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European Journal of Pharmacology 559 (2007) 32–37
www.elsevier.com/locate/ejphar

Altered antinociceptive efficacy of tramadol over time in rats with painful peripheral neuropathy

Aldric Hama *, Jacqueline Sagen

University of Miami Miller School of Medicine, The Miami Project to Cure Paralysis, Miami, FL 33136, USA

Received 12 October 2006; received in revised form 16 November 2006; accepted 21 November 2006
Available online 1 December 2006

Abstract

Pain due to peripheral nerve injury or disease is a dynamic process, such that the mechanism that underlies it alters over time. Tramadol has been reported to be analgesic in clinical neuropathic pain, with varying levels of efficacy due to a patient population that has had neuropathic pain for a wide range of time. In order to address and examine the issue, the antinociceptive efficacy of tramadol over time was tested in rats with a chronic constriction injury (CCI) of the left sciatic nerve. Rats developed a robust hind paw hypersensitivity to innocuous mechanical stimulation ipsilateral to CCI surgery. Subcutaneous injection of tramadol in rats two weeks after CCI surgery dose-dependently attenuated mechanical hypersensitivity, which was abolished with the μ-opioid receptor antagonist naloxone but not the α_2-adrenoceptor antagonist yohimbine. Systemic tramadol also attenuated mechanical hypersensitivity four weeks after CCI surgery, but the efficacy significantly diminished at this time point. In addition, the effect of tramadol at this later time point could be reduced with yohimbine as well as naloxone. These data demonstrate that the efficacy of tramadol depends in part on the duration of nerve injury-evoked nociception, and that its antinociceptive mechanism changes over time. Alteration in antinociceptive mechanism over time may explain the inconsistency in efficacy of this and other analgesic drugs in chronic pain patients.

Keywords: Allodynia; Chronic pain; Consistency; Dynamic process; α_2-Adrenoceptor; μ-Opioid receptor

1. Introduction

Neurological disorders, including chronic pain, evolve over time (Burchiel and Slavin, 2000; Chen et al., 2002; DeKosky and Marek, 2003; Gilman, 2006; Marchettini et al., 2004). Thus, a drug treatment that is efficacious in early stages may be less efficacious in later stages of the disease. Alternatively, a change in drug efficacy may be due to alterations in a drug-related mechanism over time, such as a decrease in receptor function over time due to continuous exposure to the drug (Bailey and Connor, 2005). Data from rat models of chronic neuropathic pain have demonstrated nerve injury-related changes in CNS receptors and neuropeptides over time (Cameron et al., 1997; Goff et al., 1998). It is presumed that analgesics tested at different times in such rats will show an alteration in efficacy because of the neuropathy-related changes.

Pre-clinical studies that have evaluated analgesic treatments for neuropathic pain have tested them within a relatively early time frame, for example, between one to two weeks following nerve injury (Jett et al., 1997; Joshi et al., 2006). Clinicians have noted that early diagnosis and intervention will lead to better pain relief than in patients with late-stage neuropathic pain, thus, the positive outcomes in pre-clinical drug studies may be due to early testing periods (Bonica, 1990). In contrast, neuropathic pain patients present with pain months or years following nerve injury or disease, so it is not clear how the early testing time following rat neuropathic surgery extrapolates to clinically presented pain (Bonica, 1990).

A previous study demonstrated changes in responsiveness to antinociceptive drugs over time in a rat model of neuropathic pain, by measuring changes in potency and efficacy (Hama and Borsook, 2005). The drug efficacies of gabapentin and

* Corresponding author. University of Miami Miller School of Medicine, The Miami Project to Cure Paralysis, 1095 NW 14th Terrace (R-48), Miami, FL 33136, USA. Tel.: +1 305 243 6038; fax: +1 305 243 3923.
E-mail address: ahama@med.miami.edu (A. Hama).

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doi:10.1016/j.ejphar.2006.11.047

imipramine significantly varied depending on when the drug was tested after nerve injury. The mechanism underlying the pharmacologic properties of these drugs, however, is unclear.

Tramadol, which may be a particularly promising agent for neuropathic pain owing to its multiple complementary pharmacologic mechanisms of action, is a codeine analogue that is FDA approved for the treatment of moderate to moderately severe pain. Tramadol and its *O*-desmethyl metabolite exhibit several analgesic mechanisms, including inhibition of norepinephrine and serotonin reuptake, and activity as a μ-opioid receptor agonist (Pandita et al., 2003). Although tramadol is not FDA approved specifically for neuropathic pain, clinical evidence suggests analgesic efficacy that is comparable to anticonvulsants (Hollingshead et al., 2006; Wiffen et al., 2005). Despite having activity at opioid receptors, a lack of tolerance in pre-clinical studies suggests that tramadol may be indicated for long-term use such as in persistent neuropathic pain (Franceschini et al., 1999). However, since patients will present at varying stages of neuropathic pain, an important issue is whether tramadol analgesia will be consistent. The aim of the current study was to evaluate the consistency of tramadol efficacy in a rat model of peripheral neuropathic pain, and whether or not pharmacological mechanisms underlying antinociceptive effects are altered over time following injury.

2. Materials and methods

2.1. Animals

Procedures were reviewed and approved by the University of Miami Animal Care and Use Committee. Following arrival, male Sprague Dawley rats (100–124 g; Harlan, IN) were allowed at least five days to acclimate to the animal facility, which was on a 12 h light–dark cycle. Rats were allowed access to food and water before and following surgery.

2.2. Chronic constriction injury (CCI)

A loose ligation of the left sciatic nerve was performed as previously described (Bennett and Xie, 1988). Rats were anesthetized with isoflurane in oxygen. Using aseptic technique, the left sciatic nerve was exposed and loosely ligated with four 4–0 chromic gut ligatures. The muscles overlying the nerve were sutured closed and the skin wound sealed with veterinarian grade cyanoacrylate.

2.3. Systemic tramadol in rats with a CCI

Following CCI surgery, rats (N=28) were tested during the early post-injury period (two weeks after CCI surgery) and tested again during the late post-injury period (four weeks after CCI surgery). To construct a dose–response curve, the doses of systemic (subcutaneous, s.c.) tramadol tested were 3, 10 and 30 mg/kg. Following the second test, rats were humanely euthanized.

2.4. Sensitivity to von Frey filaments

Prior to drug injection, baseline withdrawal thresholds of the hind paw ipsilateral to the CCI surgery were measured with von Frey filaments. Using the up–down method, a set of eight filaments was used to determine the withdrawal threshold (in g) (Chaplan et al., 1994). A threshold of 15 g was assigned if there was a lack of response to the highest filament. Conversely, a threshold of 0.25 g was assigned if the rat responded to the lowest filament. Following drug injection, rats were tested again once every 30 min up to 120 min post-injection.

2.5. Effect of antagonists on tramadol antinociception

Following baseline withdrawal threshold measurement, CCI rats were s.c. injected with either naloxone (5 mg/kg), yohimbine (2 mg/kg) or vehicle (1 ml/kg). Thirty minutes following antagonist (or vehicle) injection, thresholds were again measured and then either tramadol (30 mg/kg) or vehicle was s.c. injected. Rats were tested 60 min following tramadol (or vehicle) injection. There were four treatment groups in this phase of the experiment (pre-treatment/post-treatment): vehicle/vehicle, vehicle/tramadol, antagonist/vehicle and antagonist/tramadol. A total of 68 rats was used in the antagonist experiments and was used at both two weeks and four weeks post-CCI. Following baseline testing at four weeks, rats were assigned to a treatment group other than the group they were assigned to at two weeks.

2.6. Drugs

Tramadol HCl ((±)-*cis*-2-(dimethylaminomethyl)-1-(3-methoxyphenyl)cyclohexanol) and yohimbine HCl were obtained from Sigma-Aldrich Co. (St. Louis, MO) and naloxone HCl was obtained from Spectrum Chemicals (New Brunswick, NJ). All drugs were dissolved in normal saline (vehicle). The drugs were s.c. injected in a volume of 1 ml/kg.

2.7. Statistical analysis

Data are expressed as mean±S.E.M. Statistical analysis was performed using SigmaStat (v. 3.1; Point Richmond, CA). A two-way repeated measure ANOVA was used to analyze the effects of treatment over time. A Student–Newman–Keuls test was used for post-hoc comparisons. To calculate a 50% analgesic dose (A_{50}), the thresholds were converted to a percent maximum possible effect (MPE):

$$(\text{Drug Threshold} - \text{Baseline Threshold}) \div (15\ \text{g} - \text{Baseline Threshold}) \times 100.$$

The 50% analgesic dose (A_{50}) was calculated from the linear portion of the log-dose–response curve at 60 min post-injection, since maximum efficacy in the current study was noted at that time point (Tallarida and Murray, 1981). A two-way ANOVA was used to evaluate the effect of antagonists on tramadol's efficacy and a Student–Newman–Keuls test was used for post-

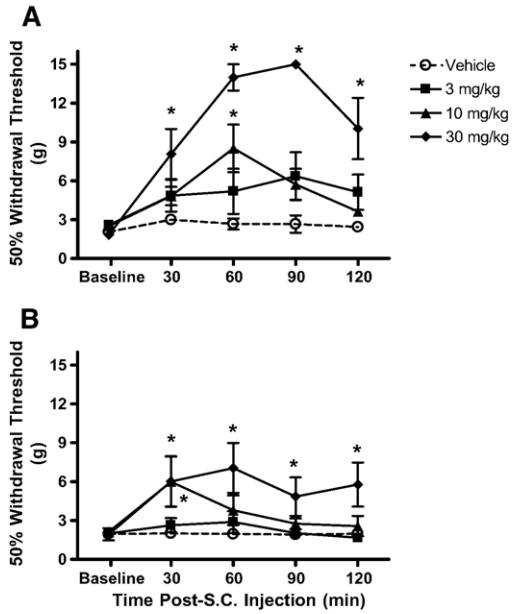

Fig. 1. Time course of the effect of tramadol in rats with a chronic constriction injury. The abscissa is time post-subcutaneous (s.c.) injection (in minutes) and the ordinate is withdrawal threshold (grams). The efficacy of s.c. tramadol was measured in rats with a chronic constriction injury (CCI) two weeks (A) and four weeks (B) after surgery. A dose-dependent increase in withdrawal threshold was observed in rats two weeks after CCI and a modest increase in threshold was observed in rats four weeks after CCI. The 50% analgesic dose of tramadol in rats 2 weeks after CCI was 8.2 mg/kg and the 50% analgesic dose in rats 4 weeks after CCI was >30 mg/kg. Data are expressed as mean±S.E.M. N=6–7 rats/treatment group. *P<0.05 vs. baseline and vehicle.

hoc comparisons. The level of statistical significance was taken at P<0.05.

3. Results

3.1. Effect of tramadol on mechanical hypersensitivity

Prior to CCI surgery, rats did not respond to the highest filament, so withdrawal thresholds were 15 g. Two weeks following CCI surgery, withdrawal thresholds decreased to 2.3±0.2 g. Subcutaneous tramadol doses of 10 mg/kg and 30 mg/kg significantly increased withdrawal thresholds (Fig. 1A; P<0.05 vs. baseline). The highest dose tested (30 mg/kg) nearly reversed the withdrawal thresholds to pre-injury cut-off levels during the time of peak effect (60–90 min), and an antinociceptive effect was still apparent at 120 min The A_{50} (±95% C.L.) at 60 min post-injection was 8.2±4.2 mg/kg. Injection of vehicle did not alter withdrawal thresholds.

Four weeks following CCI surgery, withdrawal thresholds were 2.0±0.2 g. At 60 post-injection, 30 mg/kg tramadol increased withdrawal thresholds (Fig. 1B; P<0.05 vs. baseline). However, the percent MPE of tramadol four weeks following CCI (39%) was significantly decreased compared to tramadol two weeks after CCI (93%). The A_{50} for tramadol four weeks after CCI was shifted to the right 11-fold and estimated to be 93 mg/kg. Injection of vehicle in these rats did not affect withdrawal thresholds.

3.2. Effect of antagonists on tramadol antinociception

Antagonist pre-treatments were done in order to evaluate contributing underlying antinociceptive mechanisms. Prior to s.c. injection of either naloxone or vehicle, the baseline withdrawal threshold of rats two weeks after CCI was 2.1±0.1 g (Fig. 2A). This was not significantly altered at 30 min following injection of either naloxone or vehicle pre-treatments. Either tramadol (30 mg/kg, s.c.) or vehicle was then administered, and animals were tested again 60 min later, during the range of peak effect of tramadol previously observed (see Fig. 1). At 60 min after the second injection of vehicle, the withdrawal threshold of the vehicle/vehicle group was 2.5±0.3 g. In contrast, in the vehicle/tramadol group, the withdrawal threshold increased to 11.2±1.3 g (P<0.05 vs. vehicle/vehicle group), similar to the effects of tramadol alone (see Fig. 1). However, following naloxone pre-treatment, in the naloxone/tramadol group, the mean withdrawal was 3.3±0.6 g, which was not significantly different from the withdrawal threshold of the vehicle/vehicle group. Thus, naloxone pre-treatment blocked the onset of a tramadol-induced antinociception. The withdrawal threshold of the naloxone/vehicle group was not significantly different from the withdrawal threshold of the vehicle/vehicle group 1 h after the second injection of vehicle.

In contrast with naloxone, pre-treatment with yohimbine did not significantly affect the tramadol-induced antinociception at two weeks post-CCI (Fig. 2B). Following pre-treatments (at 30 min), mean withdrawal threshold of rats pre-treated

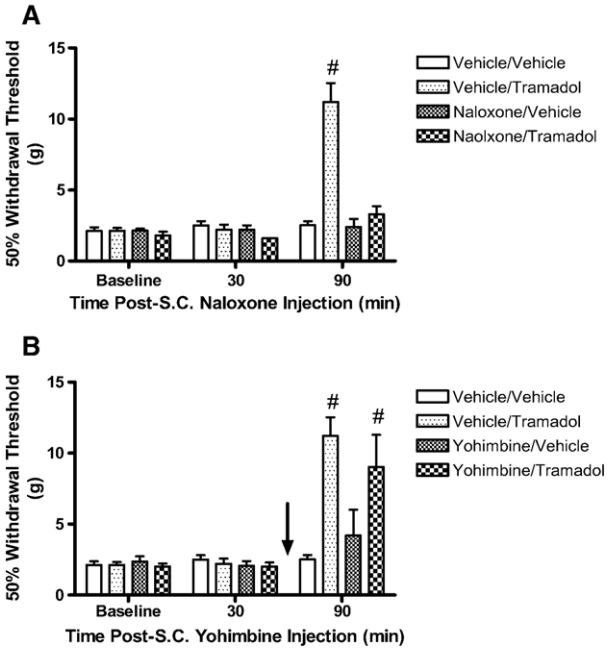

Fig. 2. Effect of antagonists on tramadol antinociception in rats two weeks following a chronic constriction injury. The abscissa is time post-subcutaneous (s.c.) injection (in minutes) after the antagonist and the ordinate is withdrawal threshold (grams). After baseline testing, rats were injected with either vehicle, naloxone (s.c. 5 mg/kg, A) or yohimbine (s.c. 2 mg/kg, B). Following the 30 min testing time point, either vehicle or 30 mg/kg of tramadol was s.c. injected. Rats were tested 60 min following tramadol injection (90 min following antagonist injection). Data are expressed as mean±S.E.M. N=7 rats/treatment group. #P<0.05 vs. vehicle/vehicle.

with yohimbine was not significantly different from vehicle-treated rats. Following pre-treatment, at 60 min after injection of tramadol, the mean withdrawal threshold of the yohimbine/tramadol group (9.0±2.3 g) was significantly higher compared to the threshold of the vehicle/vehicle group ($P<0.05$ vs. vehicle/vehicle). This was not significantly different, however, from the vehicle/tramadol group, indicating no significant block of tramadol-induced antinociception by yohimbine. One hour after vehicle injection, the withdrawal threshold of the yohimbine/vehicle group was 4.2±1.8 g, which was not significantly different from the threshold of the vehicle/vehicle group.

In order to evaluate whether pharmacologic mechanisms of tramadol antinociceptive effects remained consistent at later times following peripheral nerve injury, the antagonist study was repeated four weeks after CCI. Findings are shown in Fig. 3. Prior to injection of either antagonist or vehicle, the baseline withdrawal threshold of rats at four weeks after CCI was 2.1±0.1 g. Thirty minutes following injection of either antagonist or vehicle, withdrawal thresholds were not significantly different compared to baseline. One hour after the second injection of vehicle, the withdrawal threshold of the vehicle/vehicle group was 2.7±0.6 g. In contrast, 1 h after injection of tramadol, the withdrawal threshold of the vehicle/tramadol group was significantly increased (8.5±1.5 g) compared with the vehicle/vehicle group ($P<0.05$). Pre-treatment with either naloxone (Fig. 3A) or yohimbine (Fig. 3B) attenuated the tramadol-induced antinociception ($P<0.05$ vs. vehicle/tramadol; $P>0.05$ vs. vehicle/vehicle).

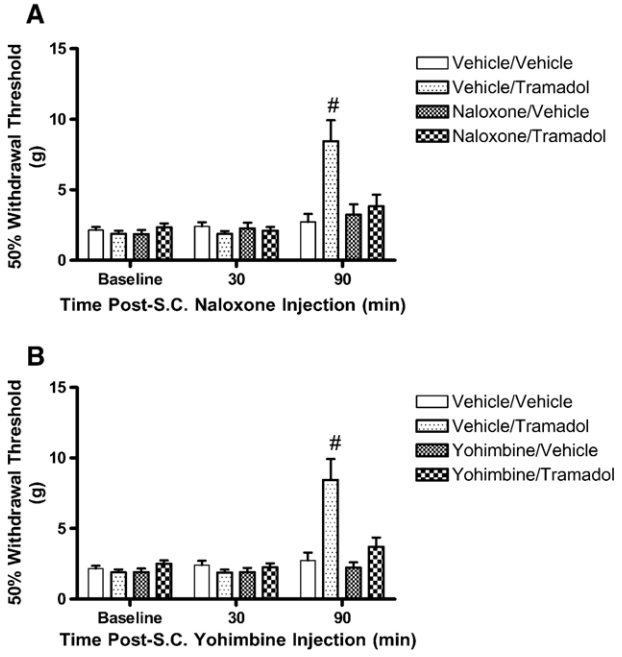

Fig. 3. Effect of antagonists on tramadol antinociception in rats four weeks following a chronic constriction injury. The abscissa is time post-subcutaneous (s.c.) injection (in minutes) of the antagonist and the ordinate is withdrawal threshold (grams). After baseline testing, rats were injected with either vehicle, naloxone (s.c. 5 mg/kg, A) or yohimbine (s.c. 2 mg/kg, B). Following the 30 min testing time point, either vehicle or 30 mg/kg of tramadol was s.c. injected. Rats were tested 60 min following tramadol injection (90 min following antagonist injection). Data are expressed as mean±S.E.M. $N=8–9$ rats/treatment group. $^{\#}P<0.05$ vs. vehicle/vehicle.

4. Discussion

The data support the contention that the antinociceptive efficacy of tramadol in neuropathic pain is influenced by the duration of the neuropathy, such that a long duration of time between CCI surgery and tramadol testing resulted in a marked decrease in antinociception, compared with testing relatively early following surgery. Tramadol antinociception was attenuated with the μ-opioid receptor antagonist naloxone but not with the α_2-adrenoceptor antagonist yohimbine in rats two weeks after CCI surgery. However, it appeared that both antagonists suppressed tramadol antinociception in rats four weeks after CCI surgery. The change in antagonist effect, along with the decreased efficacy of tramadol, suggests a change in tramadol's mechanism of action during the progression of neuropathic pain to a chronic stable state.

Tramadol (and its metabolites) has been shown to be effective in a variety of acute and chronic pain models (Apaydin et al., 2000; Combe et al., 2004; Raffa et al., 1992). The clinical efficacy has been ascribed to several mechanisms, including activity at the μ-opioid and the α_2-adrenoceptor (Desmeules et al., 1996). It has been previously noted that the activation of either of these receptors alone by prototypic agonists will lead to antinociception. However, combining sub-effective doses of agonists to both receptors leads to an enhanced antinociception that is greater than either agonist alone (Monasky et al., 1990). Rather than function as a α_2-adrenoceptor agonist, however, tramadol decreases re-uptake of norepinephrine and serotonin (Pandita et al., 2003). Robust antinociception has been previously observed by combined intrathecal injection of the tricyclic antidepressant amitriptyline, which blocks reuptake of norepinephrine and serotonin, with a sub-effective dose of opiate (Taiwo et al., 1985). In addition, an α_2-adrenoceptor agonist combined with morphine diminishes tolerance observed with long-term morphine use (Plummer et al., 1995). It appears that there is no decrement of the efficacy of tramadol taken over an extended period of time (Malonne et al., 2005). Thus, given the analgesic activity with fewer opiate-related side-effects, tramadol is ideal for long-term application for various pain states, including neuropathic pain.

One problem underlying the development of effective treatments for chronic neuropathic pain lies in the heterogeneity of mechanisms within a given patient population, which, furthermore, is due in part to changes in the pain state over time. Since CNS changes occur over time during disease or injury progression, it is likely that responses to a given drug will change over time as well (Cameron et al., 1997; Eaton et al., 1999; Goff et al., 1998; Maihofner et al., 2003). The utility of testing drugs at different times following injury would not only to indicate which treatments may be of use at either early or late times (or both) after nerve injury but also to pharmacologically point out changes in a drug-related mechanism.

The markedly reduced efficacy of tramadol in "late" CCI neuropathy compared with "early" CCI neuropathy supports

previous findings of altered efficacy in a different neuropathic pain model (Hama and Borsook, 2005). In the current study, later treatment times in established CCI resulted in over a 10-fold rightward shift of the dose–response curve of tramadol. The rats at either time point post-CCI surgery had the same degree of mechanical hypersensitivity. It is possible that metabolism of tramadol may have changed between the early and late-stage CCI rats. Such a change in metabolism, as well as in efficacy, of codeine has been observed in very young rats, but a similar alteration has not been reported for tramadol (Williams et al., 2004). Thus, more likely underlying the change in tramadol efficacy is an alteration in the opiate and/or monoaminergic components of tramadol activity occurring between the testing periods.

To evaluate this possibility, rats were pre-treated with either μ-opioid receptor antagonist naloxone or α_2-adrenoceptor antagonist yohimbine. These antagonists have been previously found to block the effects of tramadol in rat models of pain as well as in humans (Desmeules et al., 1996; Raffa et al., 1992). At two weeks after CCI surgery, the antinociceptive effect of tramadol was completely blocked by naloxone pre-treatment. However, there was no effect on antinociceptive efficacy with yohimbine pre-treatment. The data suggest that at fairly early stages following peripheral nerve injury, most of the tramadol efficacy is opiate-mediated. Although there did not appear to be an adrenergic component to the antinociceptive effect of tramadol, it is possible that other neurotransmitter systems may be involved (e.g. serotonin).

In contrast to the early post-injury antagonist studies, pre-treatment with either naloxone or yohimbine reduced the efficacy of tramadol in rats at four weeks post-CCI. These data suggest an increased contribution of the catecholaminergic component of tramadol's purported mechanism in the more developed stages of peripheral neuropathic pain. The antagonist data also suggest that changes over time in particular receptors and/or re-uptake mechanisms mediate the antinociceptive effect of tramadol.

The spinal dorsal horn appears to be a likely source of these changes since intrathecal injection of tramadol in naïve, uninjured rats has been shown to be antinociceptive, in addition to the prominent involvement of spinal opiate and adrenergic receptors in analgesia (Bernatzky and Jurna, 1986). However, there was a lack of significant antinociception with the intrathecally injected dose of 100 μg tramadol in these rats, whether tested "early" or "late" after CCI (data not shown). A 10-fold smaller dose led to a 50% antinociceptive effect that was naloxone sensitive in uninjured rats (Bernatzky and Jurna, 1986). There may be several explanations behind the lack of efficacy of intrathecally injected tramadol in neuropathic rats. First, the metabolite has a higher affinity for the μ-opioid receptor and tramadol may not have been metabolized in sufficient quantities in the intrathecal space. Second, intrathecally injected opiates tend to show decreased efficacy in rats with neuropathic pain such that the dose–response curve of intrathecal morphine is significantly shifted to the right in CCI rats (Granados-Soto and Arguelles, 2005; Mao et al., 1995). Alternatively, the lack of antinociception following intrathecal injection of tramadol in the current study suggests the possibility that the spinal dorsal horn has a negligible role in antinociceptive effects of tramadol. In fact, clinically, intrathecal tramadol does not appear to be effective on post-operative pain (Alhashemi and Kaki, 2003). It is possible that supraspinal sites, rather than the spinal dorsal horn, mediate the effect of systemic tramadol in the neuropathic state (Kovelowski et al., 1998). Changes in supraspinal function in response to neuropathy, which may have consequences on drug efficacy, have been described (Ozaki et al., 2002). Further evaluation with microinjections of tramadol into pain-related brain areas or evaluating changes in tramadol effects in brain areas with non-invasive imaging techniques should confirm this hypothesis.

The current data demonstrate that the antinociceptive efficacy of drugs may be dependent on the development and duration of the neuropathic state. In addition, the underlying mechanism may also change over time, which is consistent with the overall changes in CNS and PNS processing of nociception following a peripheral nerve injury. These data suggest that good (or no) efficacy may be observed depending on when they are tested in existing rat models of neuropathic pain. The present findings may be extended to the clinic, in that the best efficacy of drugs may be within a narrow time frame. A criterion that should be applied to developing better analgesics is that it be efficacious throughout the time course of neuropathic pain, and certainly should be evaluated for sustained effects during chronic pain stages in pre-clinical models.

Acknowledgements

We would like to thank Mr. Adam Basler for his technical assistance. This study is supported by the Miami Project to Cure Paralysis and by NIH grant NS 51667.

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