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Short communication

N-palmitoylethanolamide, an endocannabinoid, exhibits antidepressant effects in the forced swim test and the tail suspension test in mice

Hai-Ling Yu[1], Xian-Qing Deng[2], Ying-Jun Li[1], Ying-Chun Li[1], Zhe-Shan Quan[2], Xian-Yu Sun[3]

[1]College of Basic Medicine, Yanbian University, Yanji, Jilin, 133000, China

[2]College of Pharmacy, Yanbian University, Yanji, Jilin, 133000, China

[3]College of Animal Science and Technique, Bayi Agriculture University, Daqing, Heilongjiang, 163319, China

Correspondence: Zhe-Shan Quan, e-mail: zsquan@ybu.edu.cn; Xian-Yu Sun, e-mail: sxianyu@sohu.com

Abstract:
The antidepressant-like effects of N-palmitoylethanolamide (PEA), a putative endocannabinoid, was investigated in mice using the tail suspension test (TST) and the forced swimming test (FST). In TST, PEA (10, 20, and 40 mg/kg) produced a statistically significant reduction in immobility (50, 32, and 34%, respectively, *vs.* the control group), whereas fluoxetine (20 mg/kg) reduced immobility by 38%. In FST, PEA (5, 10, and 20 mg/kg) produced a statistically significant reduction in immobility (15, 21, and 36%, respectively), whereas fluoxetine (20 mg/kg) reduced immobility by 18%. Moreover, PEA (20 mg/kg) did not significantly change motor activity in a spontaneous behavioral test. In conclusion, PEA (dose range of 5–40 mg/kg) administered orally reduced immobility in TST and FST, comparable to the antidepressant effect of fluoxetine, and had no effect on spontaneous activity in mice.

Key words:
N-palmitoylethanolamide, forced swimming test, tail suspension test, open-field test

Introduction

N-palmitoylethanolamide (PEA; Fig. 1), an endocannabinoid, belongs to a family of endogenous lipid amides [22, 31]. It is secreted by human adipocytes and possesses anti-inflammatory [18], analgesic, possibly vasomodulation properties [10, 19], a significant antiepileptic effect [15, 22, 31] and may be used in treatment of anxiety disorders [29].

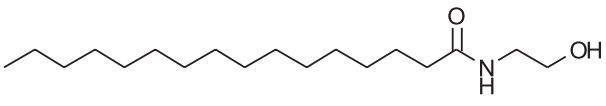

Fig. 1. Structure of N-palmitoylethanolamide

The central endocannabinoid system is a neuroactive lipid signalling system in the brain which acts to control neurotransmitter release. The expression patterns of this system throughout limbic regions of the brain ideally situate it to exert regulatory control over emotional behavior, mood and stress responsivity [16, 17]. Malfunctions in the endocannabinoid system may promote the development and maintenance of psychiatric disorders such as depression and panic disorder [3, 30]. A growing body of evidence une-

quivocally demonstrates that deficits in endocannabinoid signalling may result in depressive and anxiogenic behavioral responses, while pharmacological augmentation of endocannabinoid signalling can produce both antidepressive and anxiolytic behavioral responses [3, 10, 16, 24].

Depression is a major mental disorder associated with symptoms such as regular negative moods, decreased physical activity, feelings of helplessness, and sluggish thought and cognitive dysfunction [11, 24], and the prevalence of depression is rising every year. The current antidepressant drugs can only alleviate some symptoms, and side effects are common. Therefore, the research and development of more effective and less toxic antidepressants has attracted significant attention in recent years [13, 17, 23]. Reduced functionality might be considered a predisposing factor for major depression, boosting endocannabinoid tone might be a useful alternative therapeutic approach for depressive disorders [3, 24, 31]. So in the present study, we examined the antidepressant effect of PEA.

Materials and Methods

Drugs

The tested compound PEA (synthesized at the College of Pharmacy, Yanbian University, Jilin Province, China) and fluoxetine (Western Shanghai Pharmaceutical Co., Ltd., China) were suspended in 0.3% methyl cellulose (Loba-Chemie, Shanghai, China). All doses were expressed as milligrams per kilogram body weight of the respective drugs.

Animals

Male adult Kunming mice (Laboratory Animal Centre, College of Basic Medicine, Yanbian University, Jilin Province, China), weighing 20–24 g, were used. Animals were housed 5 per cage (32 × 18 × 16 cm) under a normal 12 h/12 h light/dark schedule with lights on at 07:00 a.m. They had free access to tap water and food pellets. Ambient temperature and relative humidity were maintained at 22 ± 2°C and 55 ± 5%, respectively. Mice were allowed at least 3 days to adapt to the laboratory environment before experiments. Experiments were performed by observers, who were unaware of the treatment that mice had received, and were carried out between 9:00 a.m. and 11:00 a.m. All studies were conducted in accordance with the Institutional Animal Care Committee at Yanbian University.

Tail suspension test (TST)

Seventy five mice were taken to the laboratory to adapt for 3 days and were randomly divided into 5 groups, 15 animals per group. Food, but not water, was withdrawn from the animals 1 h prior to drug administration. Five groups of mice were treated with a vehicle (0.3% methyl cellulose, 20 ml/kg, *po*), PEA (10, 20, and 40 mg/kg, *po*), or fluoxetine (20 mg/kg, *po*), at 8:00–9:00 a.m. for 7 consecutive days once daily. One hour after the last administration, the mice were submitted to the TST. TST was performed according to the method described by Steru et al. [21, 33], with slight modifications [27, 34, 35]. Briefly, the mice were individually suspended by the tail, using medical tape 2 cm away from the tail tip, to a fixed metal rod so that the head of the mouse hung down in the box (30 × 30 × 25 cm) to isolate the animal's attention; the head was 5 cm away from the bottom of the box. Initially, the mouse would move up and down around his head in an attempt to climb out. Mice were observed for 6 min, and the cumulative immobility time during the final 5-min interval of the test was recorded. The total duration of immobility (in s) was measured during the 5 min. 'Immobility' was defined as when they hung passively and were completely motionless.

Forced swimming test (FST)

First, 75 mice were randomly divided into 5 groups, 15 animals per group. Five groups of mice were treated with a vehicle (0.3% methyl cellulose, 20 ml/kg, *po*), PEA (5, 10, and 20 mg/kg, *po*), or fluoxetine (20 mg/kg, *po*), at 8:00–9:00 a.m. for 7 consecutive days once daily. The experiment was performed according to the procedure described by Porsolt et al. [27], with slight modifications [25, 32, 34]. Briefly, mice were individually forced to swim in a transparent glass cylinder (22-cm high, 14-cm diameter) filled 10-cm high with water (25 ± 0.5°C). All animals were forced to swim for 6 min, and the duration of immobility was observed and measured during the final 4-min interval of the test. The immobility period was regarded as the time spent by the mouse floating in the water without struggling and making only those movements neces-

sary to keep its head above the water. The water was changed after every other trial. The test was conducted 1 h after the last drug treatment, and groups of mice were tested in parallel. Each test was conducted in a quiet and warm environment.

Open-field test in mice

The open-field test was used to evaluate the exploratory activity of the animals [12, 34]. The spontaneous locomotor activities included different types of movements such as locomotion, rearing, and grooming. The investigated compound was administered 60 min before the experiment. The study was carried out in mice according to the method of Archer [2, 35] with slight modifications [7, 14, 33]. Each mouse was placed individually in the center of the open-field apparatus, and locomotor activity was assessed. The open-field apparatus was a nontransparent plastic container (80 × 60 × 30 cm); the underside was divided into equal-size 10 × 10 cm squares of 48 units without walls. The animals were gently placed in the center of the platform and were allowed to explore the surroundings. Hand-operated counters were used for 3 min to score locomotion (ambulation, number of line crossings with all four paws), rearing frequencies (number of times an animal stood on its hind legs), and grooming frequencies (number of modifications). The researchers, blind to the treatment groups, scored the behaviors in the open field. Experiments were performed in a dark room, and the apparatus was illuminated by a 60-W bulb giving a yellowish light, positioned 1 m above the center of the apparatus. The walls and floor surfaces were thoroughly cleaned with 10% ethanol between the tests.

Statistical analysis

The data are expressed as the means ± SEM and were evaluated by one-way analysis of variance followed by Tukey *post-hoc* test; $p < 0.05$ was considered to be statistically significant.

Results and Discussion

Based on the clinical association of depressive episodes with stressful life events, many of the animal models for the evaluation of antidepressant drug activity assess stress-precipitated behaviors [1, 7, 10, 35]. FST and TST in mice induce a state of despair in animals and have good reliability and predictive validity [9, 27, 28, 32, 33]. The main advantages of the two procedures are the use of a simple, objective test situation, the concordance of the results with the validated "behavioral despair" test, and the sensitivity to a wide range of drug doses [14, 28]. In the two models, mice are restricted and cannot escape, inducing a characteristic behavior of immobility. This behavior, reflecting a state of despair, is reduced by several agents that are therapeutically effective in human depression [10]. This immobility, or behavioral despair, is claimed to reproduce a condition similar to human depression [4, 6, 28].

In the FST or TST model, total immobility time is reduced by the majority of antidepressants when administered acutely or subchronically to animals [2, 21, 26, 35]. Fluoxetine (an SSRI) was found to decrease the amount of immobility and increase the prevalence of active behavior in the FST [8].

Post-hoc analysis revealed that a 7-day administration of 10, 20, or 40 mg/kg PEA induced a significant reduction in immobility time in mice during the TST as compared to the control group [$F(4,70) = 15.37$, $p < 0.001$]; the positive control, fluoxetine (20 mg/kg), also induced a significant change in immobility time ($p < 0.001$) as compared to control group (Fig. 2), and the results indicated that the activity of PEA in the TST was more effective at a lower dosage (10 mg/kg), while treatment with 40 mg/kg caused a decline in activity. Consequently, the mice were treated with PEA at 5, 10, or 20 mg/kg for 7 days in FST. In the FST,

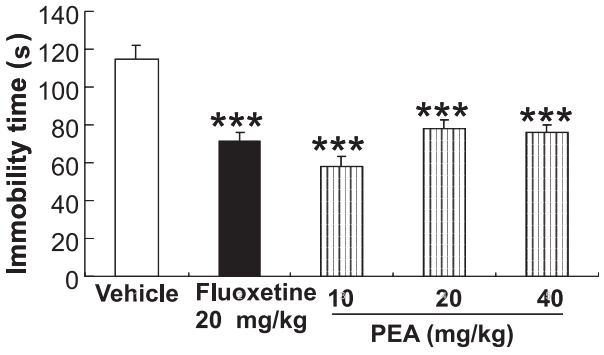

Fig. 2. Effect of N-palmitoylethanolamide (PEA) on immobility time in the tail suspension test (TST) in mice. Test solutions were administered by gastric gavage 60 min prior to the test. Values represent the mean ± SE. (n =15). *** $p < 0.0001$ *vs.* control (vehicle) group

PEA (5–20 mg/kg) treatment significantly decreased the duration of immobility as compared to the control group [F (4,70) = 6.857, p < 0.001, Fig. 3]. PEA exhibited a dose-dependent reduction in the immobility time of mice in FST, demonstrating that PEA possessed antidepressant-like activity in the behavioral models TST and FST. This study also demonstrated that PEA appears to have an effect comparable to the same dose of fluoxetine (Figs. 2, 3).

Some compounds may give false positive/negative effects in the FST and TST, in particular psychomotor stimulants, which decrease immobility time by stimulating locomotor activity, and drugs enhancing motor activity [2, 20]. Thus an additional measurement, the open-field test, was carried out with the specific aim of observing motor activity. Spontaneous locomotor activity was evaluated for 3 min, according to the procedure described above, and the effect of PEA was evaluated in the open-field test, a classical animal model for evaluating the autonomic effects of drugs and the general activity of animals [14, 32]. As shown in Figure 4, 7 days treatment with PEA did not change significantly the exploratory activity of mice, the amount of crossing [F (2,42) = 2.225, p > 0.05], rearing [F (2,42) = 2.051, p > 0.05], and grooming [F (2,42) = 0.778, p > 0.05] in the mice as compared to the vehicle-treated mice. The results demonstrated that PEA (20 mg/kg), after 7 days of treatment, did not significantly change the motor activity in mice and did not affect the body weight of the animals in any of the groups as compared with the controls (data not shown). Therefore, it is unlikely that these effects of PEA observed in the FST and TST were based on the stimulation of general motor activity.

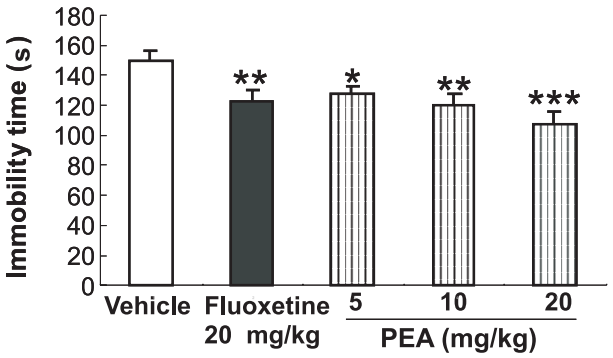

Fig. 3. Effects of N-palmitoylethanolamide (PEA) on the total duration of immobility in the forced swim test (FST) in mice. The drugs were administered 60 min before the test. The values represent the mean ± SEM (n = 15). * p < 0.05; ** p < 0.01; *** p < 0.0001 *vs.* control (vehicle) group

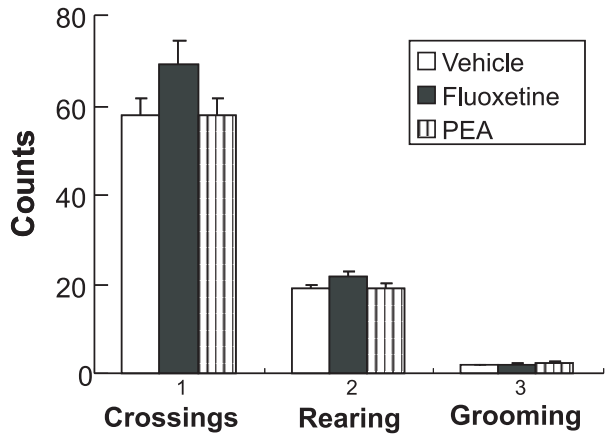

Fig. 4. Exploratory activity (counts) in the open field test. The behavioral parameters were recorded for 3 min. Locomotion, number of line crossings; Rearing, number of times seen standing on hind legs; Grooming, number of modifications; N-palmitoylethanolamide (PEA) was administered 60 min before the test. The values represent the mean ± SEM (n = 15)

The endocannabinoid system is a neuromodulatory system which is known to regulate emotional, cognitive, neurovegetative and motivational processes [17, 29]. Some scholars believe that cannabinoid-derived drugs potentiate monoaminergic neurotransmission and hippocampal neurogenesis through distinct pathways compared to classical antidepressants; they may represent an alternative drug class in the pharmacotherapy of mood and other neuropsychiatric disorders [3, 16]. So pharmacological augmentation of endocannabinoid signaling could be a novel target for the pharmacotherapy of depression [16, 17, 24].

Conclusion

In this study, the antidepressant-like effect of the endocannabinoid PEA was evaluated using TST and FST in mice. The results provide evidence that PEA possesses an antidepressant-like effect comparable to the reference drug – fluoxetine. With regard to the application of these results, PEA deserves more attention as a potential antidepressant. Therefore, endogenous cannabinoid compounds may play an important role in the near future in the treatment of depression.

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