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Short communication

Rats with chronic post-ischemia pain exhibit an analgesic sensitivity profile similar to human patients with complex regional pain syndrome — type I

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Abstract

Chronic post-ischemia pain was induced in anesthetized rats by placing a tourniquet at the ankle joint for 3 h, and removing it to allow reperfusion. The effectiveness of standard analgesic drugs to attenuate mechanical allodynia was assessed 2 and 7 days after ischemia/reperfusion. Only high doses of morphine, dexamethasone and pregabalin partially reduced mechanical allodynia 2 days post-ischemia/reperfusion, while other treatments (ibuprofen, acetaminophen, amitriptyline) were not effective. Furthermore, only the highest dose of pregabalin reduced mechanical allodynia 7 days post-ischemia/reperfusion. These results are consistent with findings that complex region pain syndrome-I pain is refractory to most standard analgesic treatments.

Keywords: Ischemia; Reperfusion; Analgesia; Inflammation; Neuropathic pain

1. Introduction

Hind limb ischemia reperfusion injury induces well-characterized physiological changes within affected muscles, nerves or skin. During the reperfusion, the synthesis of free radicals and pro-inflammatory cytokines leads to inflammatory responses and vascular injury in the ischemic tissue (for review, see Blaisdell, 2002). Poor blood flow had been also shown in muscles through leukocyte aggregation (Fitzal et al., 2002) and no-reflow (Blaisdell, 2002) in muscle blood vessels.

Despite the extensive literature on ischemia/reperfusion injury, the sensorial aspect had been poorly studied. Twenty minutes ischemia of the tail induces an acute, 1 h-lasting hyperalgesia in rats (Lin et al., 2000). Coderre et al. (2004) showed that a 3 h-ischemia/reperfusion of the hind paw induced long-term (4 weeks) mechanical and cold hypersensitivity (known as chronic post-ischemia pain), which was reduced by free radical scavengers. Mechanical hypersensitivity is also observed after 3 h-occlusion of the femoral artery (Ludwig et al., 2006), although sham rats were also affected.

The features occurring after ischemia/reperfusion injury (vascular injury, chronic ischemia, mechanical hypersensitivity) are similar to those described in human patients with complex region pain syndrome-I. Complex region pain syndrome limbs exhibit increased pro-inflammatory cytokines in blister fluids (Groeneweg et al., 2006), vascular abnormalities (Wasner et al., 1999), leukocytes aggregation (Tan et al., 2005), chronic ischemia (Koban et al., 2003) and small fiber nerve degeneration (Albrecht et al., 2006; Oaklander et al., 2006).

The aims of this study were to assess whether hypersensitivity in rats with chronic post-ischemia pain could be relieved with

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mild analgesic, anti-inflammatory and anti-neuropathic treatments, and to determine whether these rats exhibit a similar analgesic profile as patients with complex region pain syndrome-I.

2. Materials and methods

2.1. Animals

Male Long Evans rats (275–300 g, Charles River, Quebec) arrived 7 days before experiments. All treatments and testing was performed blindly by a single experimenter using a randomized block design. Methods were approved by the Animal Care Committee at McGill University, and conformed to ethical guidelines of the Canadian Council on Animal Care.

2.2. Drugs

Drugs used included sodium pentobarbital (Ceva Santé Animal, Libourne, France), morphine (Sabex, Montreal, QC) and pregabalin (Pfizer/Warner Lambert, Ann Arbor, Michigan), as well as acetaminophen, ibuprofen, dexamethasone, and amitriptyline (all obtained from Sigma-Aldrich, St. Louis, MO).

2.3. Hind paw ischemia/reperfusion

Chronic post-ischemia pain was generated following exposure to prolonged hind paw ischemia/reperfusion. Rats were anesthetized over a 3 h period with a bolus (55 mg/kg, i.p.) and chronic i.p. infusion of sodium pentobarbital for 2 h (27.5 mg/kg/h for two first hours). After induction of anesthesia, a Nitrile 70 Durometer O-ring (O-rings West, Seattle, WA) with 7/32 in. internal diameter was placed around the rat's left ankle joint for 3 h (see Coderre et al., 2004).

2.4. Mechanical sensitivity

The plantar surface of the contralateral then ipsilateral hind paws were tested for mechanical allodynia. Filaments were applied in either ascending (after negative response) or descending (after positive response) force as necessary to determine the filament closest to the threshold of response. Each filament was applied for 10 s or until a flexion reflex occurred. The minimum stimulus intensity was 0.25 g and the maximum was 15 g. Based on the response pattern, and the force of the final filament (5th stimulus after first direction change), the 50% threshold (grams) was calculated as $(10^{[X_f + k\delta]})/10000$ where X_f = value (in log units) of the final von Frey hair used, k = value for the pattern of positive/negative responses and δ = mean difference in log unit between stimuli (here, $\delta = 0.224$, for more detail see Chaplan et al., 1994). Baselines sensitivities were assessed before ischemia/reperfusion injury, and prior to and following several pharmacological treatments. Since the response to the ischemia/reperfusion injury is variable, only animals that exhibited a >30% reduction from baseline of their post-ischemia/reperfusion ipsilateral mechanical threshold were included in the study (82.8% and 74.1% of animals were used 2 and 7 days post-ischemia/reperfusion, respectively).

2.5. Pharmacological trials

Two and seven days after ischemia/reperfusion injury, rats were subjected to pre-treatment von Frey testing, and then received either acetaminophen, ibuprofen, dexamethasone, morphine, amitriptyline and pregabalin, or their vehicles. Acetaminophen was given orally (4 ml/kg) and all other drugs were given i.p. (2 ml/kg). Dosages and post-treatment testing times for each drug were based on established *in vivo* potencies and pharmacokinetics, and are indicated in the figures. Saline vehicle controls were matched for each treatment with the same volume and timing of administration as listed for each respective drug. The same animals were used at 2 and 7 days, after a 5 day wash-out period, and treatments were randomly assigned after the pre-treatment assessment.

2.6. Statistics

All data are plotted as mean 50% von Frey threshold ± S.E.M. The time course of the mechanical sensitivity was analyzed with repeated measures ANOVA followed by a Bonferroni/Dunn post-hoc test comparing each post-drug threshold to its pre-drug value. The delta area under the curve (δAUC), relative to the value before the systemic treatment, was calculated for each group over the period of observation. δAUCs were analyzed with a one-way ANOVA followed by a Fisher's PLSD test.

3. Results

The baseline von Frey thresholds for ipsilateral and contralateral paws were similar (13.9±0.1 g and 14.1±0.1 g, respectively). Mechanical thresholds of the contralateral hind paws were not significantly affected by ischemia/reperfusion injury, (data not shown or included in the analysis; 13.6±0.1 g and 13.9±0.1 g, 2 and 7 days post-ischemia/reperfusion, respectively), unlike the ipsilateral paw which exhibited hypersensitivity (4.5±0.1 g and 5.0±0.1 g, 2 and 7 days post-ischemia/reperfusion, respectively). Values above are calculated for rats from all drug groups, with $n = 228$, $n = 189$, and $n = 169$ for baseline, 2 or 7 days post-ischemia/reperfusion, respectively.

3.1. Anti-inflammatory drugs

The effects of the mild analgesic acetaminophen (A) or the anti-inflammatory drugs (B, ibuprofen; and C, dexamethasone) on von Frey thresholds in the ipsilateral hind paw are depicted in Fig. 1 (left panel: day 2 and right panel: day 7). Significant main effects of time were observed on day 2 ($F_{4,969} = 242.3$, $P < 0.0001$) and day 7 ($F_{4,781} = 195.3$, $P < 0.0001$) post-ischemia/reperfusion for acetaminophen, on day 2 ($F_{4,341} = 85.2$, $P < 0.0001$) and day 7 ($F_{4,421} = 105.3$, $P < 0.0001$) post-ischemia/reperfusion for ibuprofen, and on day 2 ($F_{4,333} = 83$, $P < 0.0001$) and day 7 ($F_{4,399} = 99.9$, $P < 0.0001$) post-ischemia/reperfusion for dexamethasone. However, no main effect of treatment or treatment × time interaction was observed for any of these drugs.

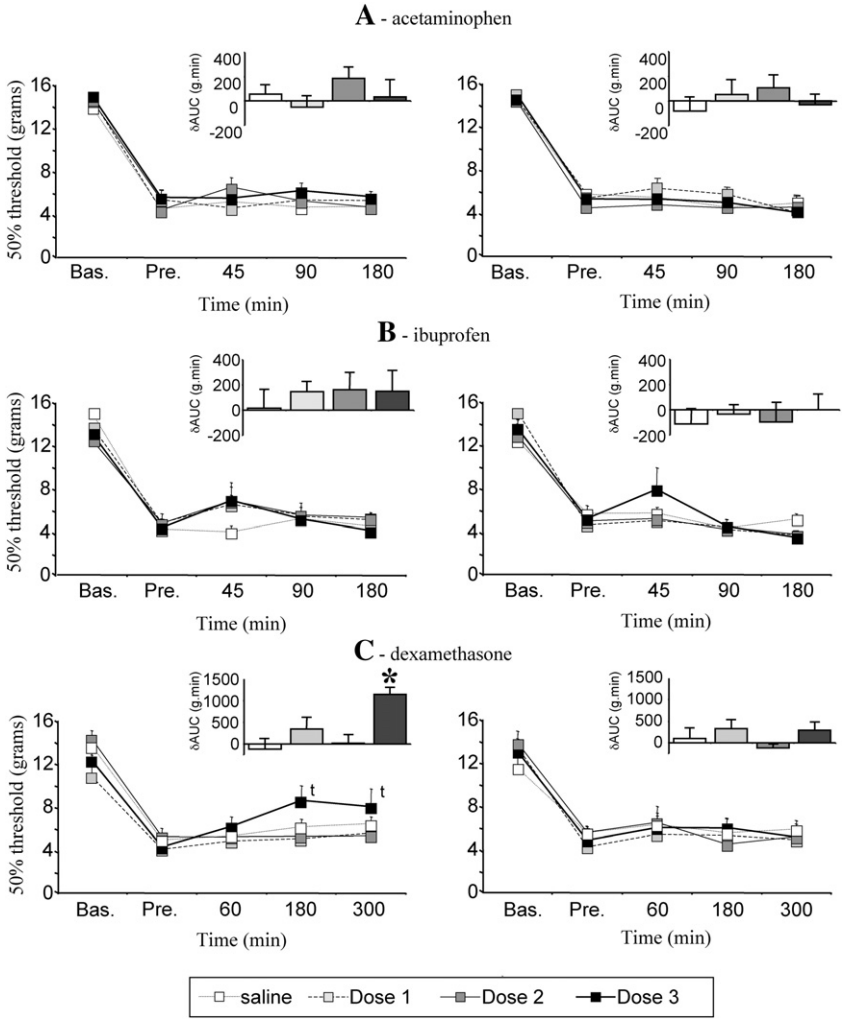

Fig. 1. Mechanical (von Frey) thresholds (grams) were assessed on the ipsilateral hind paw prior to ischemia/reperfusion injury (Bas), pre-drug treatment (Pre) and at various times post-drug treatment, 2 or 7 days after the ischemia/reperfusion-injury (left and right panels, respectively). Animals received (A) saline or acetaminophen (1, 2 and 3 = 100, 200 and 400 mg/kg, respectively), (B) saline or ibuprofen (1, 2 and 3 = 50, 100 and 200 mg/kg, respectively), (C) saline or dexamethasone (1, 2 and 3 = 1, 5 and 10 mg/kg, respectively). The δAUCs (inset histograms) were calculated relative to the mechanical threshold measured pre-treatment (Pre) over the full period of observation. $N = 6$ to 9/group (t: vs. Pre, corrected P-value $P < 0.05$, repeated measures Bonferroni/Dunn test; *: vs. saline, δAUC, $P < 0.05$, Fisher's PLSD).

All post-ischemia/reperfusion von Frey thresholds were significantly lower than baselines for all groups, except with dexamethasone at the highest dose (10 mg/kg, i.p.), 2 days post-ischemia/reperfusion only. Despite the lack of a treatment effect, compared to pre-drug values, von Frey thresholds were increased at 180 and 300 min post-dexamethasone.

Two or seven days post-ischemia/reperfusion, none of the calculated δAUCs showed a significant difference, except for dexamethasone at 2 days post-ischemia/reperfusion ($F_{3,22} = 7.467$, $P < 0.001$): the δAUC for the highest dose (10 mg/kg) was significantly greater than all other treatment groups.

3.2. Anti-neuropathic drugs

The effects of anti-neuropathic (including opioid, monoamine reuptake inhibitor and gabapentinoid) drugs (A, morphine; B, amitriptyline; and C, pregabalin) on von Frey thresholds in the ipsilateral hind paw are depicted in Fig. 2 (left panel: day 2 and right panel: day 7). Significant main effects of time were observed on day 2 ($F_{3,259} = 86.4$, $P < 0.0001$) and day 7 ($F_{3,946} = 315.5$, $P < 0.0001$) post-ischemia/reperfusion for morphine, day 2 ($F_{4,562} = 140.4$, $P < 0.0001$) and day 7 ($F_{4,408} = 102.0$, $P < 0.0001$) post-ischemia/reperfusion for amitriptyline, and day 2 ($F_{4,292} = 73.0$, $P < 0.0001$) and day 7 ($F_{4,227} = 56.7$, $P < 0.0001$) post-ischemia/reperfusion for pregabalin. A significant main effect of treatment was observed for the morphine experiment, 2 days post-ischemia/reperfusion-procedure ($F_{3,9} = 2.9$, $P < 0.05$), and significant time × treatment interactions were obtained for the amitriptyline experiment, 7 days post-procedure ($F_{12,22} = 2.9$, $P < 0.05$), and the pregabalin experiments, 2 and 7 days post-procedure ($F_{12,27} = 2.2$, $P < 0.05$ and $F_{12,22} = 1.8$, $P < 0.05$, respectively).

All post-ischemia/reperfusion von Frey thresholds were significantly lower than baselines for all groups, except for the highest doses of morphine (day 2: 3 mg/kg, 30 min post-treatment) and pregabalin (day 2: 100 mg/kg, 90 and 180 min post-treatment, and day 7: 50 and 100 mg/kg, from 45 min post-treatment).

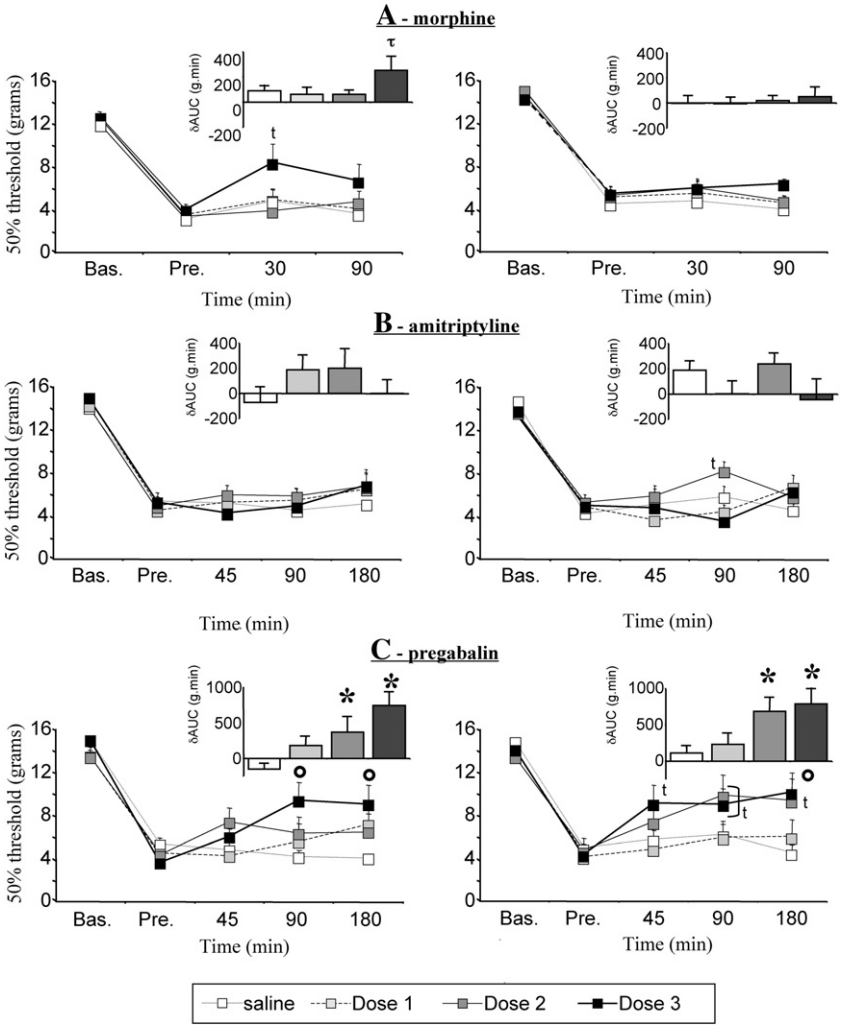

Fig. 2. Mechanical (von Frey) thresholds (grams) were assessed on the ipsilateral paw prior to ischemia/reperfusion (Bas), pre-drug treatment (Pre) and at various times post-drug treatment, 2 or 7 days after ischemia/reperfusion-injury (left and right panels, respectively). Animals received (A) saline or morphine (1, 2 and 3=0.3, 1 and 3 mg/kg, respectively), (B) saline or amitriptyline (1, 2 and 3=3, 10 and 30 mg/kg, respectively), (C) saline or pregabalin (1, 2 and 3=25, 50 and 100 mg/kg, respectively). The δAUCs (inset histograms) were calculated relative to the mechanical threshold measured pre-treatment (Pre) over the full period of observation. N=6 to 9/group. (°: vs. Pre $P<0.01$, t: vs. Pre, $P<0.05$ (repeated measure Bonferroni/Dunn test); *: $P<0.05$, τ (trend): vs. saline δAUC, Fisher's PLSD).

Two or seven days post-ischemia/reperfusion-procedure, none of the calculated δAUCs showed a significant difference, except for the day 2 and 7 pregabalin experiments, with the two higher doses (although a trend was observed for the highest dose of morphine on day 2).

4. Discussion

The results show that after a 3 h-ischemia/reperfusion injury of the hind paw a large majority of rats exhibited mechanical allodynia of the plantar skin, 2 and 7 days after the procedure. Unlike previous studies (Coderre et al., 2004; Ludwig et al., 2006), in the present study we did not observe mechanical allodynia in the contralateral hind paw. This observation could be due to the fact we always tested the contralateral hind paw prior to the ipsilateral hind paw and may have avoided acute spinal sensitization following mechanical stimulation of the hypersensitive hind paw. In rats, we have observed again that ischemia/reperfusion injury induces robust and persistent mechanical allodynia in the ipsilateral hind paw. Since cold hypersensitivity is less robust, and heat hyperalgesia is not observed, in these rats (Coderre et al., 2004), these measures were not examined here.

Systemic treatments with anti-inflammatory drugs did not alleviate the mechanical hypersensitivity at any time tested, except with dexamethasone, at the higher dose and during the earlier stage of the pathology (i.e. 10 mg/kg at 2 days post-ischemia/reperfusion). Previous studies have shown that systemic treatment with anti-inflammatory drugs (indomethacin, diclofenac, ibuprofen, dipyrone and acetaminophen) had no effect on pain behaviour during 20 min of ischemia, but attenuated hyperalgesia during the first hour of reperfusion in rats (Gelgor et al., 1992). Dexamethasone (10 mg/kg) produces a non-specific reduction of inflammatory processes and exhibits anti-nociceptive effects in neuropathic rats (Caram-Salas et al., 2004), without producing motor side effects (which appear at 80 mg/kg, Danilczuk et al., 2005).

Systemic treatments with anti-neuropathic drugs exhibited better effects than anti-inflammatory treatments in rats with chronic post-ischemia pain. Morphine (3 mg/kg) had a slight, but significant anti-allodynic effect 2 days post-ischemia/reperfusion. In rats, intrathecal morphine reduces allodynia after spinal cord ischemia/reperfusion injury (von Heijne et al., 2001), and in humans, experimental ischemic pain is reduced by i.v. morphine (Segerdahl et al., 1994). However, it is well established that morphine is more potent analgesic on inflammatory than neuropathic pain (Przewlocki and Przewlocka, 2001).

Pregabalin (50 and 100 mg/kg) was particularly effective at 2 and 7 days post-ischemia/reperfusion, which is consistent with its effectiveness in various pain conditions. Thus, pregabalin, and its analogue gabapentin, have been shown to be effective in reducing inflammatory (Hanesch et al., 2003), ischemic (Gustafsson et al, 2003) and neuropathic (Hama and Borsook, 2005) pain in various animal models. Finally, amitriptyline was not effective at any time or dose. The lack of effectiveness of amitriptyline is surprising, since it has often been shown to reduce hypersensitivity in neuropathic and inflammatory animal pain models (Bomholt et al., 2005). To our knowledge there is no pre-clinical study of the effectiveness of amitriptyline on ischemic pain.

After a 3 h-ischemia/reperfusion injury, animals exhibited robust, persistent, mechanical allodynia resistant to most treatments we tried. Three distinct painful conditions can be generated after a 3 h-ischemia/reperfusion injury, including: 1) acute inflammation (2 days post-procedure, sensitive to morphine, dexamethasone and pregabalin) when tissues exhibit oxidative injury (Nagamatsu et al., 1996), and a local production of inflammatory mediators (Nukada et al., 2000); 2) a persistent ischemic condition, which is a direct consequence of the ischemia/reperfusion insult to blood vessels, and is evidenced by poor blood flow (Fitzal et al., 2002); and 3) secondary neuropathic injury due to nerve fiber degeneration as a consequence of the poor blood flow in nerves (Nagamatsu et al., 1996). The latest phase of chronic post-ischemia pain (7 days post-ischemia/reperfusion) is probably a combination of these 3 painful conditions. The only drug that typically shows efficacy in these 3 painful conditions, pregabalin, was also the only one to display anti-allodynic effects 7 days post-ischemia/reperfusion.

In complex region pain syndrome-I patients, early glucocorticoid therapy can be useful, whereas non-steroidal anti-inflammatory drugs are typically not effective (Harden, 2005). Analgesics (including opioids) and anticonvulsants have limited effects in complex region pain syndrome-I. Although several controlled trials suggest that opioids may be effective for complex region pain syndrome-I, the only randomized placebo-controlled trial showed no significant differences after 8 days of treatment (Harke et al., 2001). Gabapentin and carbamazepine have a modest beneficial effect (Harden, 2005) in complex region pain syndrome patients. No randomized controlled trials of tricyclic antidepressants have been performed in complex region pain syndrome-I patients (for review see Berthelot, 2006).

Responses of rats with chronic post-ischemia pain to classical pharmacological treatments exhibit a profile very similar to the profile in human complex region pain syndrome-I patients, with an early period sensitive to anti-inflammatory drugs and morphine, and a later period similar to neuropathic pain patients, in which a resistance develops to these treatments. Other studies in our laboratory have shown allodynia in rats with chronic post-ischemia pain is also relieved by early treatment with guanethidine (sympathetic block) or alpha$_1$-adrenergic antagonists (Xanthos and Coderre, unpublished data). The pathology of chronic post-ischemia pain may evolve such that the major problem shifts from inflammation to secondary neuropathic injury produced by persistent nerve ischemia.

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