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Antinociceptive effects induced by intra-periaqueductal grey injection of the galanin receptor 1 agonist M617 in rats with morphine tolerance

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HIGHLIGHTS

- We report antinociception induced by M617 in the PAG in morphine-tolerant rats.
- M1145 in the PAG does not induce antinociception in morphine-tolerant rats.
- Activation of GalR1 induces antinociception in rats with morphine-tolerance.

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ABSTRACT

The present study was performed to investigate the antinociceptive effects of M617, a selective galanin receptor 1 agonist, and M1145, a selective galanin receptor 2 agonist, in the periaqueductal grey (PAG) in rats with morphine tolerance. Intra-PAG injection of 0.1 nmol, 0.5 nmol and 1 nmol of M617 induced dose-dependent increases in hindpaw withdrawal latencies (HWLs) to noxious thermal and mechanical stimulations in rats with morphine tolerance. Nevertheless, intra-PAG injection of 5 nmol of the selective galanin receptor 2 agonist M1145 showed no significant influences on HWLs to noxious thermal and mechanical stimulations in rats with morphine tolerance. The results demonstrated that it is the selective galanin receptor 1 agonist M617, not the selective galanin receptor 2 agonist M1145, induced significant antinociceptive effects in morphine-tolerant rats, indicating that galanin receptor 1 is involved in nociceptive modulation in the PAG of morphine-tolerant rats.

Morphine is a powerful analgesic drug, but also a potent inducer of tolerance and dependence. Morphine-induced tolerance describes the need for an increasing dose of morphine to achieve the same analgesic effect [2,13]. Previous studies show that development of morphine tolerance may influence the antinociceptive effects induced by several kinds of neuropeptides in the brain [4,19,21]. For example, development of morphine tolerance even attenuates substance P-induced antinociception [4].

Galanin, a 29 (30 in human)-amino-acid neuroendocrine peptide, is intensively investigated in pain modulation in the central nervous system [19,20]. The periaqueductal grey (PAG) is known to be one of the important brain regions involved in pain modulation [1], and in the PAG galanin induces antinociception in opioid-naïve animals [17]. However, it is still unknown whether galanin still induce antinociception in rats with morphine tolerance. Galanin acts upon three subtypes of galanin receptors (Gal R1, Gal R2, and Gal R3) [3,8,12]. Chemical M617 is a selective agonist of galanin receptor 1 [11], and M1145 is a selective agonist of galanin receptor 2 [16]. Our previous studies show that M617 induces antinociception in the central nucleus of amygdala or intrcerebroventricular of rats [5,7,9], showing that Gal R1 is involved in the pain modulation in the brain. In the present study, we explored the antinociceptive effect of galanin in the PAG in morphine-tolerant rats.

In our present study, the antinociceptive effects of M617 and M1145 in the PAG of rats with morphine tolerance were explored to make clear which type of galanin receptor was involved in antinociception in morphine-tolerant animals.

Experiments were carried out on male Sprague-Dawley rats weighting 200–250 g (The Experimental Animal Center, Academy of Military Medical Sciences, Beijing, China). The rats were housed in cages which maintained in a room temperature of 20 ± 2 °C with a 12 h light–dark cycle. All experiments were conducted according to the guideline of the International Association for the Study of Pain [22] and every effort was made to minimize both the animal suffering and the number of animals used.

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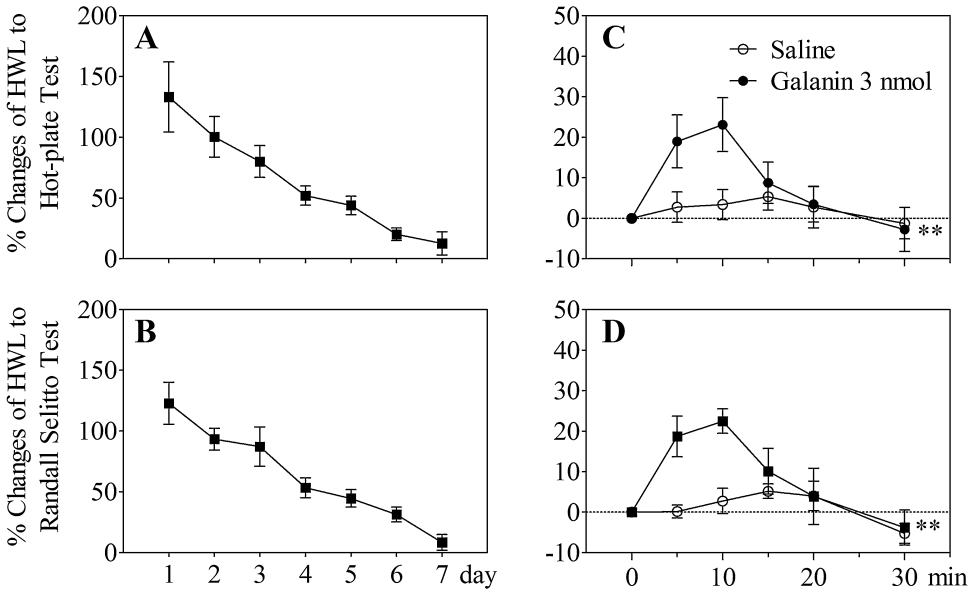

Fig. 1. The development of morphine tolerance and effects of intra-PAG injection of galanin on HWLs in rats with morphine tolerance. A and B, the percentage changes of HWLs to thermal (A) or mechanical (B) stimulation after morphine treatment from the 1st day to the 7th day. C and D, effects of intra-PAG injection of 3 nmol of galanin on the percentage changes of HWLs to thermal (C) and mechanical (D) in rats with morphine tolerance. Intra-PAG injection of 1 μl of 0.9% saline is as a control. Data are presented as mean ± S.E.M. The statistical difference between groups was determined by two-way ANOVA, $^{**}P < 0.01$. PAG, periaqueductal grey; HWL, hindpaw withdrawal latency.

The hindpaw withdrawal latencies (HWLs) during thermal and mechanical stimulation were measured as described previously [6,9,10]. The Hot-plate Test was used to assess the HWL to noxious thermal stimulation. The entire ventral surface of the rat's hindpaw was placed manually on a hot-plate which was maintained at a temperature of 52 °C (51.8–52.4 °C). The time to hindpaw withdrawal was measured in seconds and referred to as the HWL to stimulation. The Randall Selitto test (Ugo Basile, Type 7200, Italy) was used to assess the HWL to mechanical stimulation. A wedge-shaped pusher at a loading rate of 30 g/s was applied to the dorsal surface of a hindpaw. The latency required to initiate the withdrawal response was assessed and expressed in seconds. The HWL were tested and regarded as the basal HWLs. The HWLs recorded during subsequent experiments were expressed as percentage changes from the basal level (% change of HWL). The HWLs were tested before and repeated at 5, 10, 15, 20 and 30 min after intra-PAG injection. Each rat was tested by both types of stimulation. Each measurement took 2–15 s, thus the HWLs of one rat in every time point were measured within 1–2 min. A cut-off limit of 15 s was set up to avoid tissue damage. Data for left hindpaws was close to that for right hindpaws in each rat, and we only show data for right hindpaws.

Animals were anesthetized by intraperitoneal (i.p.) injection of sodium pentobarbital (45 mg/kg) and were mounted on a stereotaxic instrument. Two stainless steel guide cannulas of 0.8 mm outer-diameter were vertically inserted into the region of 1.5 mm above the PAG (B, −5.5 mm; LR, 0.5 mm; V, 6.0 mm. B, anterior (+) or posterior (−) to Bregma; L or R, left or right to midline; V, ventral to the surface of skull) according to Paxinos and Watson [14] and were fixed to the skull by dental acrylic. There were more than 3 days for rats to recover from the operation. On the day of intra-PAG injections, a stainless steel tube with a 0.4 mm out-diameter was vertically inserted into the guide cannula into the PAG. One microliter of solution was infused into the PAG over 1 min though a microsyringe. At the end of the experiment rats received a high dose of sodium pentobarbital (90 mg/kg) and the rat heads were cut and fixed in 10% formalin for 24 h with the injecting tube in situ before section. The location of the tip of the injecting tube was verified and all the tips of the injecting tube were in the PAG of rats in the present study.

Solutions for intra-PAG injection were prepared with sterilized saline (0.9%), each with a volume of 1 μl containing: (1) 1 nmol of galanin (rat galanin, Tocris, UK) (2) 0.1, 0.5, or 1 nmol of M617 (Selective Gal R1 agonist; Tocris, UK); (3) 5 nmol of M1145 (selective Gal R2 agonist; a gift from Dr. Johan Runesson and Professor Ülo Langels). Data from the experiment were expressed as mean ± S.E.M. Statistical difference between groups was determined by two-way analysis of variance (ANOVA). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ were considered as significant differences.

After recovery from surgery, all rats were conditioned to the nociceptive tests for 3 days. All the rats ($n = 12$) received i.p. injections of 10 mg/kg of morphine twice/day (at 8:00 AM and 8:00 PM) for 7 days. The HWLs to either thermal or mechanical stimulation were daily detected 10 min after the second morphine injection. The HWLs measured prior to the morphine injection were taken as the basal HWLs. As shown in Fig. 1A and B, the percentage changes of HWLs to thermal or mechanical stimulation descended from the 1st day to the 7th day after morphine treatment, indicating morphine-induced antinociception apparently decreased after chronic morphine treatment. It demonstrated that the rats became tolerance to morphine-induced antinociception.

Then we assessed the antinociceptive effect of galanin in the PAG in morphine-tolerant rats. Two groups of morphine-tolerant rats received intra-PAG injection of 3 nmol of galanin ($n = 6$), at a dose used in our previous work [17], or 1 μl of 0.9% saline as a control ($n = 6$). As shown in Fig. 1C and D, the HWLs to thermal and mechanical stimulations increased significantly after intra-PAG injection of 3 nmol of galanin (Hot-plate Test: $F(1,6) = 3.63$, $P < 0.01$; Randall Selitto Test: $F(1,6) = 3.27$, $P < 0.01$) compared to the control, suggesting that galanin in the PAG induces antinociceptive effects in morphine-tolerant rats.

We further explored the antinociceptive effects of M617 in rats with morphine tolerance. Four groups of morphine-tolerant rats received intra-PAG injection of 0.1 nmol ($n = 6$), 0.5 nmol ($n = 6$) or 1 nmol ($n = 6$) of M617, or 1 μl of 0.9% saline as a control ($n = 6$). As shown in Fig. 2A and B, the HWLs to thermal and mechanical stimulations increased significantly in a dose-dependent manner after intra-PAG injection of 0.1 nmol of M617 (Hot-plate Test: F