Preemptive levetiracetam decreases postoperative pain in rats

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Abstract

OBJECTIVES: Preemptive versus therapeutic effects of levetiracetam were investigated in a model of postoperative incisional pain in rats.
METHODS: Levetiracetam (250, 500, and 1000 mg/kg intraperitoneal (i.p.) or morphine (5 mg/kg i.p.) was administered either 1 h before (preemptive administration) or 1 h after (therapeutic administration) incisional surgery to the hind paw of rats. The effects of levetiracetam were evaluated based on thermal hyperalgesia measured by the plantar test.
RESULTS: All preoperatively treated levetiracetam groups showed a significant, dose dependent, increase in paw withdrawal latency. However, post-incisional administration of levetiracetam produced no antihyperalgesic effect at any dose or at any time. In contrast, post-incisional administration of morphine reduced thermal hyperalgesia, while preemptive administration of morphine did not produce any significant antihyperalgesic effects.
CONCLUSION: The present results suggest that levetiracetam might possess preemptive analgesic activity.

INTRODUCTION

It is well established that some anticonvulsants (e.g. carbamazepine, gabapentin) are effective in the treatment of neuropathic pain (Mcquay *et al.*, 2008; Wiffen *et al.*, 2005). Moreover, recent meta-analyses has showed that perioperative administration of gabapentinoids (gabapentin and pregabalin) effectively reduce postoperative pain and opioid consumption (Hurley *et al.*, 2006; Tiippana *et al.*, 2007). Preemptive administration of gabapentin has recently been reported to reduce postoperative pain in patients undergoing surgery (Al Mujadi *et al.*, 2006; Prabhakar *et al.*, 2007; Van Elstraete *et al.*, 2008).

Levetiracetam is a newer, second-generation, broad-spectrum anticonvulsant. It is a structural analogue of piracetam with a very favorable pharmacokinetic and tolerability profile (Carreno, 2007). The mechanism of action of levetiracetam is not well understood. Levetiracetam was found to have unique brain-specific binding sites (the synaptic vesicle protein SV2A) which probably plays a major role in its anti-epileptic properties (Lynch *et al.*, 2004).

The analgesic activity of levetiracetam has not been studied as extensively as that of gabapentin.

Levetiracetam did not show any analgesic effects in the tail flick and hot plate tests, but has been shown to induced antihyperalgesic effects in models of neuropathic pain in rats (Ardid *et al.*, 2003; Beyreuther *et al.*, 2006). Levetiracetam has also been recently reported to reduce anesthetic-induced hyperalgesia in rats (Archer *et al.*, 2007). Data on the analgesic activity of levetiracetam in humans is very limited. Levetiracetam has been shown to significantly increase pain tolerance thresholds to electrical stimulation of the sural nerve in healthy volunteers (Enggaard *et al.*, 2006). Effective therapy of neuropathic pain, with a low incidence of adverse events, has been reported in three case studies (Price, 2004).

Considering the favorable response to perioperative gabapentin in surgery patients and a lack of reports on the effects of levetiracetam on postoperative pain, we thought it was worthwhile to study the perioperative effects of levetiracetam in more detail. Therefore, the aim of the present study was to investigate preemptive versus therapeutic (post-incisional) effects of levetiracetam in a model of postoperative incisional pain in rats.

MATERIALS AND METHODS

Experimental animals

Adult male Wistar albino rats weighing 250–300 g were used (provided by VUFB Konarovice, Czech Republic). The animals were housed under standard laboratory conditions (in a temperature-controlled (22 ± 1 °C) room in groups of 6 with a normal 12 h light/dark cycle). Animals were fed standard rat chow (Pelet, St1; VELAZ, Czech Republic) with water available ad libitum.

All experiments were reviewed and approved by the Committee for Protection of Laboratory Animals of the 3rd Faculty of Medicine, Charles University and were concordant with the IASP Committee for Research and Ethical Issues requirements (Zimmermann, 1983).

Experimental study design

Two experiments were performed: one involving preemptive administration, the other involving therapeutic administration. In both experiments rats were randomly allocated to five experimental groups (6–8 animals per group): 1. control animals – saline 1.0 ml/kg i.p.; 2. levetiracetam – 250.0 mg/kg i.p.; 3. levetiracetam – 500.0 mg/kg i.p.; 4. levetiracetam – 1000.0 mg/kg i.p.; 5. morphine – 5.0 mg/kg i.p. All drugs were applied one hour prior to surgery, in the case of preoperative treatment, or one hour after surgery, in the case of postoperative treatment. The dose of levetiracetam was chosen on the basis of our pilot experiments and with respect to a previously published study involving the effects of levetiracetam on pain in rodents (Ardid, Lamberty, Alloui, Coudore-Civiale, Klitgaard, and Eschalier).

Paw incision

As described by Brennan (Brennan *et al.*, 1996), rats were anesthetized with ketamine 100 mg/kg i.p. and xy-lazine 10 mg/kg i.p. and a 1.0 cm longitudinal incision was made through skin, fascia and muscles of the plantar surface of the right hind paw; starting 0.5 cm from the proximal edge of the heel and extending toward the toes. All tendons remained intact. The skin was then sutured closed and animals were allowed to recover in their cages.

Paw withdrawal testing

The response to a noxious thermal stimulus was determined using a thermal plantar device (Plantar test 7371, Ugo Basile, Italy), according to the procedure described by Hargreaves (Hargreaves *et al.*, 1988). Rats were placed into transparent plastic chambers (22 cm wide x 17 cm long x 14 cm high) for 15 minutes prior to the start of the each experiment to allow the animal to acclimate to their new environment before testing. The animals were without restraint. A movable infrared radiant heat source (maintained at 60 mW) was placed directly under the plantar surface of the hind paw and the reaction time necessary to elicit licking of the hind paw was measured and taken as the nociceptive threshold. A cut-off time of 15 s was used in all experiments. Following baseline measurements, the incision to the right hind paw was made. At 2, 6, 12, and 24 hours after surgery, paw withdrawal latencies (PWL) were once again measured in these animals.

Data analysis

Statistical analysis was carried out using two-way repeated measures ANOVA (analysis of variance) with a post-hoc Bonferroni's *t-test*. All results are expressed as mean values ± SEM (standard error of mean). Significance was accepted at the 0.05 level.

RESULTS

Preoperative (preemptive) treatment

A two-way repeated measures ANOVA, with treatment as one factor and time after surgery as the second factor, showed a significant treatment effect (F 4.199 = 2.710, $p = 0.046$) and time after the surgery (F 4.199 = 27.997, $p < 0.001$) on the PWL. The subsequent Bonferroni's *t-test* showed a statistically significant decrease of PWL in the control and morphine treated rats at 6, 12, and 24 hours after surgery (t = 3.996, $p < 0.001$, t = 7.595, $p < 0.001$, t = 3.268, $p = 0.014$ for the control and t = 4.005, $p < 0.001$, t = 4.699, $p < 0.001$, t = 3.212, $p = 0.016$ for morphine treated rats). A significant decrease in the PWL was also seen in rats treated with the lowest dose of levetiracetam (250 mg/kg) at 6 and 12 hours after surgery (t = 3.467, $p = 0.07$ and t = 3.992, $p < 0.001$). Treatment with 500 and 1000 mg/kg of levetiracetam completely restored PWL when compared to baseline values and no decrease was observed at 6 and 12 hours after surgery. The only significant decrease in PWL was seen in the 1000 mg/kg-treated group at 24 hours post-surgery (t = 3.167, $p = 0.019$) (Table 1). When compared

Table 1.

Preemptive treatment

	Baseline	2 hours	6 hours	12 hours	24 hours
Control	10.36	8.15	6.77***	4.94***	7.86*
S.E.M. (n = 9)	0.34	0.65	0.45	0.76	1.21
Morphine 5 mg/kg	9.28	8.54	5.44***	5.04***	6.05*
S.E.M. (n = 6)	0.27	0.31	0.37	0.46	0.34
Levetiracetam 250 mg/kg	9.73	9.16	7.18**	6.84***	8.16
S.E.M. (n = 12)	0.54	0.59	0.69	0.65	0.92
Levetiracetam 500 mg/kg	9.98	8.97	8.04	7.07	6.83
S.E.M. (n = 6)	0.56	0.26	0.36	0.62	0.21
Levetiracetam 1000 mg/kg	10.95	9.12	7.80	7.77	7.49*
S.E.M. (n = 7)	0.84	0.74	0.52	0.28	0.80

The latency of paw withdrawal (s) in levetiracetam (250, 500 and 1000 mg/kg i.p.) and morphine (5 mg/kg) preemptively treated rats; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ for the difference between baseline and time after surgery, a two-way repeated measures ANOVA with a post-hoc Bonferroni's t-test.
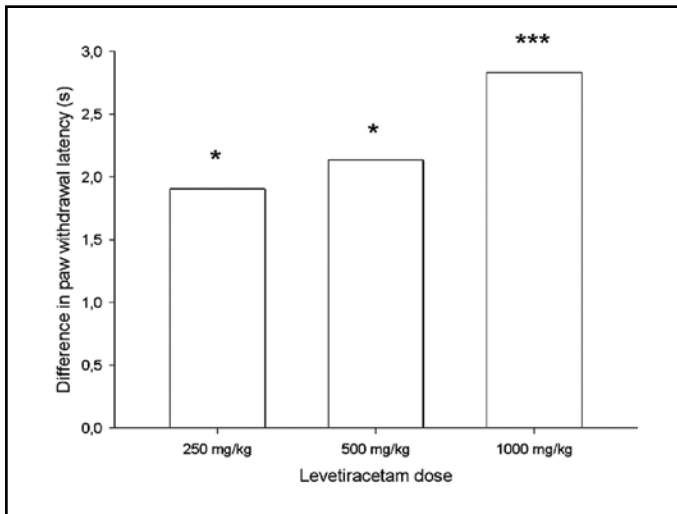

Figure 1. The dose-dependent increase of paw withdrawal latency in levetiracetam-pretreated rats compared to control rats 12 hours after surgery. The ordinate shows paw withdrawal latency expressed as the difference between levetiracetam-pretreated rats and control rats; *$p < 0.05$, ***$p < 0.001$ when compared to control rats.

with the control group of rats, significant differences in PWL were found 12 hours after surgery. All levetiracetam pretreated groups of rats showed significantly longer PWL (t = 3.305, $p = 0.012$ for the 250 mg/kg, t = 3.218, p = 0.016 for the 500 mg/kg and t = 4.188, $p < 0.001$ for the 1 000 mg/kg of levetiracetam) (Figure 1).

Postoperative (therapeutic) treatment

A two-way repeated measure ANOVA, with treatment as one factor and time after the surgery as the second factor, showed a significant treatment effect (F 4.179 = 3.371, $p = 0.021$), time after the surgery (F 4.179 = 30.278, $p < 0.001$) and [the interaction treatment × time after the surgery] (F 16.179 = 4.213, $p < 0.001$) of the PWL. The subsequent Bonferroni's *t-test* showed a statistically significant decrease in the PWL in the control rats at all time points (t = 7.577, $p < 0.001$, t = 7.844, $p < 0.001$, t = 6.416, $p < 0.001$ and t = 4.175, p < 0.001

at 2, 6, 12, and 24 hours after surgery, respectively). Morphine completely restored PWL with no significant differences between the baseline value and those after surgery. A significant decrease in the PWL was seen in rats treated with all doses of levetiracetam at 2 hours (t = 4.049, $p < 0.001$, t = 5.093, $p < 0.001$, and t = 3.342, $p = 0.011$ for 250, 500, and 1000 mg/kg, respectively), 6 hours (t = 2.863, $p = 0.049$, t = 5.264, $p < 0.001$, and t = 4.388, $p < 0.001$ for 250, 500, and 1 000 mg/kg, respectively), 12 hours (t = 3.497, p = 0.007, t = 4.273, $p < 0.001$, and t = 4.167, $p < 0.001$ for 250, 500, and 1 000 mg/kg, respectively) and 24 hours (t = 2.923, $p = 0.041$, and t = 2.995, $p = 0.033$ for 250, and 1000 mg/kg, respectively) after surgery. There were no statistically significant differences in the PWL between the control and levetiracetam-treated rats at any of the time points (Table 2).

Table 2.

Postoperative treatment

	Baseline	2 hours	6 hours	12 hours	24 hours
Control	10.65	5.37***	5.18***	6.18***	7.74***
S.E.M. (n = 8)	0.23	0.54	0.62	0.56	0.55
Morphine 5 mg/kg	9.06	11.07	7.33	7.45	7.57
S.E.M. (n = 6)	0.71	0.60	0.75	0.66	0.60
Levetiracetam 250 mg/kg	8.96	5.94***	6.83*	6.36**	6.78*
S.E.M. (n = 7)	0.83	0.48	0.44	0.24	0.44
Levetiracetam 500 mg/kg	9.75	5.96***	5.83***	6.57***	7.76
S.E.M. (n = 7)	0.86	0.60	0.24	0.36	0.58
Levetiracetam 1000 mg/kg	9.01	6.68*	5.95***	6.11***	6.93*
S.E.M. (n = 8)	1.09	0.47	0.34	0.40	0.33

The latency of paw withdrawal (s) in levetiracetam (250, 500 and 1000 mg/kg i.p.) and morphine (5 mg/kg) postoperatively treated rats; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ for the difference between baseline and time after surgery, a two-way repeated measures ANOVA with a post-hoc Bonferroni's t-test.

DISCUSSION

The preemptive administration of levetiracetam significantly and dose dependently inhibited postoperative hyperalgesia to a thermal stimulus, measured by paw withdrawal latency, after incisional hind paw surgery in rats; while morphine, administered preemptively, had almost no effect. Conversely postoperative (therapeutic) administration of levetiracetam did not reduce postoperative hyperalgesia while morphine was effective when given postoperatively.

The present results support the supposition that preemptive analgesia, at least at the level of preclinical trials, is possible. The concept of preemptive analgesia emerged in the early 1980s, when the important role of the central nervous system, in the development of hyperalgesia, was first stressed (Woolf, 1983). However, the utility of preemptive analgesic interventions in managing acute postoperative pain is still under discussion and is often a matter of debate. The conclusion of an earlier meta-analysis of preemptive analgesia for postoperative pain relief, which reviewed a wide range of treatment modalities (nonsteroidal anti-inflammatory drugs or paracetamol, opioids, epidural analgesia, caudal blocks), was negative with regard to the potential beneficial effects of pre-emptive analgesia on post-operative pain (Moiniche *et al.*, 2002). The conclusions of another, more recently published meta-analysis (Ong *et al.*, 2005) assessing efficacy of preemptive analgesia for acute postoperative pain management in five types of analgesic interventions (epidural analgesia, local anesthetic wound infiltration, systemic N-methyl-D-aspartic acid receptor antagonists, systemic nonsteroidal anti-inflammatory drugs, and systemic opioids), was also not particularly encouraging.

However, even more recent meta-analyses (Hurley, Cohen, Williams, Rowlingson, and Wu; Tiippana, Hamunen, Kontinen, and Kalso) have shown evidence that perioperative gabapentinoids effectively reduce postoperative pain, opioid consumption, and opioid-related adverse effects after surgery. Several recent studies reported that preemptive administration of gabapentin reduced postoperative pain and morphine demand in patients undergoing surgery (Al Mujadi, Refai, Katzarov, Dehrab, Batra, and Al Qattan; Prabhakar, Arora, Bithal, Rath, and Dash; Van Elstraete, Tirault, Lebrun, Sandefo, Bernard, Polin, Vally, and Mazoit). Thus, it seems that preemptive analgesia can be effective at least with certain anticonvulsants.

Apart from selection of the appropriate drug, immediate action of the drug, from the beginning of the surgical noxious input and during early development of hyperalgesia, may also be important. The major difference in terms of time of action between effective preemptive and ineffective therapeutic levetiracetam treatment in the present study was a period of 60 minutes relative to the incision; pretreatment 60 minutes before surgery, levetiracetam, with a half-life of several hours, had time to affect development of hyperalgesia, in contrast to postoperative treatment given 1 hour after surgery. Thus, early spontaneous afferent activity appears to be the trigger of neuropathic pain in rats and effective pre-emptive analgesia can be achieved only when the nerve block was administered shortly after injury (Xie *et al.*, 2005). An increased production of cytokines starts at the time of tissue injury and pre-emptive epidural analgesia has been shown to attenuate production of proinflammatory cytokines after hysterectomies (Beilin *et al.*, 2003).

In our experiments, we did not observe any signs of adverse effects of levetiracetam, even at the highest dose. In another study (Klitgaard *et al.*, 1998), levetiracetam produced a minor reduction in spontaneous activity and abdominal tone in rats, but only at the highest dose tested (1700 mg/kg, i.p.) while doses impairing rotarod performance in 50% of rats were within the range of 1060–1119 mg/kg i.p. Even though the highest dose of levetiracetam in our experiment is very close to this value, preemptive analgesia was produced at lower doses as well. Moreover, we do not believe that the longer latencies in the paw withdrawal reflex in rats, preemptively treated with the highest dose of levetiracetam, were due to levetiracetam toxicity, since the PWL in rats given the same dose of levetiracetam postoperatively were similar to the PWL of control rats.

The lack of preemptive effects of morphine may be due to a different mechanism of action, as well as due to different pharmacokinetic properties when compared to levetiracetam (especially the shorter half-life of morphine).

In conclusion, levetiracetam administered prior to surgery, significantly reduced the pain response, as measured with the plantar test, in rats; and the effect was dose-dependent. In contrast, the same doses of levetiracetam administered postoperatively were ineffective. Further studies are warranted to assess the preemptive analgesic effects of levetiracetam.

Acknowledgement

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