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Antinociceptive effects induced by injection of the galanin receptor 1 agonist M617 into central nucleus of amygdala in rats

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HIGHLIGHTS

- Intra-central nucleus of amygdala injection of M617 induces antinociception.
- No significant differences between the M617- and galanin-induced antinociception.
- Galanin receptor 1 is involved in galanin-induced antinociception in the brain.

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ABSTRACT

The present study was performed to explore the antinociceptive effects of M617, a selective galanin receptor 1 agonist, in the central nucleus of amygdala (CeA) of rats. Intra-CeA 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. Furthermore, rats received intra-CeA administration of M617 and galanin. The HWL to noxious thermal and mechanical stimulations increased markedly, and there were no significant differences in HWLs of rats received intra-CeA administration of M617 and galanin. The results demonstrated that intra-CeA injection of M617 induced significant antinociceptive effects in CeA of rats, indicating that galanin receptor 1 may be involved in M617-induced antinociception in the CeA of rats.

The central nucleus of amygdala (CeA) is known to be one of the important brain structures involved in multiple physiological functions [1,2,4]. Studies have demonstrated that the CeA is involved in pain modulation [4,8,15,25]. There are high densities of galanin-ergic fibers and galanin receptor 1 in the CeA [11–13]. Galanin is demonstrated to be involved in pain modulation in the central nervous system, both in spinal cord levels and in the brain [5,8,18,6,21,20,23]. Our previous study demonstrated that intra-CeA injection of galanin induced dose-dependent increases in HWLs to noxious thermal and mechanical stimulations in rats [8].

It is known that there are three subtypes of galanin receptor (GalR 1, GalR 2, and GalR 3), which all belong to the family of G-protein coupled receptors [3,14]. Our previous study demonstrated that the galanin-induced antinociception in CeA was blocked by galanin receptor antagonist galantide, indicating that the galanin-induced antinociception is mediated by galanin receptors [8]. It has been reported that the chemical M617 is a new selective agonist of galanin receptor 1 [10,17]. The present study was performed to investigate the antinociceptive effects of M617 in the CeA of rats.

Experiments were carried out on male Sprague-Dawley rats weighting between 210 and 280 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 $20 \pm 2\,^{\circ}C$ with a 12 h light–dark cycle. All experiments were conducted according to the guideline of the International Association for the Study of Pain [27] and every effort was made to minimize both the animal suffering and the number of animals used.

The hindpaw withdrawal latencies (HWLs) during thermal and mechanical stimulation were measured as described previously [5,8]. 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 $50 \pm 2\,^{\circ}C$. The time to hindpaw withdrawal was measured in seconds and referred to as the HWL to thermal

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stimulation. The experimenter was asked to be well practiced in conducting the Hot-plate Test so as to be able to keep the ventral surface of the hindpaw in careful contact with the hot-plate. 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. All rats were conditioned to the nociceptive test conditions 5 days before the experiment was actualized. Before intra-CeA injection, the HWL were tested 3 times and regarded as the basal HWLs. The HWLs recorded during subsequent experiments were expressed as percentage changes from the basal level (% change of HWL) for each rat. The HWLs were tested before and repeated at 5, 10, 15, 20, 30, 45 and 60 min after intra-CeA injection. Each rat was tested by both types of stimulation. The procedures for making four measurements at each time point were as follows: first, the HWL to thermal stimulation was tested, and then the HWL to mechanical stimulation was tested. Generally, 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.

Animals were anesthetized by intraperitoneal injection of sodium pentobarbital (45 mg/kg) and were mounted on a stereotaxic instrument. A stainless steel guide cannula (0.8 mm outer diameter) was directed to the CeA (B, −2.2 mm; LR, 4.0 mm; V, 8.0 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 [16] and was fixed to the skull by dental acrylic. There were more than 3 days for rats to recover from the operation. On the day of experiment, a stainless steel needle with 0.4 mm diameter was directly inserted into the guide cannula with 1.5 mm beyond the tip of the latter. At the end of the experiments the rats were injected 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 CeA of rats in the present study.

Solutions for intra-CeA injection were prepared with sterilized saline (0.9%), each with a volume of 1 μl containing: (1) 0.1, 0.5, or 1 nmol of M617 (Selective Gal R1 agonist; Tocris, UK); (2) 1 nmol of galanin (rat galanin, Tocris, UK).

Data from the experiment were expressed as mean ± S.E.M. Statistical difference between groups was determined by two-way analysis of variance (ANOVA) or by Student's t test (two-tailed). $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ were considered as significant differences.

Four groups of rats received intra-CeA injection of 0.1 nmol ($n=9$), 0.5 nmol ($n=9$), or 1 nmol ($n=9$) of M617, or 1 μl of 0.9% saline as a control ($n=9$). As shown in Fig. 1, the HWLs to thermal and mechanical stimulations increased significantly in a dose-dependent manner after intra-CeA injection of 0.1 nmol of M617 (Hot-plate Test: $F_{\text{left/left}}(1,9)=18.84$, $P<0.001$; $F_{\text{right/right}}(1,9)=65.15$, $P<0.001$. Randall Selitto Test: $F_{\text{left/left}}(1,9)=11.9$, $P<0.001$; $F_{\text{right/right}}(1,9)=19.23$, $P<0.001$), 0.5 nmol of M617 (Hot-plate Test: $F_{\text{left/left}}(1,9)=55.89$, $P<0.001$; $F_{\text{right/right}}(1,9)=106.7$, $P<0.001$. Randall Selitto Test: $F_{\text{left/left}}(1,9)=32.16$, $P<0.001$; $F_{\text{right/right}}(1,9)=23.51$, $P<0.001$) or 1 nmol of M617 (Hot-plate Test: $F_{\text{left/left}}(1,9)=42.45$, $P<0.001$; $F_{\text{right/right}}(1,9)=149.23$, $P<0.001$; Randall Selitto Test: $F_{\text{left/left}}(1,9)=44.72$, $P<0.001$; $F_{\text{right/right}}(1,9)=67.63$, $P<0.001$),

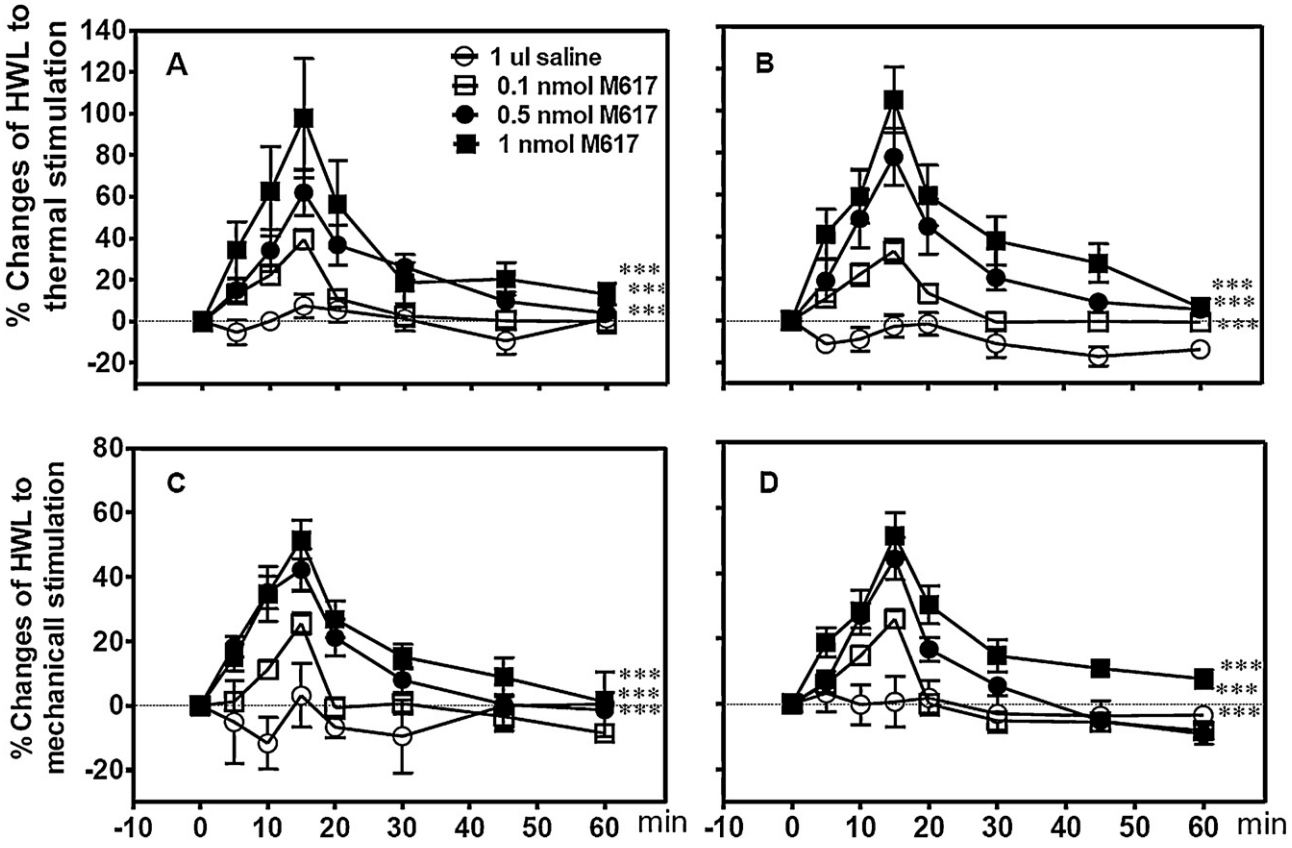

Fig. 1. Effects of intra-CeA injection of 0.1 nmol, 0.5 nmol, 1 nmol of M617 on the HWLs to thermal (A and B) and mechanical stimulation (C and D) in rats. Intra-CeA injection of 1 μl of 0.9% saline is as a control. Data are presented as mean ± S.E.M. The statistical difference between groups was determined by two-way ANOVA. CeA, the central nucleus of amygdala; HWL, hindpaw withdrawal latency.