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Research report

Sensitivity during the forced swim test is a key factor in evaluating the antidepressant effects of abscisic acid in mice

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HIGHLIGHTS

- FST sensitive and insensitive mice were screened out based on the FS pre-test.
- ABA showed antidepressant-like effects in FST sensitive mice but not insensitive.
- The two substrains mice performed differently during forced swimming.

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ABSTRACT

Abscisic acid (ABA), a crucial phytohormone, is distributed in the brains of mammals and has been shown to have antidepressant effects in the chronic unpredictable mild stress test. The forced swim test (FST) is another animal model that can be used to assess antidepressant-like behavior in rodents. Here, we report that the antidepressant effects of ABA are associated with sensitivities to the FST in mice. Based on mean immobility in the 5-min forced swim pre-test, ICR mice were divided into short immobility mice (SIM) and long immobility mice (LIM) substrains. FST was carried out 8 days after drug administration. Learned helplessness, as shown by increased immobility, was only observed in SIM substrain and could be prevented by an 8-day ABA treatment. Our results show that ABA has antidepressant effects in SIM substrain and suggest that mice with learned helplessness might be more suitable for screening potential antidepressant drugs.

1. Introduction

Major depression is a stress-related disorder that affects approximately 11% of the population, and it will become the second leading illness in the world by 2020 [1,2]. Antidepressant medications using specific monoamine reuptake inhibitors are frequently used for treating depression, but at least 50% of patients are poor responders, suggesting that other mechanisms are involved [3]. Therefore, a major emphasis in modern psychiatric research is to uncover the underlying aetiology of depression. A key component of the research is the use of animal models, which are predictive of antidepressant activity [4]. To date, a number of animal models for depression are currently available including the forced swim test (FST) [5], tail suspension test [6], chronic unpredictable mild stress [7], learned helplessness [8,9], and extinction-induced depression [10].

The rat FST originally developed by Porsolt et al. is highly valuable for assessing the antidepressant-like effects of the majority of currently-available antidepressants [11,12]. Lucki modified some parameters of the traditional FST in order to increase the reliable detection of selective serotonin reuptake inhibitors [13,14]. The modified FST has the additional benefit of determining whether a novel pharmacological agent predominantly activates the monoamine systems [4,15]. For both versions, a pre-test of 15 min or 10 min is included, as this accentuates the different behaviors in the 5-min swim test following drug treatment. Interestingly, for unknown reasons, only a test exposure (i.e., no pre-swim required) is sufficient in mice to ensure a stable immobility baseline, which can be reduced with a broad range of acute antidepressant treatment [4]. In this study we thus used a 5-min pre-test to replace the 15 min pre-test, screening the susceptibility to forced swimming first, and then use the 5-min FST to detect the effects of drug 8 days later.

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Several reviews have documented strain and gender differences in the FST in both rodents and mice [16,17]. Besides this, there are documented variations in FST behavior with day schedule at which the test is done [18]. The FST has been used to breed two separate lines, namely swim-test-susceptible and swim-test-resistant rats. After an uncontrollable stress procedure, rats that showed a large decrease in climbing behavior 90 min after stress were designated as swim-test susceptible, whereas those that did not differ from non-stress controls were designated as swim-test resistant [4,19]. Notably, animals from the 12th to 15th generations were shown to have selective differences in response to antidepressant administration. Specifically, antidepressant administration prevented the stress-induced decrease in climbing behavior in the susceptible line, whereas no effect of treatment was observed in the resistant line [20]. The influence of the strain of rodent on behavior in the FST has been more extensively characterized in mice than rats [4]. In female mouse, FST behavior also varies with estrous cycle stage [21]. But no one has documented differences in male mouse behavior in the FST with all these factors controlled for. Therefore, it is necessary to investigate the contribution of FST performance to the predictability of screening for potential antidepressant drugs.

Clinical reports have suggested that excess retinoic acid (RA) is associated with depression [22], and findings of our group suggest that the RA signaling pathway might be involved in the pathophysiology of depression [23–25]. Interestingly, abscisic acid (ABA), which is a phytohormone that regulates fundamental physiological functions in plants [26], shares a similar molecular structure with RA [27]. Moreover, it has been discovered that ABA exists and functions in a wide range of mammals [28–32]. In humans, ABA has been shown to regulate different cell functions including inflammatory processes, insulin release and glucose uptake [33]. Furthermore, ABA is considered to be an anti-inflammatory factor in mammals that down-regulates the inflammatory and immune responses in mouse models of obesity, diabetes, colitis and pulmonary disease [34–36]. In our recent studies, we demonstrated that ABA could improve spatial memory in rats [37] and is a potential antidepressant that acts by regulating the activity of the hypothalamic-pituitary-adrenal (HPA) axis, as shown by its antidepressant effects in the context of the chronic unpredictable mild stress test [38]. In the present study, we investigated whether ABA administration could improve depression-like behaviors in the FST and studied how the sensitivity of mice to the FST affects the evaluation of the antidepressant effects of drugs.

2. Materials and methods

2.1. Animals

Male ICR mice, an outbred colony, were purchased from Shanghai Laboratory Animal Center, Chinese Academy of Sciences, Shanghai, China. The mice were given free access to food and water and were maintained on a 12-h light/dark cycle (lights on 0700 h) at 22 ± 1 °C and 50–60% relative humidity. The mice were housed in regular cages (30 × 20 × 12 cm, 4 in one cage) without enrichment environment in the specific pathogen free mouse facilities. Mice were handled for 5 min daily for 7 days before behavioral testing. All animal experiments and procedures were approved by the Animal Care and Use Committee of the University of Science and Technology of China and were in accordance with the guide for Care and Use of Laboratory Animals of the National Institutes of Health (NIH publication No. 85–23, revised 1985).

2.2. Experimental design and drug administration

Seventy-two ICR mice (2 months of age) were subjected to a 5-min forced swim pre-test and then divided into SIM substrain and LIM substrain according to their mean immobility time (see below). They were further divided randomly into three groups (n = 12 for each): ABA group mice were administered 60 mg/kg (±)-*cis*, *trans*-ABA (Sigma, St. Louis, USA), mifepristone group mice were treated with 2.5 mg/kg mifepristone (Tocris Bioscience, Bristol, United Kingdom), and control mice were treated with vehicle. ABA and mifepristone were dissolved in a vehicle of sterile saline solution (0.9% w/v sodium chloride) with dimethyl sulphoxide (DMSO) at a ratio of 1:1 (v/v). All treatments were given by intraperitoneal (i.p.) injections between 0800 and 0900 every day for 8 days. The 5-min FST was conducted 1 h later on the final day of administration, and then the brain tissues were collected immediately (Fig. 1). The dose of ABA was based on the reports of Guri et al. and our previous findings [34,38].

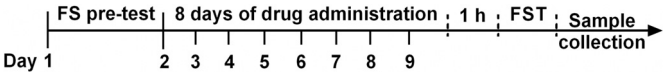

Fig. 1. Schedule of drug administration.

2.3. FST

The FST was performed according to previous reports [4,39]. The behavioral apparatus was a cylindrical tank with water in which mice cannot touch the bottom of the tank or escape. The tank is made of transparent Plexiglas, 30 cm high and 20 cm in diameter, and filled with water at 22 ± 2 °C to a depth of 19 cm. The mice were placed in the cylinder for 5 min between 1000 h and 1500 h, and the session was videotaped. Moreover, the water was replaced with cleaned one after each test. Three predominant behaviors are observed in the FST: immobility (the mouse floats in the water without struggling and only moves enough to keep its head above water), swimming (the mouse moves horizontally in the swim cylinder, including crossing into another quadrant), and climbing (upward-directed movement of the forepaws, usually against the side of the swim cylinder) [4,40]. Scoring was performed by an independent observer who was blind to the treatment conditions. The total time spent engaged in each activity was analyzed.

2.4. RNA isolation and quantitative real-time PCR

Hypothalamus and hippocampus were rapidly dissected after death and frozen quickly in liquid nitrogen and stored at −80 °C. The total RNA was extracted using the TRIzol (Pufei, Shanghai, China). Total RNA (500 ng) was then reverse transcribed (TaKaRa, Dalian, China) into cDNA and analyzed via Q-PCR using a SYBR Green PCR Master Mix (TaKaRa, Dalian, China) on a QuantStudio 7 platform (Applied Biosystems, Foster, USA). A Q-PCR system was applied in a 10-μl volume for 40 cycles (15 s at 95 °C and 1 min at 60 °C). The following primers were used in our study were as follows: mouse β-actin: 5′-ggctgtattcccctccatcg-3′ and 5′-ccagttggtaacaatgccatgt-3′; mouse CRH: 5′-catgttaggggcgctctc-3′ and 5′-aggaggcatcctgagagaagt-3′; mouse c-fos: 5′-ctacgaggcgtcatcctccc-3′ and 5′-tccgttccccttcggattctc-3′; mouse synapsin I: 5′-cacagctggcccagaaac-3′ and 5′-ttggtcagagactgggatttg-3′. The gene expression levels were evaluated using the $2^{-\Delta\Delta Ct}$ method.

2.5. Data analyses

Data analyses were performed using SPSS Statistics 19.0 (IBM, NY, USA). The values are expressed as the means ± SEM. The differences between two groups were tested using Student's t-test. The differences between three groups were tested using a one-way analysis of variance (ANOVA), followed by an LSD post hoc test. The