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Chronic administration of amitriptyline and caffeine in a rat model of neuropathic pain: multiple interactions

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Abstract

This study was designed to determine (1) whether chronic amitriptyline administration was effective in alleviating symptoms of neuropathic pain in a rat model of spinal nerve injury, and (2) whether the effect of amitriptyline involved manipulation of endogenous adenosine, by determining the effect of caffeine, a non-selective adenosine A_1 and A_2 receptor antagonist, on its actions. Nerve injury was produced by unilateral spinal nerve ligation of the fifth and sixth lumbar nerves distal to the dorsal root ganglion, and this resulted in stimulus-evoked thermal hyperalgesia and static tactile mechanical allodynia. Animals received pre- and post-surgical intraperitoneal doses of amitriptyline (10 mg/kg) and caffeine (7.5 mg/kg), alone or in combination, and following surgery, were administered amitriptyline (15–18 mg/kg/day) and caffeine (6–8 mg/kg/day), alone or in combination, in the drinking water. Rats were tested for thermal reaction latencies and static tactile thresholds at 7, 14 and 21 days following surgery. In the paw *ipsilateral* to the nerve ligation, chronic amitriptyline administration consistently decreased the thermal hyperalgesia produced by spinal nerve ligation over a 3-week period, and this effect was blocked by concomitant caffeine administration at all time intervals. In the contralateral paw, thermal withdrawal latencies were more variable, with the most reproducible finding being a reduction in thermal thresholds in the amitriptyline–caffeine combination group. There was no effect by either drug or the drug combination on the static tactile allodynia produced by spinal nerve ligation in the *ipsilateral* paw. However, chronic amitriptyline administration induced a tactile hyperaesthesia in the *contralateral* paw at all time intervals, and this effect was exacerbated by concomitant chronic caffeine administration. The results of this study indicate that chronic administration of amitriptyline is effective in alleviating thermal hyperalgesia, but not static tactile allodynia, in the hindpaw ipsilateral to nerve injury, and the block of this effect by caffeine suggests that this effect is partially achieved through manipulation of endogenous adenosine systems. Additionally, chronic amitriptyline administration induces contralateral hyperaesthetic responses that are augmented by caffeine. Both the symptom-specific effect, and adenosine involvement in amitriptyline action may be important considerations governing its use in neuropathic pain.

Keywords: Amitriptyline; Caffeine; Adenosine; Neuropathic pain; Chronic administration

1. Introduction

Antidepressants are widely used in the treatment of neuropathic pain (Onghena and Van Houdenhove, 1992; McQuay et al., 1996). This condition involves changes at various levels of the transmission of pain information (including peripheral nerves, spinal and supraspinal sites), and these contribute both to the development and maintenance of the condition (Coderre and Katz, 1997; Attal and Bouhassira, 1999; Baron, 2000). The analgesic efficacy of antidepressants occurs irrespective of mood altering effects (Sindrup, 1997; Eschalier et al., 1999). When antidepressants are administered systemically, their actions are expressed at a number of sites. Initial studies focussed on supraspinal and spinal sites of action (e.g. Spiegel et al., 1983; Hwang and Wilcox, 1987; reviewed Eschalier et al., 1999), but more recent studies have demonstrated an additional peripheral site of action for antidepressants (Sawynok et al., 1999; Esser and Sawynok, 1999).

One antidepressant commonly used in the treatment of neuropathic pain is amitriptyline, an agent with multiple actions, some of which are still incompletely understood (Sindrup, 1997; Eschalier et al., 1999). While this multiplicity of action may afford amitriptyline its unique analgesic effect in chronic pain, in that it simultaneously interacts with a number of mechanisms that contribute to the condition, it may also be a major dose-limiting factor in its use (MacFarlane et al., 1997). In a previous study, we demonstrated a thermal anti-hyperalgesic action of acutely administered amitriptyline in the spinal nerve liga-

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tion model of neuropathic pain, and discussed the potential mechanisms involved (Esser and Sawynok, 1999). These actions include blocking reuptake of noradrenaline and serotonin, acting as an antagonist at NMDA, histamine receptors, α-adrenoceptors and muscarinic receptors, as well as blocking various cation channels. There is also evidence that suggests involvement of an adenosine component in this action (Esser and Sawynok, 2000). While these actions are important to the overall effect of amitriptyline in neuropathic pain, they have usually been considered in the context of acute drug administration. It is important to recognize the long-term effects of chronic antidepressant treatment, since these agents produce a number of actions that are uniquely expressed following chronic administration paradigms, and many of these could also influence the neuroplastic changes that occur with neuropathic pain. These include downregulation of β-adrenoceptors and $GABA_B$ receptors, upregulation of α_1-adrenoceptors, decreased activity of α_2-adrenoceptors and dopamine autoreceptors, alterations in 5-hydroxytryptamine (5-HT) mechanisms (reviewed Blier and De Montigny, 1995; Leonard, 1996), downregulation of NMDA receptors and mechanisms (reviewed Skolnick, 1999) and enhancement of cyclic AMP, protein kinase A, cyclic AMP response element binding protein and brain-derived neurotrophic factor (BDNF) (reviewed Duman et al., 1997). A number of these actions are potentially relevant to neuropathic pain mechanisms which involve multiple changes at central synapses (reviewed Woolf and Doubell, 1994; Attal and Bouhassira, 1999; Woolf and Decosterd, 1999).

A number of animal studies have examined the acute effects of antidepressants in chronic pain models (Eschalier et al., 1999), but a much more limited number have looked at effects of chronic administration of antidepressants in the treatment of chronic pain (Ardid and Guilbaud, 1992; Lang et al., 1996). In the present study, we examined the effect of a non-invasive method of chronically administering amitriptyline (by inclusion in the drinking water) on two behavioral manifestations of neuropathic pain using a rat model of spinal nerve injury (Kim and Chung, 1992). We also sought to determine whether the action of chronic amitriptyline involved adenosine-based mechanisms by using concomitant chronic administration of caffeine, a non-selective adenosine A_1 and A_2 receptor antagonist, in the drinking water. Since both drugs are administered chronically via the oral route in humans, drug delivery using this method was considered to be of greatest relevance to the human situation.

2. Methods

2.1. Animals

Experiments were conducted on male Sprague–Dawley rats (100–120 g) from Charles River, Quebec, Canada, using procedures approved by the Dalhousie University Committee on Laboratory Animals. Animals were housed in pairs, maintained on a 12/12-h light/dark cycle at 22 ± 1 °C and given ad libitum access to food and water.

2.2. Spinal nerve ligation

Experiments were performed with adherence to the guidelines of the IASP on animal experimentation in pain research (Zimmerman, 1983), and were designed to limit the number of animals used. Animals were rendered neuropathic through unilateral tight ligation of the fifth and sixth lumbar spinal nerves (L5 and L6), in accordance with the technique of Kim and Chung (1992). Animals were anaesthetized with 1.5–2.0% halothane. Rats were administered Ringers lactate solution (5 ml; subcutaneous; s.c.), atropine (0.6 ml/kg; intramuscular), penicillin (Penlong; 1.0 ml/kg; s.c.) and topical eye ointment (Lacri-Lube; Allergen). The surgical area was aseptically scrubbed with alcohol and iodine, and a 3-cm dorsal incision was made using the ischium as the midline. Using blunt dissection and partial removal of the articular facet and the L4 transverse process, the L5 and L6 spinal nerves were exposed under a dissecting microscope. The nerves were then tightly ligated with sterilized 6-0 silk. After ensuring hemostasis, the wound was closed in layers with subcutaneous and cutaneous suturing (3.0 Novophil). Animals were then placed in a heated area for surgical recovery and monitoring. Following a 48-h recovery period, animals were again housed in pairs, and standard environmental enrichments added to the cages.

2.3. Behavioral assessment

All behavioral tests were conducted between 08:00 and 14:00. Following a recovery period of 7 days, animals were moved from the Animal Care Center and acclimated to the testing room for 40 min. After this initial period, the animals were placed in the respective testing apparatus for 30–40 min or until exploratory behavior ceased. At 7, 14 and 21 days, thermal thresholds (Section 2.3.1) and static tactile thresholds (Section 2.3.2) were determined. These thresholds were obtained over a 2-day period (one behavioral test per day) to minimize the stress and potential effects of repetitive testing.

2.3.1. Thermal withdrawal thresholds

To test for thermal withdrawal thresholds, a paw thermal stimulator (UARDG, Dept. of Anesthesiology, UCSD) was used to direct a focused beam of light at the paw (either ipsilateral or contralateral), and measure the latency of response as described by Hargreaves et al. (1988). Rats were placed in pairs in a clear Plexiglass box ($22 \times 19 \times 25$ cm) on top of a temperature maintained glass surface (30 ± 0.1 °C). Groups of six animals were tested at a time. A photoelectric light source was directed at each hind paw,

in random order, and the threshold to withdrawal determined manually or automatically.

2.3.2. Static tactile thresholds

Following weighing and acclimatization to the testing room, rats were placed in pairs in a clear plexiglass box ($30 \times 22 \times 26$ cm) on an elevated wire mesh platform to allow access to the ventral surface of the hind paws. Rats were further acclimated to the testing chambers and the static mechanical withdrawal threshold determined (once every 30 min) using Semmes–Weinstein monofilaments (Stoelting, Wood Dale, IL). The 50% withdrawal threshold was determined using the Dixons up–down method (Chaplan et al., 1994). Briefly, filaments were applied to the ventral surface of the paw, starting with the 4.31 filament (2.04 g) and the response noted. Following a positive response (paw withdrawal, with characteristic pain behavior), a lower filament was then applied. Conversely, a negative response (no response) to a filament would mean application of a higher filament. This pattern of application was repeated until a series of six responses was obtained. The 50% withdrawal threshold was then determined from the tabular value for the pattern of six responses (k), the final monofilament value (X, log units), and interpolated using the formula:

$$50\%\,\text{g threshold} = (10^{[X+k]})/10{,}000$$

where is the mean difference between stimuli.

2.4. Chronic drug administration

As amitriptyline hydrochloride is subject to degradation when in aqueous solution and exposed to light for periods of 3 to 4 days (Buckles and Walters, 1976), drugs were administered in opaque bottles and fresh solutions were given every 48 h.

Five groups of animals were included in this study. One group served as age matched non-ligation controls (naïve). Rats receiving spinal nerve ligation were divided into four groups: no drug (control), caffeine alone, amitriptyline alone, and amitriptyline–caffeine combination. All drugs were dissolved in water normally provided to animals in the Animal Care Centre. Thirty minutes prior to the spinal nerve ligation surgery, animals were injected with drug (i.p.) based on their assigned treatment group of caffeine (7.5 mg/kg), amitriptyline (10 mg/kg), or a combination of amitriptyline (10 mg/kg) and caffeine (7.5 mg/kg). Control rats were given a systemic injection (i.p.) of saline in an equivalent volume to the drug treatment groups. The animals then underwent spinal nerve ligation surgery. Following surgery, animals received a subsequent i.p. administration of drug based on the respective assignment group. After an initial observation period, the animals were housed individually for 48 h to allow for initial wound healing. Animals were then paired back with their original cagemate in an enriched environment for the duration of the experiment. The experimenter responsible for assessment of thermal thresholds and static tactile mechanical thresholds was blinded to the treatment groups.

2.4.1. Measurement of weight, fluid consumption and drug concentration

Handling of animals was limited to the experimenters during the study. Every 48-h, the animals were weighed and fluid levels measured and changed. The dose of drug consumed by each rat over the 48-h period was estimated by determining the volume consumed, multiplying the concentration of the drug in the water by the individual volume of fluid, and dividing by the weight of the rat.

2.5. Data analysis

The raw values of withdrawal thresholds of the paws for thermal (s) and tactile (grams) stimuli were recorded and the values were expressed as mean ± standard error of the mean (S.E.M.). Comparisons between groups for the specific measure (ipsilateral or contralateral paw withdrawal thresholds) were made using an analysis of variance (ANOVA) with Bonferroni's method of post hoc correction for thermal hyperalgesia and with Mann–Whitney Rank Sum for allodynia.

3. Results

3.1. Weight gain, fluid consumption, and drug dosing

No statistical significance was observed between any of the groups in terms of the average weight gain or fluid

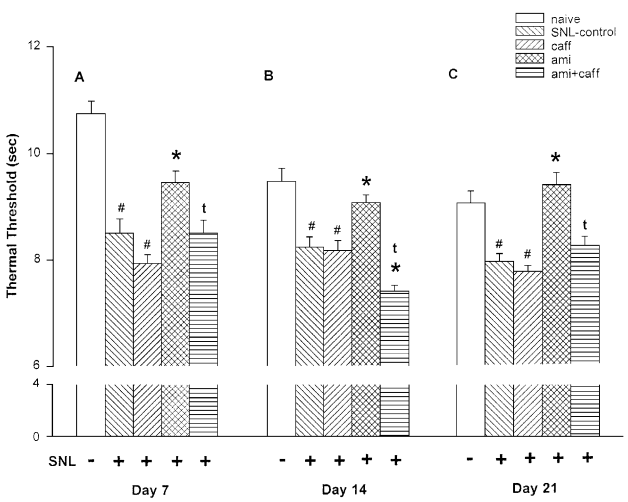

Fig. 1. Histograms depicting the thermal withdrawal thresholds of the ipsilateral (ligated) paw at 7 (A), 14 (B) and 21 (C) days following unilateral spinal nerve ligation (SNL) compared to a naïve group, and effects of chronic drug administration on such thresholds. Naïve and SNL-control groups had normal drinking water during the 21-day assessment period. At each of the time points, the histograms depict the mean + S.E.M. for each group ($n = 4$ for naïve, $n = 6$ for control, $n = 8$ for caffeine (caff), amitriptyline (ami) and amitriptyline + caffeine (ami + caff)) for this and each subsequent figure. $^{\#}P < 0.05$ compared to naïve; $^{*}P < 0.05$ compared to SNL-control; t: $P < 0.05$ compared to amitriptyline.

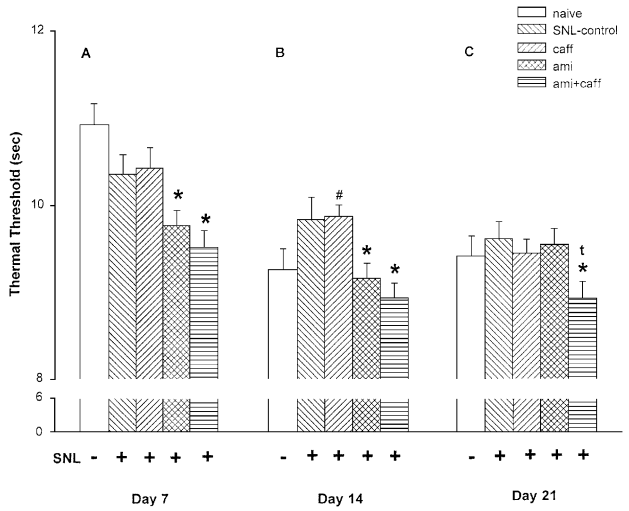

Fig. 2. Histograms depicting the thermal withdrawal thresholds of the contralateral (non-ligated) paw at 7 (A), 14 (B) and 21 (C) days following unilateral spinal nerve ligation (SNL) compared to a naïve group, and effects of chronic drug administration. See legend to Fig. 1 for further details. $^{\#}P < 0.05$ compared to naïve; $^{*}P < 0.05$ compared to SNL-control; *t*: $P < 0.05$ compared to amitriptyline.

consumption at any of the time points. All groups gained weight at approximately the same rate (5 g/day). The approximate dose of drug received by the respective groups based on the weight, fluid consumption, and initial concentrations was 15–18 mg/kg/day of amitriptyline and 6–8 mg/kg/day of caffeine, irrespective of whether the drugs were administered alone or in combination.

3.2. Thermal withdrawal thresholds

In the *ipsilateral* paw, the spinal nerve ligated group had thermal paw withdrawal thresholds that were significantly lower than those observed in naïve animals at all time points (indicated by #; Fig. 1A–C). Caffeine-treated animals also had response thresholds significantly lower than naïve animals at all time points, and these were no different from the nerve-ligated group (#; Fig. 1A–C). Amitriptyline-treated animals exhibited a lesser reduction in ipsilateral paw response thresholds at all three time points compared to the spinal nerve ligation group (*; Fig. 1A–C). The amitriptyline–caffeine combination group had withdrawal thresholds that were lower than those of the amitriptyline group at all time points (*t*; Fig. 1A–C), indicating blockade of the action of amitriptyline throughout this time course by the coadministration of caffeine.

In the *contralateral* paw, thermal threshold values were more variable. However, the spinal nerve ligated group had contralateral paw thermal withdrawal thresholds that did not differ from those of the naïve group at any time point (Fig. 2A–C). The chronic caffeine group had thresholds that were generally no different from those of the naïve group, although at day 14 values were slightly elevated (#; Fig. 2B). Chronic amitriptyline-treated animals had lower thresholds compared to spinal nerve ligation controls at days 7 and 14 (*; Fig. 2A,B). The amitriptyline–caffeine combination group had contralateral paw withdrawal thresholds lower than those of the spinal nerve ligation group at all time points (*; Fig. 2A–C), and lower than the amitriptyline group at day 21 (*t*; Fig. 2C).

3.3. Static tactile withdrawal thresholds

In the *ipsilateral* paw, all experimental groups had significantly lower static tactile withdrawal thresholds

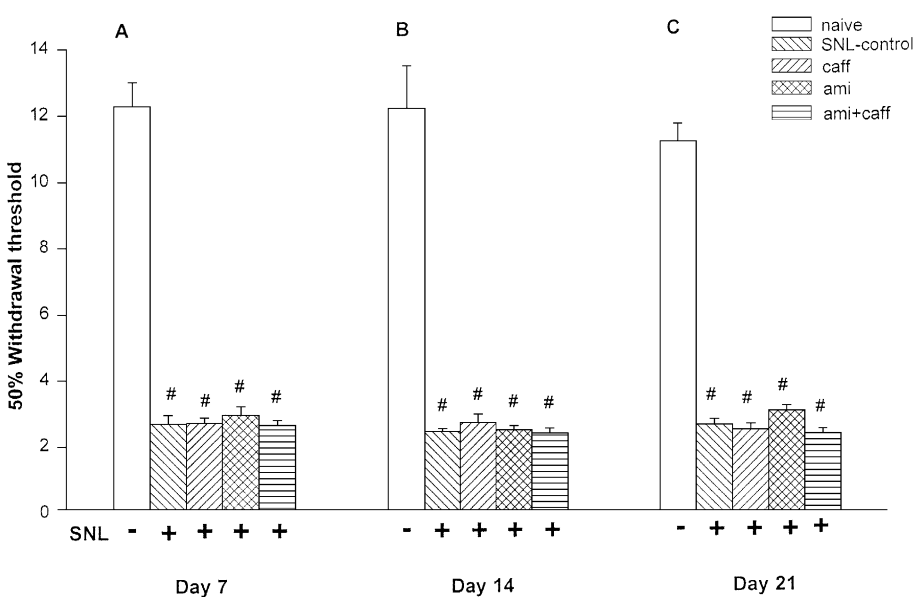

Fig. 3. Histograms depicting the tactile withdrawal thresholds of the ipsilateral (ligated) paw at 7 (A), 14 (B) and 21 (C) days following unilateral spinal nerve ligation (SNL) compared to a naïve group, and effects of chronic drug administration. See legend to Fig. 1 for further details. $^{\#}P < 0.05$ compared to naïve.

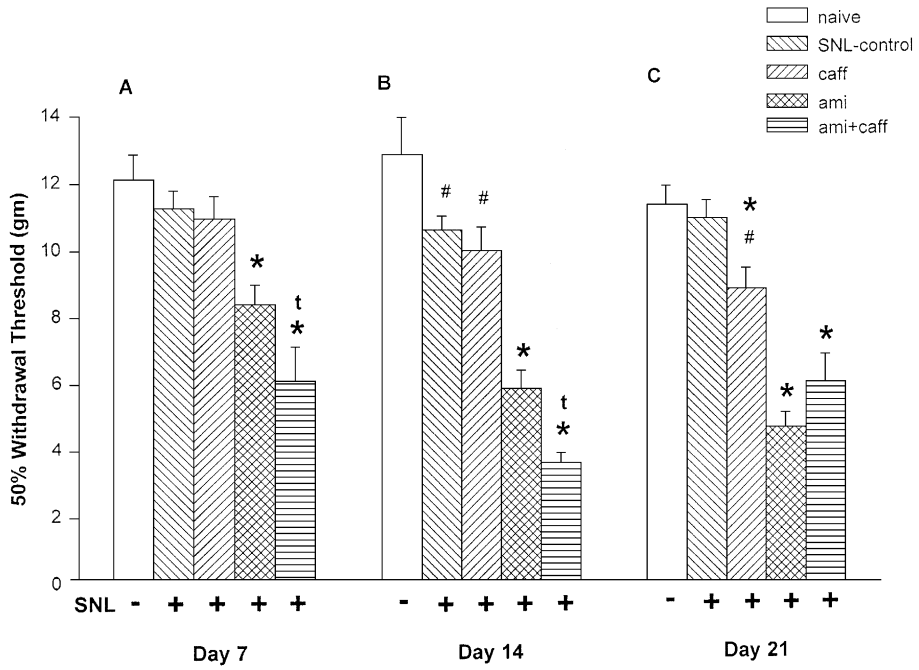

Fig. 4. Histograms depicting the tactile withdrawal thresholds of the contralateral (non-ligated) paw at 7 (A), 14 (B) and 21 (C) days following unilateral spinal nerve ligation (SNL) compared to a naïve group, and effects of chronic drug administration. See legend to Fig. 1 for further details. $^{\#}P < 0.05$ compared to naïve; $^{*}P < 0.05$ compared to SNL-control; t: $P < 0.05$ compared to amitriptyline.

compared to naïve rats at all time points (#; Fig. 3), and there were no statistically significant differences between any of the experimental groups.

In the *contralateral* paw, the static tactile mechanical withdrawal threshold for the spinal nerve ligation control was lower than that of the naïve group at day 14 only (#; Fig. 4B), and this reduction was quite modest. In the chronic caffeine group, thresholds were significantly lower than those of the naïve group at days 14 and 21 (#; Fig. 4B,C), and lower than the spinal nerve ligation group at day 21 (*; Fig. 4C), and these changes were also modest. The chronic amitriptyline group thresholds were significantly lower than those of the spinal nerve ligation control group at all time points (*; Fig. 4A–C), and this effect was quite prominent at days 14 and 21. The amitriptyline–caffeine combination group thresholds also were significantly lower than the spinal nerve ligation group at all time points (*; Fig. 4A–C), and significantly lower than those of the chronic amitriptyline group at days 7 and 14 (t; Fig. 4A,B). These results indicate that chronic amitriptyline produces a contralateral hyperesthesia following spinal nerve injury, and that caffeine augments the contralateral hyperaesthesia produced by amitriptyline.

4. Discussion

The purpose of this study was to determine whether chronic administration of amitriptyline, by inclusion in the drinking water, was effective in alleviating symptoms of neuropathic pain in a rat model of spinal nerve injury, and whether this effect could be altered by coadministration of caffeine. The main findings are that (1) chronic administration of amitriptyline alleviates thermal hyperalgesia, but not static tactile allodynia, in the ipsilateral paw following spinal nerve ligation, (2) the effect of amitriptyline on thermal hyperalgesia in the ipsilateral paw is blocked by chronic administration of caffeine, (3) chronic administration of amitriptyline causes tactile hyperaesthesia in the contralateral paw following spinal nerve ligation, and (4) amitriptyline-induced contralateral hyperesthesia is exacerbated by combination with caffeine. Given that responses to thermal stimulation appear to result from peripheral and central processing initially mediated via C-fibers, while tactile allodynia arises from Aβ- and Aδ-fibres (Woolf and Doubell, 1994; Field et al., 1999), these results suggest that amitriptyline may be more effective in alleviating manifestations of nerve injury mediated by C- rather than A-fibers. Chronic amitriptyline administration may actually cause an untoward effect on some aspects of tactile physiology following nerve injury (cf. contralateral hyperaesthesia). The present results also indicate that part of the analgesic efficacy of chronic amitriptyline administration following nerve injury involves endogenous adenosine. The ability of caffeine to essentially abolish the beneficial anti-hyperalgesic action of amitriptyline, while augmenting a potential adverse consequence (contralateral hyperaesthesia), suggests that some caution may need to be exercised

in patients using amitriptyline for the treatment of neuropathic pain who regularly consume caffeine from various sources. This possibility needs to be verified in controlled clinical trials.

In this study, we found that chronic amitriptyline administration (15–18 mg/kg/day) alleviated the thermal hyperalgesia that normally occurs in the ipsilateral paw following spinal nerve ligation. This effect was observed over the complete 21-day observation period, and no waning of effect was observed throughout this interval. Acute administration of amitriptyline had previously been shown to produce a thermal anti-hyperalgesic action in this model (Esser and Sawynok, 1999). Thermal thresholds following nerve injury are mediated by C-fibres (Shir and Seltzer, 1990; Field et al., 1999; Ossipov et al., 1999). Mechanisms potentially involved in the relief of thermal hyperalgesia by acutely administered amitriptyline are considered elsewhere, and include block of the uptake of noradrenaline and 5-HT, block of NMDA receptors, interactions with endogenous opioid systems and block of histamine, cholinergic or serotonergic receptors or cation channels (Esser and Sawynok, 1999). Chronic administration of antidepressants downregulates β-adrenoceptors, $GABA_B$ and $5\text{-}HT_{2A}$ receptors, decreases functional activity of $5\text{-}HT_{1A}$, $5\text{-}HT_{2A}$, α_2-adrenoceptors and dopamine autoreceptors, upregulates α_1-adrenoceptors, modulates NMDA receptors, and upregulates intracellular signalling involving the cyclic AMP cascade and BDNF (reviewed Blier and De Montigny, 1995; Leonard, 1996; Duman et al., 1997; Skolnick, 1999). Many of these chronic actions may potentially modify systems that are involved in pain processing following nerve injury. However, it is difficult to ascertain a specific contribution of such actions to the relief of neuropathic symptoms when the effects of chronic amitriptyline administration are qualitatively the same as the effect of acute amitriptyline administration.

In contrast to hyperalgesia, chronic amitriptyline did not have any effect on the nerve injury-induced reduction in static tactile thresholds (allodynia) in the ipsilateral paw. It did, however, produce a reduced tactile threshold in the contralateral paw following nerve injury. The hyperesthetic response to tactile stimulation in the contralateral hindpaw was significant at day 7, and was even more pronounced at days 14 and 21. This response had previously been noted following acute administration of amitriptyline (Esser and Sawynok, 1999). There was also some contralateral hypersensitivity to the thermal stimulus at days 7 and 14 with amitriptyline, which had not been observed following acute administration. While this was statistically significant, contralateral thermal thresholds were in general more variable than other responses. It is important to note that the response to tactile stimulation in the contralateral paw is qualitatively different from that seen in the ipsilateral paw, in that the response involved a brisk withdrawal from the stimulus without the secondary behavioural responses associated with allodynia (biting, licking and guarding of the paw). Static tactile mechanical allodynia following spinal nerve ligation is mediated by Aδ-fibres, while dynamic mechanical allodynia is mediated by Aβ-fibres (Field et al., 1999; Ossipov et al., 1999). Peripheral nerve injury is recognized to produce changes that affect the contralateral, non-lesioned side, and these are thought to be mediated by the actions of various growth factors (Koltzenburg et al., 1999). The more prominent expression of the hyperaesthesia at later time intervals would be consistent with chronic amitriptyline administration enhancing BDNF (Duman et al., 1997), which then contributes to the contralateral response, as BDNF can contribute to hypersensitivity responses (Mannion et al., 1999). Whether there is a direct clinical correlate to this observation following chronic amitriptyline administration in humans would need to be determined by the use of quantitative sensory testing which discriminates between static and dynamic mechanical allodynia (Ochoa and Yarnitsky, 1993) in a condition involving a clear unilateral nerve injury.

A significant observation from the present study is that chronic administration of caffeine can change the expression of the effects of chronic amitriptyline following spinal nerve ligation, with differing effects on thermal hyperalgesia versus tactile allodynia, and in the ipsilateral versus contralateral paws. Thus, caffeine blocks the beneficial effect of amitriptyline on thermal hyperalgesia in the ipsilateral paw, and this block is relatively complete at all time intervals. There was no intrinsic effect of caffeine on thermal hyperalgesia at any time interval. Caffeine block of the ipsilateral thermal antihyperalgesic action of amitriptyline was observed previously when both drugs were administered acutely (Esser and Sawynok, 2000). In the contralateral paw, caffeine combination with amitriptyline augmented the contralateral tactile hyperaesthesia at 7 and 14 days. At 21 days, caffeine itself had a slight effect on static tactile thresholds, and facilitated the contralateral thermal hyperresponsiveness produced by amitriptyline. The ability of chronic caffeine to attenuate some actions of amitriptyline (ipsilateral thermal hyperalgesia) but to augment others (contralateral tactile hyperaesthesia) at the same time suggests that the amitriptyline–caffeine interaction is based on pharmacodynamic rather than pharmacokinetic mechanisms.

At low doses, caffeine acts primarily by antagonism at adenosine receptors, while at higher doses, it exerts additional pharmacological actions such as inhibition of phosphodiesterase (Fredholm, 1995). The doses of caffeine used in this study are quite modest, and it is likely that an amitriptyline–adenosine interaction accounts for much, but perhaps not all, of the effects of caffeine. Previous studies had reported that acute administration of methylxanthines could block the acute analgesic actions of antidepressants given systemically in nociceptive (Pareek et al., 1994; Sierralta et al., 1995), inflammatory (Sawynok et al., 1999) and neuropathic (Esser and Sawynok, 2000) pain models. Antidepressants inhibit the uptake of adenosine in neuronal

preparations (Phillis and Wu, 1982), and enhance the ability of adenosine to inhibit neuronal activity in the cortex (Stone and Taylor, 1979; Phillis, 1984) and hippocampus (Zahorodna and Bijak, 1999). A recent study has demonstrated that amitriptyline can increase the peripheral availability of adenosine following spinal nerve injury (Liu et al., 2000). Collectively, these observations indicate that antidepressants in general, and amitriptyline in particular, can enhance the availability and/or activity of adenosine. Adenosine has been shown to alleviate manifestations of neuropathic pain in a number of preclinical nerve injury models (Sjölund et al., 1996; Lee and Yaksh, 1996; Cui et al., 1997), as well as in clinical studies (reviewed Segerdahl and Sollevi, 1998), such that the enhanced availability/action of adenosine could well be a significant component of the beneficial effect of amitriptyline in neuropathic pain. Chronic administration of caffeine can produce additional effects such as upregulation of adenosine A_1, 5-HT, cholinergic and $GABA_A$ receptors, downregulation of α-adrenoceptors and alterations in Ca^{2+} channel function (Shi et al., 1993). While these actions could potentially contribute to amitriptyline–caffeine interactions when these agents are given chronically, the amitriptyline/adenosine analgesia hypothesis appears likely, since the amitriptyline–caffeine interaction occurs both acutely and chronically.

Despite the attractiveness of the above hypothesis, there are some elements to the interpretation that are puzzling. Thus, exogenous or endogenous adenosine is effective in alleviating static mechanical allodynia in nerve injury models, in particular the spinal nerve ligation model used in this study (Lee and Yaksh, 1996; Lavand'homme and Eisenach, 1999), yet amitriptyline does not alleviate this particular manifestation in this or a previous study using an acute administration paradigm (Esser and Sawynok, 1999). The ability of caffeine to augment the facilitatory effect of amitriptyline on contralateral tactile thresholds also appears to run counter to this interpretation. However, it should be noted that in some experiments, spinal application of adenosine analogs can facilitate responses evoked by Aδ fibres, while at the same time suppressing C-fibre activity (Reeve and Dickenson, 1995), suggesting that the actions of adenosine on mechanisms involved in chronic pain may be more complex than is currently appreciated. Both amitriptyline and caffeine have multiple components to their actions, especially following chronic administration, and it appears that these are still incompletely understood.

From a clinical standpoint, while the results of this study extend those of previous studies demonstrating a symptom-related effect of amitriptyline on stimulus-evoked pain following peripheral nerve injury, they are even more relevant in that they involve the non-invasive chronic oral administration of amitriptyline which parallels the human situation. The finding that chronic amitriptyline appears to be effective in alleviating pain symptoms mediated through C-fibres (thermal hyperalgesia), while it is not effective in alleviating neuropathic pain mediated through Aδ fibres (static mechanical allodynia), is in line with the hypothesis that different types of neuropathic pain symptoms may be mediated by different mechanisms (Woolf and Decosterd, 1999; Field et al., 1999; Baron, 2000). A differential symptom-related effect of chronic amitriptyline may help to explain why the degree of pain relief that occurs with amitriptyline in treating neuropathic pain is only partial (MacFarlane et al., 1997). Thus, patients presenting with predominant symptoms mediated by C-fibres (e.g., hyperalgesia) may be the ones most likely to receive benefit from amitriptyline. However, since patients more often present with a composite of symptoms (e.g., hyperalgesia, allodynia and spontaneous pain) involving different mechanisms, the effect of amitriptyline may appear only partial if symptoms mediated by non C-fibre mechanisms are prominent. This study also provides further evidence that the thermal antihyperalgesic effect of amitriptyline may be due to manipulation of endogenous adenosine. The antagonist effect of caffeine may be clinically relevant, as it may also contribute to the limited effectiveness of amitriptyline in neuropathic pain, since caffeine is widely consumed from various sources. The mean daily consumption rate of caffeine can vary widely between countries, but can reach 300–400 mg/day (equivalent to 4–7 mg/kg/day) (Fredholm et al., 1999). While there needs to be caution in the direct extrapolation of caffeine dosages from rodents to humans due to species and kinetic differences, the possibility that chronic caffeine intake limits the benefit derived from amitriptyline in neuropathic pain merits consideration in clinical trials. Finally, there remains a possibility that chronic caffeine intake could actually exacerbate some aspects of amitriptyline actions (cf. contralateral hyperaesthesia), and this might contribute further to limiting the benefit derived from amitriptyline.

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