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Preliminary evidence that abscisic acid improves spatial memory in rats

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HIGHLIGHTS

- ABA administered peripherally was rapidly taken up into the rat brain.
- ABA improved long-term spatial memory in young rats.
- ABA increased arm entries in a Y-maze test of short-term memory in young rats.

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ABSTRACT

Abscisic acid (ABA) is a crucial phytohormone that exists in a wide range of animals, including humans, and has multiple bioactivities. As direct derivatives of carotenoids, ABA and retinoic acid (RA) share similar molecular structures, and RA has been reported to improve spatial memory in rodents. To explore the potential effects of ABA on spatial learning and memory in rodents, 20 mg/kg ABA was administered to young rats for 6 weeks, and its effects on behaviour performance were evaluated through a series of behavioural tests. ABA pharmacokinetic analysis revealed that the exogenous ABA was distributed widely in the rat brain, characterised by rapid absorption and slow elimination. The behavioural tests showed that ABA increased both the duration spent in the target quadrant and the frequency it was entered in the probe test of the Morris water maze (MWM) and decreased the latency to locate the target quadrant. Moreover, ABA decreased the latency to enter the novel arm in the Y-maze test, accompanied by increases in the total entries and distance travelled in the three arms. However, there were no significant differences between the ABA-treated and control rats in the open field test and elevated plus-maze test. These results preliminarily indicate that ABA improves spatial memory in MWM and exploratory activity in Y-maze in young rats.

1. Introduction

Abscisic acid (ABA) is one of the "classical" plant hormones and regulates many aspects of plant growth, development and environmental stress response [1,2]. Recently, it was discovered that ABA functions as an endogenous signal hormone not only in plants but also in a wide range of animals. In addition to its roles in regulating stem cell expansion [3], pro-inflammatory factor release [4–6], insulin release [7], and glucose uptake [8], ABA is also considered an anti-inflammatory factor in mammals, as evidenced by its ability to down-modulate the inflammatory and immune responses in mouse models of obesity, diabetes, colitis and pulmonary disease after chronic dietary ABA [9–11]. Moreover, Le Page-Degivry et al. reported that ABA concentrations are significantly higher in the brain than in other tissues of pigs and rats, and they confirmed that ABA is produced and released by the brain itself (e.g., microglial cells) [12,13]. However, it remains unclear whether exogenous ABA can permeate into the brain and affect behavioural performance.

ABA and retinoic acid (RA) are direct derivatives of carotenoids acting as hormones [14,15], and both substances share a similar molecular structure; i.e., with a free carboxyl group at the end of the isoprene-composed side-chain, which is the critical part of their bioactivity [16,17]. Chronic RA administration has been found to rescue memory deficits and neuropathological changes in murine models of Alzheimer's disease [18,19], reverse spatial memory deficits in vitamin-A deprived rats [20], and alleviate the age-related deficit in the CA1 long-term potentiation efficacy of aged mice [21]. Moreover, chronic isotretinoin treatment (13-cis-retinoic acid, reaches bioactivity primarily via isomerisation to RA) was found to improve impaired memory in models of ageing, experimentally induced amnesia, and Alzheimer's disease [22–24]. Although RA can ameliorate learning and memory in rodents, it is a known teratogen, and its physical side effects are well documented; i.e., adverse effects on mood, liver function, and the mucocutaneous,

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ophthalmic, and musculoskeletal systems [25,26]. In the present study, we investigate whether ABA, a naturally occurring phytochemical molecule, can be used as an alternative to RA to improve spatial learning and memory.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley (SD) rats were provided with free access to food and water and maintained on a 12-h light/dark cycle (lights on at 0700 h) at 22 ± 1 °C and 50–60% relative humidity. Rats were handled daily for 5 min for at least 4 days before initiating ABA treatment. All experimental procedures in this study were approved by the Animal Care and Use Committee at the University of Science and Technology of China, which complies with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication no. 85-23, revised 1985).

2.2. Pharmacokinetic study design

Forty-eight rats weighing 250 to 300 g (7 weeks old) were administered a dose of 20 mg/kg ABA. Four rats were sacrificed at each of the following time points: 0 min, 5 min, 10 min, 20 min, 30 min, 40 min, 1 h, 2 h, 3 h, 4 h, 8 h, and 12 h after intraperitoneal (i.p.) injection. Blood samples were collected and centrifuged at 4 °C (4000 rpm, 10 min). The hypothalamus, hippocampus, cortex and cerebellum were isolated immediately after the death. All samples were stored at −80 °C until ABA assay.

2.3. ABA purification and determination

Rat brain tissues were weighed (wet weight) and homogenised in 80% methanol (extract buffer) containing 1 mM 2,6-dibutyl-4-methyl phenol (Sigma, St. Louis, USA) and held at 4 °C overnight. The samples were centrifuged twice at 4 °C (5000 rpm, 5 min), and the supernatants were collected and dehydrated using nitrogen blowing.

ABA levels were measured using radioimmunoassay (RIA) as described previously [27,28]. The reaction volume contained 100 μl of sample (dissolved in 50% PBS) or serum, 150 μl 50% PBS, 100 μl diluted antibody solution (Mac252, Abcam, Cambridge, USA), and 100 μl [^{3}H]-ABA solution (approximately 16,000 cpm; ARC, St. Louis, USA). The reaction mixture was incubated at 4 °C for 45 min, and then 500 μl saturated ammonium sulphate (Sigma, St. Louis, USA) was added at room temperature for 30 min to end the reaction. The samples were centrifuged (12,000 rpm, 5 min) to obtain the precipitated pellet, which was then washed with 50% saturated ammonium sulphate and centrifuged again. The pellet was dissolved in 250 μl distilled water and 1.5 ml of scintillation liquid (Sigma, Waltham, USA). Finally, bound radioactivity in the precipitated pellets was measured using a liquid scintillation counter (PerkinElmer, Waltham, USA).

2.4. Chronic ABA administration

Four-week-old SD rats received daily intraperitoneal (i.p.) injections of 20 mg/kg (±)-*cis*, *trans*-ABA (ABA, n = 7) (Sigma, St. Louis, USA) or vehicle (control, n = 6). ABA was dissolved in the vehicle of sterile saline solution (0.9% w/v sodium chloride) with dimethyl sulphoxide (DMSO) at a ratio of 1:1 (v/v). The dose of 20 mg/kg ABA was based on the findings of Guri et al. [11], modified according to the formula for dose translation based on body surface area. All rats were treated between 1100 h and 1200 h from 6 weeks before the start of behavioural tests until the completion of the behavioural tests.

2.5. Behavioural tests

Behavioural tests were performed in a soundproof room with a neutral environment. Each test was conducted during the light phase of the light/dark cycle in the following order: prehensile traction test, beam balance test, walk test, open field (OF) test, elevated plus-maze (EPM) test, Y-maze test, and Morris water maze (MWM) test (Fig. 1). All tests were conducted between 0830 h and 1100 h after a 30-min habituation period and video-recorded for subsequent analysis. The experimental observer was blind to the treatment group being scored.

2.6. Prehensile traction test

This test measured muscle strength and equilibrium [29,30]. A 55-cm in length and 0.3-cm in diameter rope was placed horizontally 50 cm above a foam pad. The forepaws of the test subject were placed on the centre of the horizontal rope, and the rat was then released. The rat's performance was scored as follows: 0, hangs on 0 to 2 s; 1, hangs on 3 to 4 s; 2, hangs on 5 s, no third limb up to the rope; and 3, hangs on 5 s and brings hind limb up to the rope. The suspension time of each rat before it fell down (up to 60 s) was also recorded.

2.7. Beam balance and beam walk tests

These two tests assessed gross and fine motor function, respectively [30,31]. The beam balance test consisted of placing the animal perpendicularly on an elevated (50 cm) narrow wooden beam (2.3 × 120 cm) for a maximum of 60 s and scoring performance as described previously [30]. The beam walk test was performed using the same elevated beam marked at 5-cm intervals. The number of segments crossed was recorded over three trials.

2.8. Open field test

The OF test was used to analyse anxiety-like behaviour [32] and the locomotor activity of animals in a novel environment [33]. The floor of a 100 × 100 cm square area surrounded by a 50-cm high wall was divided into 16 equally sized squares by white lines. Rats were placed individually in one of the four corners, facing the wall, and permitted to explore the environment for 5 min. The total number of squares crossed, the

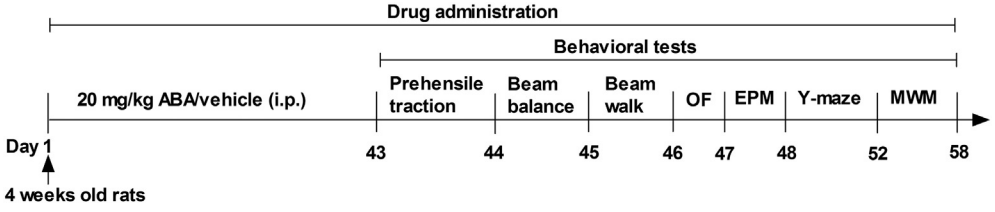

Fig. 1. The schedule of chronic ABA administration in the experimental design.