresholds to mechanical stimulation on the right side as compared to ipsilateral injection (Fig. 3A).

3.4. Autotomy behaviour

Contralateral treatment with 0.3 mg U-50,488H significantly reduced autotomy behaviour measured at 3, 4, 5 and 6 weeks compared to saline-treated mononeuropathic rats (Table 1). Contralateral co-administration of U-50,488H with an equimolar dose of naloxone methiodide significantly reduced autotomy scores compared to the rats treated with the opioid receptor agonist alone. No difference in autotomy score was found between different sites of administration, i.e. contralateral, ipsilateral and bilateral, of U-50,488H (Table 1).

4. Discussion

In the present study, we report data on the antinociceptive effect of the κ-opioid receptor agonist U-50,488H in a well-established rat model of unilateral peripheral neuropathy (chronic constriction of the common sciatic nerve) (Bennett and Xie, 1988). Administration of a low dose (0.3 mg) of U-50,488H into the contralateral hindpaw produced a significant attenuation in pain-related behaviour in the nerve-injured hindpaw by increasing withdrawal latencies to mechanical but not thermal stimulation. No difference in pain-related behaviour was found between different peripheral (contralateral, ipsilateral and bilateral) treatments with 0.3 mg U-50,488H. Autotomy behaviour was also reduced for 6 weeks after sciatic nerve ligation in rats treated with the opioid receptor agonist into the contralateral hindpaw.

Based on our recent findings on the efficacy of a low dose (0.3 mg) of U-50,488H in producing an antinociceptive response after contralateral administration in rats with unilateral carrageenan-induced hindpaw inflammation (Bileviciute-Ljungar and Spetea, 2001), the same dose was tested using a similar approach in mononeuropathic rats. We have reported that the effect of 0.3 mg U-50,488H was due to a local and not to a systemic action in inflammatory conditions, being abolished by sciatic nerve ligation on the contralateral non-inflamed side (Bileviciute-Ljungar and Spetea, 2001).

Our present results are in agreement with the previous studies reporting on the antinociceptive action of other κ-opioid receptor agonists in chronic constriction injury in neuropathic rats (Keita et al., 1995; Catheline et al., 1998; Walker et al., 1999). Administration of the peripherally selective κ-opioid receptor agonists, ICI 204448 ((*R*,*S*)-*N*-]2-(*N*-methyl-3,4-dichloro-phenylacetamido)-2-(3-carboxy-phenyl)-ethyl]-pyrrolidine hydocloride) and asimadoline, into the nerve-injured paw of mononeuropathic rats elicited antinociception up to 60 min after drug injection (Keita et al., 1995; Walker et al., 1999). Similar findings were reported with another κ-opioid receptor agonist, U-69,593 (*trans*-3,4-dichloro-*N*-methyl-*N*-[7-(1-pyrrolidinyl) cycloexil]benzene-acetamide methanesulfonate) (Catheline et al., 1998). However, no effect on pain-related behaviour was detected after drug administration directly into the contralateral paw. The lack of any significant effect after contralateral treatment might be due to different testing paradigms used for evaluation of nociceptive reflexes (i.e. vocalization threshold vs. hindpaw withdrawal reflex) and/or the fact that the nociceptive responses were measured a short time after the drug injection. Spinal antinociceptive and antiallodynic effects of δ-opioid receptors agonists, e.g. DPDPE ([D-Pen^2,D-Pen^5]enkephalin) and deltorphin II have also been reported, after a single drug administration in rats with a crushed sciatic nerve, with effects persisting up to 60 min (Mika et al., 2001). Chronic administration of DPDPE and deltorphin II resulted in significant prolongation of the reaction time on days 2, 4 and 6 post-injury. Moreover, endomorphin-1 and endomorphin-2, two μ-opioid receptor agonists, were also shown to produce antinociception at the spinal level in mononeuropathic rats (Przewlocka et al., 1999).

Previous electrophysiological studies have shown that spontaneous activity of nociceptive wide-dynamic range neurons in the spinal cord is bilaterally increased following

development of mononeuropathy in the rat (Sotgiu and Biella, 1998). Furthermore, our previous studies indicate that contralateral administration of lidocaine reduces spontaneous activity and afterdischarges of wide-dynamic range in rats with a unilateral peripheral nerve injury (Bileviciute-Ljungar et al., 2001). It remains to be investigated whether this type of neurophysiological mechanism might underlie the contralateral effect induced by U-50,488H.

In the present study, no difference has been found in the antinociceptive effect of U-50,488H between different peripheral sites of administration: contralateral, ipsilateral and bilateral. This indicates that peripheral administration of a drug which acts by reducing nerve activity might induce long-term changes in the spinal neuronal mechanisms following neuropathic pain. These results are in line with our earlier findings showing that low doses of both contralaterally administered and ipsilaterally administered xylocaine, a local anaesthetic, reduce pain-related behaviour in mononeuropathic rats, with no differences between these two sites of administration (Bileviciute-Ljungar and Lundeberg, 2000a). In contrast, antinociception after contralateral administration of the local anaesthetic bupivacaine in rats with unilateral inflammation has been reported to be long-lasting (up to 24 h) (Bileviciute-Ljungar and Lundeberg, 2000a) as compared to ipsilateral administration (up to 25 min) (Kayser and Guilbaud, 1987).

In conclusion, our results indicate that repeated contralateral treatment with the κ-opioid receptor agonist U-50,488H attenuates pain-related behaviour in mononeuropathic rats via peripheral opioid receptors. Autotomy behaviour was also reduced up to 6 weeks after sciatic nerve constriction in opioid treated rats. Administration of peripherally opioid analgesics, such as κ-opioid receptor agonists, may represent an efficacious approach for management of neuropathic pain in humans.

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