Pregabalin enhances the antinociceptive effect of oxycodone and morphine in thermal models of nociception in the rat without any pharmacokinetic interactions

Original Article
Running head: Pregabalin and opioid interaction in the rat

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What is already known about this topic?

- Gabapentin potentiates the antinociceptive effect of morphine
- Co-use of gabapentinoids and opioids is associated with increased mortality in opioid dependent individuals

What does this study add?

- Pregabalin potentiates the antinociceptive effects of both oxycodone and morphine in acute nociception
- Pregabalin does not increase the brain concentrations of either oxycodone or morphine
- Co-administration of pregabalin does not inhibit the development of tolerance to morphine

Number of words in Abstract 241 (250) in Introduction 497 (500) and in Discussion 1201 (1500)

Abstract

**Background:** Oxycodone is increasingly being used in combination with pregabalin. Pregabalin use is prevalent in opioid dependent individuals. A high number of deaths caused by the co-use of gabapentinoids and opioids occur. It is not known whether pregabalin affects concentrations of oxycodone or morphine in the central nervous system.

**Methods:** Effects of pregabalin on acute oxycodone or morphine-induced antinociception, tolerance, and sedation were studied using tail-flick, hot plate and rotarod tests in male Sprague-Dawley rats. Concentrations of pregabalin, opioids and their major metabolites in the brain were quantified by mass spectrometry.

**Results:** In the hot plate test, morphine (2.5 mg/kg, s.c.) caused antinociception of 28% maximum possible effect (MPE), whereas pregabalin (50 mg/kg, i.p.) produced 8–10% MPE. Co-administration of pregabalin and morphine resulted in antinociception of 63% MPE. Oxycodone (0.6 mg/kg s.c.) produced antinociception of 18% MPE, which increased to 39% MPE after co-administration with pregabalin. When pregabalin 10 mg/kg was administered before oxycodone (0.6 mg/kg, s.c.) or morphine (2.5 mg/kg), only the effect of oxycodone was potentiated in the tail-flick and the hot plate tests. Brain
concentrations of the opioids, their major metabolites and pregabalin were unchanged. Pregabalin co-administration (50 mg/kg, i.p., once daily) did not prevent the development of morphine tolerance.

**Conclusions:** Pregabalin potentiated antinociceptive and sedative effects of oxycodone and morphine in acute nociception. Co-administration of pregabalin with the opioids did not affect the brain concentrations of oxycodone or morphine. Pregabalin did not prevent morphine tolerance.

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**Introduction**

Gabapentinoids (gabapentin and pregabalin) are first line analgesics in neuropathic pain (Attal et al., 2010). Neuropathic pain may be difficult to treat and therefore combinations of analgesics that have different mechanisms of action are commonly used (Vranken, 2009). Co-administration of gabapentin with morphine has decreased the doses of both drugs required for adequate analgesia in neuropathic pain (Gilron et al., 2005). A combination of gabapentin and oxycodone in neuropathic cancer pain provided better pain relief and also decreased the adverse effects compared to opioid monotherapy (Keskinbora et al., 2007).

Gabapentinoids are not efficacious enough when used as the sole analgesics in nociceptive pain, but their co-administration with opioids does reduce the consumption of postoperative opioids (Zhang et al., 2011; Pesonen et al., 2011), and gabapentinoids potentiate opioid analgesia in acute pain (Eckhardt et al., 2000). Gabapentinoids are therefore increasingly used as a constituent of combination therapy in acute post-operative pain (Tiippana et al., 2007). However, clinical trials do not provide insight into the nature of the pharmacological interactions between these two drug classes.
When using high doses of opioids for long periods of time opioid tolerance may compromise analgesia. The possible effect of co-administration of pregabalin on the development of morphine tolerance is therefore a relevant question. Gabapentinoids also have anxiolytic effects (Lauria-Horner et al., 2003) and pregabalin is approved for treating generalized anxiety disorder. This may be one reason for the increasing use of gabapentinoids by individuals addicted to opioids (Kammerer et al., 2012). Concerns have also been raised regarding the abuse potential of pregabalin (Grosshans et al., 2013). The numbers of deaths that are attributed to the combination of a gabapentinoid, mainly high dose pregabalin, and an opioid are also increasing (Häkkinen et al., 2014). The mechanism of the possibly lethal interaction remains to be understood. Both pharmacodynamic and pharmacokinetic interactions must be considered.

Pregabalin, like gabapentin, exerts its antinociceptive and sedative effects by the inhibition of calcium influx that is attained when pregabalin binds to the \( \alpha_2\delta \) subunit of voltage-gated calcium channels in the neuronal presynaptic terminals (Field et al., 2006). Pregabalin differs from gabapentin by having a more favourable pharmacokinetic profile (Bockbrader et al., 2010b). Also minor pharmacodynamic differences between pregabalin and gabapentin have been suggested (McClelland et al., 2004). Most of gabapentin, 80%, and virtually all pregabalin is eliminated unchanged via the kidneys (Vollmer et al., 1986; Bockbrader et al., 2010a), and they have no known pharmacokinetic interactions with other drugs. However, we are unaware of any published studies that have investigated pharmacokinetic interactions between gabapentinoids and opioids at the central nervous system (CNS) level or addressed the role of possible transporter systems.

The aims of our study were to investigate in the rat the acute antinociceptive effects of oxycodone and morphine when combined with pregabalin compared with the individual effects, to assess whether pregabalin prevents the development of morphine tolerance, and to investigate whether pregabalin has any effects on the brain concentrations of either of the two opioids or vice versa.

**Methods**

**2.1 Animals**

Research was conducted in accordance with the guidelines of the local authorities and the International Association for the Study of Pain (Zimmermann 1983). The provincial government of Southern Finland (Uudenmaan Lääninhallitus, Hämeenlinna, Finland, ESAVI/5684/04.10.03/2011) approved the study protocol. Male Sprague-Dawley rats (Scanbur, Sollentuna, Sweden, weighing 300 g at the beginning of experiments) were used. The rats were accommodated in transparent plastic cages in light- and temperature-
controlled rooms (lights on at 7:00 a.m., off at 7:00 p.m.; temperature 23 ± 2 °C). Water and standard laboratory chow were available ad libitum. The rats were habituated to the testing conditions for three days before the experiments and they were trained in the rotarod for three min for two days before the experiments commenced. The rats were euthanized by decapitation and whole brain samples were harvested and stored at -80 °C. The blood samples collected in decapitation were let to coagulate at room temperature for 60 min, after which they were centrifuged at 4900g for 20 min at +23 °C. Serum was collected and stored at -20 °C.

2.2 Drugs

Morphine hydrochloride, oxycodone hydrochloride trihydrate solution (Oxanest®, Leiras Takeda, Helsinki, Finland) and pregabalin capsules (Lyrica®, Pfizer, New York City, U.S.A) were purchased from the University Pharmacy (Helsinki, Finland). Morphine was dissolved and oxycodone was diluted by physiological saline. Pregabalin was dissolved to 0.5% methylcellulose in physiological saline and was kept on a magnetic stir plate to maintain the suspension.

Morphine, oxycodone and their control (physiological saline) were injected subcutaneously (s.c.) in a volume of 2 mL/kg, whereas pregabalin and its control (methylcellulose) were administered intraperitoneally (i.p.) in a volume of 5 mL/kg. The opioid concentrations were given as free base amounts per unit of volume.

2.3 Behavioural tests

Tail-flick latencies were tested using a tail-flick apparatus (Ugo Basile 37360, Comerio, Italy). The rats were restrained in plastic tubes that were covered by a cloth. At each time point, three different points of the middle third of the tail were exposed to infrared light. The intensity of the infrared light was adjusted so that the baseline latency in the test was 2.6 ± 0.5 s (n = 40). A flick of the tail automatically stopped the timer of the apparatus and the mean of the results was calculated. The cut-off was set at 10 s to avoid tissue damage to the tail. When an individual measurement reached the cut-off, no further tests were performed for that particular time point.

Hot plate tests were performed using a hot plate device (Harvard Apparatus Ltd. Edenbridge, Kent, UK). The rat was put inside a transparent cage on the hot plate (52 ± 0.2 °C) in the test. Licking, stomping the hind paw or vocalization were considered as a sign of thermal nociception. The time interval from being placed on the hotplate to exhibiting the first sign of thermal nociception was measured. The animal was immediately removed from the hot plate after exhibiting a sign of thermal nociception. The baseline latency was 9.7 ± 3.8 s (n = 40). The cut-off time was set to 60 s to avoid tissue damage.
The rotarod test was performed using a rotarod apparatus (Ugo Basile 47700, Comerio, Italy). The time the animal was able to stay on a rod that was rotating at the fixed speed of 20 rpm was measured. The cut-off limit was set to 30 s. The tail-flick, hot plate and rotarod (when applicable) tests were performed at each time point.

The animals were randomized to the treatment groups and all tests of the four experiments were performed blinded to the treatment.

2.4 Experimental design

Acute experiments

Equianalgesic doses of oxycodone and morphine were used. The equianalgesic doses of morphine and oxycodone for the rat were approximated from the study of Lemberg et al (Lemberg et al., 2006). The doses were chosen to produce between a maximum possible effect (MPE) of 25 – 50% in order to enable possible potentiation of the effect when co-administered with pregabalin. The choice of the doses of pregabalin was based on those used in a published study in mouse models of acute nociception (Keyhanfar et al., 2013).

In experiment 1, the acute effects of pregabalin on oxycodone or on morphine antinociception and motor co-ordination were studied. Pregabalin 50 mg/kg (i.p.) or vehicle was administered simultaneously with either morphine 2.5 mg/kg (s.c.) or oxycodone 0.6 mg/kg (s.c.). Nociceptive and motor co-ordination (sedation) tests were performed 30 min after drug administration.

In experiment 2, the acute effects of pregabalin on oxycodone or morphine antinociception were studied by administering pregabalin 10 mg/kg s.c. before either opioid. Our aim was to evaluate whether a low pregabalin dose with no effect of its own could cause potentiation of oxycodone and morphine when given before the opioids. The blood and brain samples were collected for drug concentration analysis. However, the samples related to morphine administration were not analyzed due to the negative behavioural finding. Pregabalin 10 mg/kg (i.p.) and its vehicle were given 30 min prior to morphine 2.5 mg/kg (s.c.) or oxycodone 0.6 mg/kg (s.c.) and their respective vehicles. Antinociception was evaluated using the tail-flick and hot plate tests 30 min after the latter drugs (60 min after pregabalin). Brain and blood samples were obtained as described under 2.1.

In experiment 3, the acute effects of pregabalin on morphine antinociception were studied at 90 min and the brain and blood samples were collected for drug concentration analysis. The later time point was chosen to ensure a high concentration of pregabalin, which would enable the detection of a possible effect on the CNS drug transporter system. Morphine 2.5 mg/kg, pregabalin 50 mg/kg, and their respective vehicles were administered simultaneously. Nociception was evaluated using the tail-flick and hot plate tests after 90 min. Tissue and blood samples were obtained as described in 2.1.
**Tolerance experiments**

In experiment 4, we assessed the interactions of morphine and pregabalin for chronic administration conditions. Morphine tolerance was induced by giving two s.c. injections of morphine a day for four consecutive days. The individual doses were 10 mg/kg on day 1, 15 mg/kg on day 2, 20 mg/kg on day 3, and 30 mg/kg on day 4. In addition to the morphine tolerance treatment, pregabalin 50 mg/kg (i.p.) or vehicle was administered once daily for each of those consecutive days.

On the fifth day, rats treated with either morphine alone or morphine with pregabalin were randomized into two acute treatment groups to test the development of tolerance. One group was treated with morphine 5 mg/kg (s.c.) and the other received morphine 5 mg/kg (s.c.) with pregabalin 50 mg/kg (i.p.). The tail-flick and hot plate tests were performed 30 min after receiving the treatment injections. After the experiment, supplementary doses of pregabalin and morphine were administered to match the dosing of the tolerance scheme (when applicable). In the evening of the fifth day, morphine 30 mg/kg (s.c.) with or without pregabalin 50 mg/kg was given again. On the sixth day the acute treatments were crossed-over between the groups compared with day five. The results of the respective groups of the fifth and the sixth day were pooled.

Cross-tolerance between morphine and pregabalin was also assessed in morphine-tolerant and morphine-naïve rats. Morphine tolerance was induced without pregabalin as described above for experiment 4. On the fifth day, both the morphine-tolerant and morphine-naïve rats were given pregabalin 50 mg/kg, (i.p.) or the vehicle. The hot plate test was executed 30 min after receiving the pregabalin treatments.

**2.5 Determination of drug concentrations**

The determinations of morphine, morphine-3-glucuronide (M3G), oxycodone, noroxycodone, oxymorphine, and pregabalin were carried out using an Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany) coupled to an API 3000 tandem mass spectrometry (AB Sciex, Toronto, ON, Canada) that operated in a positive turbo ion spray mode. Prior to analysis, spiked calibration standards and quality control samples were prepared in rat brain tissue homogenates for all the treatment compounds at appropriate concentrations that ranged from 1.0 to 2250 ng/mL. The LC-MS/MS analyses of morphine, M3G and pregabalin were performed separately as previously described (Dominguez-Ramirez et al., 2006; Oertel, et al., 2009) with some modifications. The chromatographic separations were achieved on Atlantis HILIC Silica column (3 µm particle size, 100 mm × 2.1 mm I.D., Waters, Milford, MA, USA) using a gradient elution of mobile phase consisting of acetonitrile and 10-mmol/L ammonium formate in 0.2% formic acid (v/v). Oxycodone served as
an internal standard for morphine and M3G, and the limit of quantification was 1.0 ng/mL for both analytes. Gabapentin was used as an internal standard for pregabalin determinations. The determination of oxycodone and its metabolites were performed as previously described (Neuvonen & Neuvonen, 2008). The limit of quantification for oxycodone, noroxycodone and oxymorphine was 1.0 ng/mL. The day-to-day coefficients of variations (CVs) for all the methods described were below 15% for the concentrations of all analytes.

2.6 Statistical analysis

The results of the hot plate and tail-flick tests are expressed as a percentage of MPE, calculated thus MPE% = \[\frac{\text{post-drug latency} - \text{baseline latency}}{\text{cut-off time} - \text{baseline latency}}\] × 100%, which takes into account the differences in baseline nociceptive latencies. The rotarod test results are expressed as the percentage change from the baseline values. In the text and figures, data are presented as the means of the sample values (±SD).

The interactions of the main effects, or synergy between the studied doses were analyzed using 2 × 2 between-subjects factorial design in two-way analysis of variances. One-way analysis of variances with multiple-comparison-adjusted Tukey post hoc analysis was used to test for statistically significant differences in mean values. The difference was considered significant at values \( p < 0.05 \) in all tests. The data were analyzed using GraphPad Prism, version 6.0a for Mac OS X (GraphPad Software, La Jolla, CA, USA).

Results

3.1 Pregabalin increases the antinociceptive and sedative effects of oxycodone and morphine

In experiment 1, pregabalin 50 mg/kg was co-administered with oxycodone 0.6 mg/kg or morphine 2.5 mg/kg, then hot plate, tail-flick, and rotarod tests were performed 30 min after the treatments were given.

Hot plate test data (fig. 1a), reveal that oxycodone alone produced antinociception of 18% MPE \( (p < 0.05) \) and pregabalin alone 8% MPE (n.s.). Co-administration of both pregabalin and oxycodone showed an antinociceptive effect of 39% MPE \( (p < 0.05) \), but despite this change in MPE the statistical analysis did not indicate a synergistic effect between the studied doses. Morphine alone produced antinociception of 28% MPE \( (p < 0.05) \) whereas pregabalin alone produced a 10% MPE (n.s.) After co-administration of both pregabalin and morphine, an antinociceptive synergy (63% MPE) was obtained (interaction of main effects, \( p < 0.05 \), fig. 1b).
In the tail-flick test, no significant synergy was seen after the co-administration of pregabalin and oxycodone (fig. 1c) or pregabalin and morphine (fig. 1d).

In the rotarod test (fig. 1e), oxycodone alone reduced the time on the rod by 4% (n.s.) and pregabalin by 6% MPE (n.s.). Co-administration of oxycodone and pregabalin obtained 31% reduction ($p < 0.05$) in time, but the statistical analysis did not indicate a synergistic effect. Morphine alone reduced the mean time the rats remained on the rotating rod by 18% (n.s.) and pregabalin by 4% (n.s.). Co-administration of morphine and pregabalin reduced the rotarod time synergistically with the treatment doses (interaction of main effects, $p < 0.001$) by 66% (fig. 1f).

In experiment 2, a smaller dose of pregabalin, 10 mg/kg, was administered 30 min before morphine 2.5 mg/kg and oxycodone 0.6 mg/kg were administered. Tail-flick and hot plate tests were performed 30 min after the administration of the opioids.

The hot plate test (fig. 2a) revealed that pregabalin did not cause antinociception but oxycodone caused an effect of 18% MPE ($p < 0.05$). With pregabalin pretreatment, the antinociceptive effect of oxycodone was synergistically (interaction of main effects, $p < 0.05$) increased to 40% MPE with the studied treatment doses.

In the tail-flick test (fig. 2c), pregabalin produced a 9% MPE (n.s.) antinociceptive effect, whereas oxycodone produced an antinociceptive effect of 43% MPE ($p < 0.0001$). After pregabalin pretreatment the antinociceptive effect of oxycodone was 71% MPE ($p < 0.0001$, fig. 2c), but the statistical test did not indicate statistically significant synergy. When morphine and pregabalin were co-administered no statistically significant synergy could be detected in the hot plate (fig. 2b) or tail-flick (fig. 2d) tests with the studied doses.

### 3.2 Pregabalin does not change the concentrations of oxycodone or morphine in the brain or vice versa.

The brain concentrations of pregabalin, oxycodone, and its major metabolites, noroxycodone and oxymorphone were analyzed in experiment 2, when pregabalin 10 mg/kg was administered 30 min before oxycodone 0.6 mg/kg. The behavioural data were assessed (fig. 2a and 2c) and the samples were collected 30 min after the administration of oxycodone. Pregabalin did not change the brain concentrations of oxycodone (fig. 3a), noroxycodone (fig. 3b) or oxymorphone (data not shown). The brain concentrations of pregabalin were not affected by co-administration of oxycodone either (fig. 3c).

Brain morphine, M3G, and pregabalin concentrations were analyzed in experiment 3, when pregabalin 50 mg/kg was administered with morphine 2.5 mg/kg. The behavioural data were assessed and the samples were collected 90 min thereafter. The hot plate test determined that the antinociceptive effects were 43% MPE ($p < 0.0001$) for pregabalin and 20% MPE ($p < 0.05$) for
morphine. The co-administration of morphine and pregabalin caused an antinociception effect of 54% MPE ($p < 0.0001$).

The tail-flick test revealed that morphine alone produced antinociception of 11% MPE ($p < 0.05$) whereas pregabalin produced an antinociception effect of 9% MPE (n.s.). After co-administration, however, they showed an antinociceptive effect of 31% MPE ($p < 0.01$), but the test for synergy did not reach the level of statistical significance.

The brain concentrations of morphine, M3G or pregabalin were not affected by co-administration of pregabalin and morphine (figs. 3d-f), respectively.

3.3 Chronic co-treatment with pregabalin does not prevent morphine tolerance

In experiment 4, chronic morphine treatment led to significant tolerance to the antinociceptive effect of morphine 5 mg/kg as measured by the hot plate (fig. 4a) and tail-flick (fig. 4b) tests, (11% vs. 71% MPE and 31% vs. 81% MPE, respectively). Co-administration of pregabalin 50 mg/kg with morphine during the tolerance treatment did not affect the development of morphine tolerance (fig. 4a and 4b). In the tolerant animals, pregabalin 50 mg/kg did not potentiate the effect of acute morphine 5 mg/kg as measured by the hot plate test at the 30-minute time point (fig. 4a).

The effect of acute pregabalin 50 mg/kg was assessed in morphine-tolerant and naïve rats to study any possible cross-tolerance between morphine and pregabalin. Pregabalin 50 mg/kg had similar effects in morphine-tolerant and naïve animals at 120 min, in the hot plate test (data not shown).

Discussion

We found that pregabalin potentiated oxycodone- and morphine-induced antinociception in acute thermal nociceptive tests in the rat. The sedative effect of the two opioids was also potentiated by pregabalin. In addition, we showed that the behavioural data could not be explained by any changes in the brain concentrations of oxycodone, morphine, their major metabolites or pregabalin. The effects of co-administration of oxycodone and pregabalin have previously not been studied in preclinical models of acute pain. The data on brain concentrations of these drugs are novel. Our results also indicate that pregabalin administration does not prevent the development of morphine tolerance.

The present investigation supports the results from previous preclinical studies conducted on the effects of morphine, gabapentin, and pregabalin (Shimoyama et al., 1997), (Meymandi et al., 2006). In addition to confirming the antinociceptive synergy with the studied treatment doses of pregabalin and
morphine, we showed that similar synergy levels exist between pregabalin and oxycodone. Cristoph et al. previously showed that oxycodone has an additive antinociceptive effect with pregabalin in the neuropathic model in the rat (Christoph et al., 2011). Nevertheless, data obtained from chronic neuropathic pain studies cannot be directly translated to acute nociceptive pain studies such as ours. We also found increased sedation when pregabalin was combined with oxycodone, in line with the results by Cristoph and colleagues. Notably, sedation probably explains the increased latencies in the hot plate test found in our acute test when the opioids were administered with pregabalin 50 mg/kg. The doses of the opioids in our study were chosen to produce only moderate antinociception in order to enable the combination with pregabalin to produce a stronger effect within the limits of the cut-off-times of the thermal tests.

Oxycodone and morphine exert their antinociceptive effects by activating presynaptic G-protein coupled μ-opioid receptors (Narita et al., 2008), which leads to the inhibition of voltage-gated calcium channels and reduced transmission (Tsuno et al., 1986; Ostermeier et al., 2000). Moreover, μ-opioid receptors are also located postsynaptically, where they open the potassium channels, which in turn hyperpolarizes the cell (Schneider et al., 1998). Considering that also pregabalin exerts its effects by the inhibition of calcium influx, the reduced influx of calcium and the consequent reduction of released excitatory amino acids would seem to be the most plausible mechanism of interaction. Nonetheless, the hypothesis has not been empirically confirmed.

When pregabalin 10 mg/kg was administered 30 min before the opioids, oxycodone showed a pronounced potentiation compared with morphine in both the hot plate and tail-flick tests, which was rather surprising. The μ-opioid receptor has been suggested to be functionally selective (i.e. different agonists promote different downstream responses) to opioids (Morgan et al. 2014; Morse et al., 2011). In addition, bone cancer may modify μ-opioid receptor function so that morphine but not oxycodone loses its activity on the receptor (Nakamura et al., 2013). Thus, the second-messenger systems activated by morphine or oxycodone might be differently susceptible upon to the effect of pregabalin on the inhibition of the calcium currents. This speculation does not contradict the hypothesis of the alteration of calcium currents as a mechanism of action of the potentiation of opioids and pregabalin; but it does offer a plausible explanation for the differences between oxycodone and morphine found in our present study.

There are no known pharmacokinetic interactions for pregabalin. Studies of the metabolism of pregabalin in the rat have not been published, however. In humans, pregabalin is only minimally metabolized and it does not bind to plasma proteins (Bockbrader et al., 2010b). A recent study reported that pregabalin was not a substrate of P-glycoprotein, the major opioid efflux transporter protein at the blood-brain barrier (Chan et al., 2014). Novel drug transporters are continuously being identified in research. We therefore studied the disposition of pregabalin, morphine, oxycodone, and their major metabolites in the brain in vivo. We exclude the possibility of a
pharmacokinetic interaction in the CNS, thus the pharmacological potentiation appears to be solely attributed to pharmacodynamics.

Our finding that pregabalin co-treatment did not prevent morphine tolerance from developing disagrees with a previous preclinical study, which reported that systemic gabapentin prevented morphine tolerance (Gilron et al., 2003). We did not detect an attenuation of tolerance to morphine in either the hot plate or the tail-flick test. The tail-flick test was the antinociceptive test that was also used by Gilron et al. The discrepancy in findings between our study and that of Gilron et al. might be explained by the differences in the tolerance scheme, since we used increasingly larger doses of morphine.

Morphine tolerance was reported to be prevented when gabapentin and morphine were administered intrathecally (Bao et al., 2014). Intrathecally administered morphine alone or pregabalin alone did not change the levels of the anti-inflammatory cytokine interleukin 10 (IL-10) in the spinal cord. Yet, when pregabalin and morphine were administered together, the levels of IL-10 were increased. In addition, IL-10 antibody reversed the prevention of morphine tolerance by gabapentin (Bao et al., 2014).

Pregabalin did not potentiate the antinociceptive effect of morphine in morphine-tolerant animals in our study. Yet in the naïve animal the same dose of pregabalin significantly potentiated the effect of morphine in the hot plate test. We ruled out the possibility of cross-tolerance between morphine and the acute effect of pregabalin. The activation of μ-opioid receptor and the downstream signaling is known to be altered following repeated morphine administration (Macey et al. 2014). A possible explanation for our result could be that the changed intra-cellular signaling in morphine tolerant rats models no longer enables the potentiation of antinociception. Although the morphine dose given to the tolerant rats was double the dose given to naïve animals, it is possible that the morphine potentiation by pregabalin might have occurred if a larger morphine dose had been administered acutely to the tolerant rats.

One of the reasons for conducting this study was to elucidate possible mechanisms for the lethal combination of pregabalin with either oxycodone or morphine. The results indicate that there could be a significant additive or synergistic effect in both sedation and antinociception after acute administration. However, the combination had lost this synergistic effect on sedation and antinociception after the chronic administration of morphine. This could suggest that the combination of pregabalin and an opioid does not have an increased effect in opioid tolerant individuals; especially when the dose of acute morphine relative to the developed tolerance is small. However, we did not measure respiratory depression in opioid tolerance. It is also possible that the response to a high dose of pregabalin during withdrawal is different. These possible factors would be important aspects to study in the future as the clinical evidence suggests that delayed pregabalin toxicity may be particularly dangerous.

In conclusion, the results of this study characterise a clinically important drug combination in the context of acute pain analgesia. Pregabalin potentiates the
effects of both oxycodone and morphine, but the pattern of interaction between the two opioids is different. The interactions of pregabalin with the two opioids are not explained by pharmacokinetic factors. Isobolographic analysis is also needed to determine the interaction with other dose combinations. Clinical trials are needed to assess the efficacy and safety of the long-term co-administration of opioids and gabapentinoids more thoroughly.

References


**Author Contributions**

All authors discussed the results, commented on the manuscript and approved for the submission.

V.J. contributed to the design of the study, data acquisition, analysis, interpretation and manuscript preparation

T.L. contributed to the design of the study, data acquisition, analysis, interpretation and manuscript preparation

J.L. contributed to the data acquisition and analysis

M.N. contributed to the design of the study, data acquisition and interpretation

P.R. contributed to the design of the study, data acquisition, analysis, interpretation and manuscript preparation

E.K. contributed to the design of the study, data acquisition, analysis, interpretation and manuscript preparation
Fig. 1. Effects of pregabalin 50 mg/kg (P50), oxycodone 0.6 mg/kg (OXY0.6), morphine 2.5 mg/kg (MO2.5) and the combined effect of pregabalin with oxycodone (OXY0.6+P50) or pregabalin and morphine (MO2.5+P50) in the hot plate (a and b), tail-flick (c and d) and rotarod tests (e and f). The means of the maximum possible effect (MPE%) ± SD in the hot plate and tail-flick tests measured 30 min after administration are plotted. In the rotarod test, the percentage changes from the baseline time are shown, (%) ± SD. Pregabalin was administered intraperitoneally, oxycodone and morphine subcutaneously. #, ###, #### Statistically significant difference (p < 0.05, p < 0.001, p < 0.0001, respectively) as compared with the vehicle control. *, **** Statistically significant difference (p < 0.05, p < 0.0001) as compared between the indicated groups. n = 12–16 rats, except in the vehicle groups, where n = 10–15 rats.

Fig. 2. Effects of pregabalin 10 mg/kg (P10) (administered 30 min before the opioids), oxycodone 0.6 mg/kg (OXY0.6) and morphine 2.5 mg/kg (MO2.5) and the combined effect of pregabalin and morphine (MO2.5+P10) or oxycodone (OXY0.6+P10) for the hot plate (a and b) and tail-flick tests (c and d). The means of the maximum possible effects (MPE%) ± SD after 30 min of administration of the opioids (60 min after pregabalin) are plotted. Pregabalin was administered intraperitoneally, oxycodone and morphine subcutaneously. #, ###### Statistically significant difference (p < 0.05, p < 0.0001) as compared with the vehicle control. *, ** Statistically significant difference (p < 0.05, p < 0.01, respectively) as compared between the indicated groups. n = 13–14 rats, except in b and d, where n = 8 rats.

Fig. 3. The brain concentrations (ng/g tissue) of oxycodone (a), noroxycodone (b), and pregabalin (c) in experiment 2. Pregabalin 10 mg/kg, i.p. was administered 30 min before oxycodone 0.6 mg/kg s.c. and the samples were taken 30 min after the oxycodone administration. The brain concentrations (ng/g tissue) of morphine (d), morphine-3-glururonide (e), and pregabalin (f) from experiment 3. Pregabalin 50mg/kg, i.p. and morphine 2.5mg/kg, s.c. were administered at the same time and the samples were taken 90 min later. The data are expressed as means of the groups ± SD. n = 6–8 rats.

Fig. 4. Effects of pregabalin 50 mg/kg (P) on the development of morphine tolerance and the effect of pregabalin 50mg/kg on acute morphine 5 mg/kg (MO) after chronic morphine treatment in hot plate (a) and tail-flick (b) tests. The means of the maximum possible effect (MPE%) ± SD 30 min after administration of the drugs (acute treatment) are plotted. Vehicle (-). Pregabalin was administered intraperitoneally, morphine subcutaneously. #### Statistically significant difference (p < 0.0001) as compared with the vehicle control. **, **** Statistically significant difference (p < 0.01, p < 0.0001) between the indicated groups.