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Pregabalin role in inhibition of morphine analgesic tolerance and physical dependency in rats

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ABSTRACT

Pregabalin is recently proposed as analgesic or adjuvant in pain management. While previous preclinical investigations have evaluated pregabalin–opioid interactions, the effect of pregabalin on opioid tolerance and dependency has not yet been studied. Here we evaluated the effects of different doses of pregabalin (50, 100 and 200 mg/kg, *s.c.*) on morphine-induced tolerance and dependency in rats. Adult male *Wistar* rats were rendered tolerant to analgesic effect of morphine by injection of morphine (10 mg/kg, *s.c.*) twice daily for 7 days. To develop morphine dependence, rats were given escalating doses of morphine. To determine the effect of pregabalin on the development of morphine tolerance and dependence, different doses of pregabalin were administrated before morphine. The tail-flick and naloxone precipitation withdrawal tests were used to evaluate the degree of tolerance and dependence, respectively. Chronic morphine-injected rats showed significant decrements in the percentage maximum possible effect (%MPE) of morphine on the days 5 and 7 (32.5% ± 3.5, 21.5% ± 4, respectively) compared to the first day (100%) which showed morphine tolerance. Pregabalin 200 mg/kg completely prevented the development of morphine tolerance. In addition, concomitant treatment of morphine with pregabalin attenuated almost all of the naloxone-induced withdrawal signs which include weight loss, jumping, penis licking, teeth chattering, wet dog shakes, rearing, standing, sniffing, face grooming and paw tremor. These data show that pregabaline has a potential anti-tolerant/anti-dependence property against chronic usage of morphine. Therefore, pregabalin appears to be a promising candidate for the treatment of opioid addiction after confirming by future clinical studies.

1. Introduction

Morphine is a potent opioid analgesic that is widely used for acute and chronic pain control (Somogyi et al., 2007). However repeated or long term use of opioids develops opioid tolerance and dependence which reduces the therapeutic efficacy of these drugs. Recently, the pharmacotherapy of opioid tolerance and addiction has drawn intensive interest (Ueda and Ueda, 2009).

Pregabalin is a γ-aminobutyric acid (GABA) analog that belongs to gabapentinoids, the third generation of anticonvulsants. It has been also proposed to exert analgesic effect of its own or as adjuvant in different models of pain (Knotkova and Pappagallo, 2007; Moore et al., 2009). For example, it increases the efficacy of celecoxib in the treatment of low back pain (Romano et al., 2009) and enhances the action of naproxen to reverse hyperalgesia (Hurley et al., 2002). There are also several clinical and experimental studies that report the action of pregabalin in reducing the acute pain with opioids as combination therapy (Dauri et al., 2009; Meymandi and Sepehri, 2008; Meymandi et al., 2006). While previous preclinical investigations have evaluated pregabalin–opioid interactions (Eckhardt et al., 2000; Matthews and Dickenson, 2002; Shimoyama et al., 1997), the effects of pregabalin on opioid tolerance and dependency have not been studied. Thus, the present study was designed to test the protective effects of different doses of pregabalin (50, 100 and 200 mg/kg, *s.c.*) on morphine-induced tolerance and dependency in rats.

2. Materials and methods

2.1. Animals

Adult male *Wistar* rats weighing 200–250 g were obtained from the breeding colony at the Iran Pasteur Institute, Tehran. Four rats were housed per cage and were kept at a constant temperature of 20 ± 2 °C with a 12:12-h light/dark cycle (lights on at 7:00 a.m.). Food and water were available ad libitum in the home cages. The rats were habituated to the environment for at

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least one week before the start of the experiment. Animals were handled in accordance with the criteria outlined in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health (NIH) publication 86-23; revised 1985;http://www.oacu.od.nih.gov/regs/guide/guidex.htm). The protocols were also approved by the institutional ethics committee of Bu-Ali Sina University. All procedures and experiments were performed between 10:00 and 14:00.

2.2. Drugs

Morphine hydrochloride (TEMAD, Iran), pregabalin (Sigma, St. Louis, USA) and naloxone hydrochloride (Tolid Daru, Iran, 3 mg/kg) were dissolved in saline immediately before use and administered subcutaneously (*s.c.*). The drugs were given in the volume of 1 ml/kg.

2.3. Antinociceptive test

Antinociception was assessed by tail-flick (TF) test (D'Amour and Smith, 1941). The TF latency for each rat was determined thrice at 3 min intervals and mean was designated as baseline latency before morphine injection. The intensity of the beam was adjusted to produce mean control reaction time between 2 and 4 s. The cut-off time was fixed at 10 s in order to avoid any damage to the tail. The TF latencies were converted to the percentage of the maximum possible effect (%MPE) according to the following formula:

$$\%\text{Antinociception (\%MPE)} = 100\,(\text{Reaction time of test} - \text{basal reaction time})/(10 - \text{basal reaction time})$$

2.4. Morphine tolerance

To induce analgesic tolerance, morphine at twice daily dose of 10 mg/kg was injected at 8.00 a.m. and 6.00 p.m. for 7 days. To determine the effect of pregabalin on the development of morphine tolerance, pregabalin (50, 100 and 200 mg/kg, *s.c.*) or saline was injected 30 min prior to each injection of morphine. TF latencies were measured 30 min after morphine injection on days 1, 3, 5 and 7 during treatment.

2.5. Morphine dependence and induction of withdrawal syndrome

To develop morphine dependence, rats were injected subcutaneously with morphine twice daily for 7 days. The dose of morphine on days 1 and 2 was 2.5 mg/kg; this dose was doubled every day thereafter to reach a total dose of 40 mg/kg on day 6. On day 7 the animals received the last injection of morphine, 50 mg/kg. To determine the effect of pregabalin on the development of morphine dependence, pregabalin (50, 100 and 200 mg/kg, *s.c.*) or saline was given 30 min before morphine. On the seventh day, naloxone (3 mg/kg, *s.c.*) was given 5 h after the last injection of morphine. Immediately after naloxone injection, the rats were individually placed in a Plexiglas observation chamber and the following withdrawal signs were observed for 30 min after the naloxone injection: weight loss, jumping, penis licking, teeth chattering, wet dog shakes, rearing, standing, sniffing, face grooming and paw tremor. An independent investigator, who was unaware of the treatment received, evaluated the reactions of each animal.

2.6. Statistical analysis

Statistical comparisons in %MPE between groups over the time course of study were determined by two- or one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* test. The difference in withdrawal signs between experimental groups was determined by one-way ANOVA followed by Tukey's *post-hoc* test. All results are shown as the mean ± S.E.M, with statistical significance set at $P < 0.05$.

3. Results

3.1. The effect of pregabalin on the development of tolerance to analgesic effect of morphine

Chronic administration of morphine (10 mg/kg twice daily) for 7 days caused a significant decrease in its analgesic effect (tolerance). Concomitant treatment of morphine with pregabalin in doses of 100 and 200 mg/kg prevented the development of tolerance to analgesic effect of morphine. However, tolerance developed with 50 mg/kg pregabalin during the experimental time course (Fig. 1). Furthermore, concomitant treatment of saline with morphine had no anti-tolerant effect (data not shown).

3.2. The effect of pregabalin on the development of morphine dependence

Fig. 2 shows the percentage of weight loss before and after naloxone injection in the pregabalin treated and untreated morphine-dependent groups. Naloxone injection in the morphine dependent group caused a great weight loss in the animals (8.78% ± 0.32). Although the 50 mg/kg dose of pregabalin did not prevent the weight loss precipitated by naloxone administration, the higher doses (100 and 200 mg/kg) of pregabalin significantly attenuated weight loss in the treated morphine-dependent rats compared to untreated

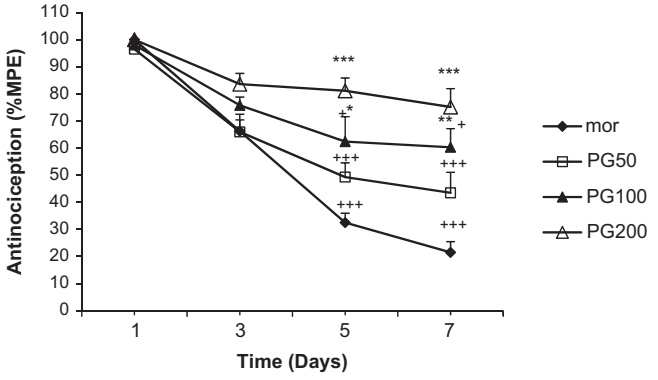

Fig. 1. The effect of pregabalin (PG) on the development of tolerance to the analgesic effect of morphine (Mor) in rats. Values represent mean ± S.E.M. ($n=7$). $^{+}P<0.01$ and $^{+++}P<0.001$ are significantly different vs. antinociception values in the first day at the same group. $^{*}P<0.05$,$^{**}P<0.01$, and $^{***}P<0.001$ as compared with morphine-injected rats at the same time.

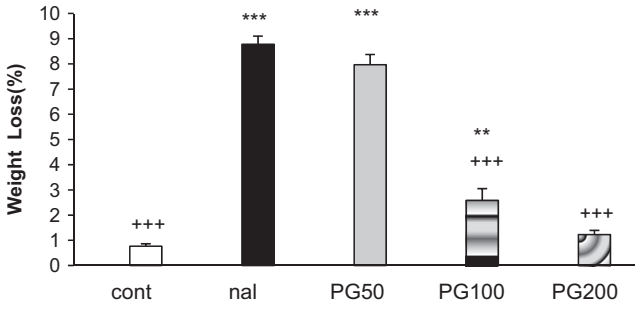

Fig. 2. The percentage of weight loss during a 30-min observation period in the experimental groups. **: $P<0.01$, and ***: $P<0.001$ compared to the control group. $^{+++}$: $P<0.001$ compared to the morphine-dependent naloxone-treated group. Cont, control group; Nal, morphine dependent naloxone-treated group; PG 50, PG 100 and PG 200, morphine-dependent groups that received pregabalin at doses of 50, 100 and 200 mg/kg before naloxone injection; ordinate, mean ± S.E.M.