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Phytohormone abscisic acid elicits antinociceptive effects in rats through the activation of opioid and peroxisome proliferator-activated receptors β/δ

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ABSTRACT

The phytohormone abscisic acid exists in animal tissues particularly in the brain. However, its neurophysiological effects have not yet been fully clarified. This study was designed to evaluate the possible antinociceptive effects of abscisic acid on animal models of pain and determine its possible signaling mechanism.

Tail-flick, hot-plate and formalin tests were used to assess the nociceptive threshold. All experiments were carried out on male Wistar rats. To determine the role of Peroxisome proliferator-activated receptor β/δ (PPARβ/δ) and opioid receptors on the induction of abscisic acid antinociception, specific antagonists were injected 15 min before abscisic acid.

The data showed that abscisic acid (5, 10 and 15 μg/rat, i.c.v.) significantly decreased pain responses in formalin test. In addition, it could also produce dose-dependent antinociceptive effect in tail-flick and hot-plate tests. Administration of PPARβ/δ antagonist (GSK0660, 80 nM, i.c.v.) significantly attenuated the antinociceptive effect of abscisic acid in all tests. The antinociceptive effects of abscisic acid were completely inhibited by naloxone (6 μg, i.c.v.) during the time course of tail-flick and hot-plate tests.

The results indicated that the central injection of abscisic acid has potent pain-relieving property which is mediated partly via the PPAR β/δ and opioid signaling.

1. Introduction

Plant hormone abscisic acid is a sesquiterpenoid (C15H20O4) which was discovered in 1960 (Finkelstein et al., 2002; Schwartz et al., 2003). In plants, it regulates physiological and defensive functions including seed development, adaptation to abiotic conditions of environment and resistance against pathogens and infection (Finkelstein et al., 2002; Mauch-Mani and Mauch, 2005). It also acts as antioxidant agents in laboratory animals (Celik et al., 2007). Surprisingly, phytohormone abscisic acid is produced in animal tissues (from sponges to mammals) particularly in mammalian brain (Le Page-Degivry et al., 1986; Zocchi et al., 2001). Endogenous abscisic acid is released from innate immune cells such as monocytes, macrophages and granulocytes and acts as an autocrine or paracrine agent in inflammatory processes (Bruzzone et al., 2007; Magnone et al., 2009, 2012). However, its possible neurophysiological roles have not yet been fully clarified.

Administration of abscisic acid has numerous pharmacological effects including anti-inflammatory, antioxidant, anti-atherosclerosis, anti-depressant, pro-cognitive and anti-anxiety, and anti-cancer effects (Guri et al., 2007, 2008, 2010a; Suzuki et al., 1998; Tan et al., 2006; Qi et al., 2014; Naderi et al., 2017; Soti et al., 2018). Recently, it has been reported that abscisic acid ameliorates cognitive impairment on high-fat diet-induced neuroinflammation in rats (Sanchez-Sarasua et al., 2016). It has been reported that abscisic acid activates several intracellular and extracellular receptors. They include lanthionine synthetase C-like protein 2 (LANCL2) located on plasma membrane and peroxisome proliferator-activated receptors (PPARs) members of the nuclear receptors superfamily (Bassaganya-Riera et al., 2011; Sturla et al., 2009). Molecular studies have shown that silencing of LANCL2 inhibits abscisic acid effects in granulocyte (Sturla et al., 2009). PPARs have three isoforms including PPARα, PPARβ/δ and PPARγ which are known to regulate the nociceptive and inflammatory responses (Freitage and Miller, 2014).

Abscisic acid is structurally similar to thiazolidinediones (as PPARγ agonist) and both compounds ameliorate insulin resistance and inhibit systemic inflammation (Bishop-Bailey and Bystrom, 2009; Guri et al., 2011). In humans, a gene network has been identified and considered as ortholog for arabidopsis abscisic acid-related genes. PPARγ receptors are involved in the abscisic acid-related human genes network (Bassaganya-Riera et al., 2010). PPARβ/δ has high capacity to bind to

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the lipophilic vitamins and also to vitamin A metabolite, retinoic acid (Bishop-Bailey and Bystrom, 2009; Shaw et al., 2003). On the other hand, abscisic acid as a lipophilic compound is the retinoic acid analogue and both of them are synthesized from β-carotene as vitamin A precursor (Finkelstein et al., 2002; Qi et al., 2015). However, in spite of the existence of abscisic acid in most parts of the brain, its interaction with PPARβ/δ has not yet been studied (Le Page-Degivry et al., 1986). It has been reported that PPARs signaling suppresses pain behaviors induced by chemical and mechanical tissue injury, nerve damage, and inflammation (LoVerme et al., 2006).

Since abscisic acid is produced in the brain and possibly can be absorbed from diet (fruits and vegetables) and the possible antinociceptive effect of it has not yet been clarified, the present study was designed to analyze the possible antinociceptive effect of this phytohormone using formalin, tail-flick and hot-plate tests in rats. In addition, the involvement of PPARβ/δ and opioid receptors signaling was analyzed by pharmacological tools using a specific antagonist.

2. Materials and methods

2.1. Animals

Male Wistar rats (230–270 gr) were obtained from the Shahid Bahonar University of Kerman Animal House. Food and water were available ad libitum. The animals were housed under a 12 h light/dark cycle in controlled condition with temperature of 22 ± 2 °C. Animals were handled daily for 4 days before the experiment day in order to adapt them to manipulation and reduce nonspecific stress responses. All experiments followed the guidelines on ethical standard for investigation of experimental pain in animals (Zimmermann, 1988) and were approved by the Animal Experimentation Ethic Committee of Kerman Neuroscience Research Center (EC/KNRC/95–26).

2.2. Surgery

Anesthetized rats with ketamine and xylazine (60 mg and 10 mg/kg, respectively) were implanted with guide cannula stereotaxically (Stoelting, USA). Guide cannulas (22-gauge stainless steel tubing) were implanted and aimed bilaterally into right and left ventricles according to the atlas of Paxinos and Watson (1998). The intended coordinates for the left and right ventricles were AP = 1.6 mm from Bregma, ml = ± 0.8 from the midline and DV = 3.4 mm from the skull surface. Guide cannulas were fixed to skull by means of two stainless steel screws and acrylic dental cement. After surgery, the animals were transferred to their home cages and given recovery at least a week before drugs injection and behavioral experiment.

2.3. Drugs

(±)-cis, trans- abscisic acid and GSK0660 were purchased from Sigma-Aldrich (USA). Sodium salicylate and naloxone were gifted by Exir Pharmaceutical Co. (Iran). Sodium salicylate was dissolved in physiological saline and injected intraperitoneally (i.p.). Naloxone was dissolved in artificial *cerebrospinal fluid* (aCSF). Abscisic acid and GSK0660 were dissolved in dimethyl sulphoxide (DMSO) then diluted with aCSF. Ratio of aCSF to DMSO was 2:1 (v/v). Abscisic acid, GSK0660 and naloxone were administrated intraventricularly (i.c.v.). The drugs were given in the volume of 1 ml/kg (i.p.) and in a total volume of 2 μl (i.c.v.).

2.4. Microinjection

The drugs were microinjected by a 27-gauge needle and polyethylene tube (PE-10) fitted to a 1.0 μl Hamilton microsyringe. The injection needle was longer than cannula and ended 1 mm below the tip of the cannula. Drugs were separately delivered in the volume 1 μl per side into ventricles. All of behavioral tests were carried out between 09:00 a.m. and 13:00 a.m.

2.5. Experimental design

Rats were divided randomly into several experimental groups (n = 6–7). Control group (Cont) which had no surgery and treatment; vehicle-treated group (Veh) which received abscisic acid vehicle; abscisic acid-treated groups which received different doses of abscisic acid (5, 10 or 15 μg/rat), GSK0660 plus abscisic acid-treated group which received 80 nM/rat GSK 15 min before abscisic acid injection, and naloxone plus abscisic acid-treated group that had naloxone (6 μg/rat) 15 min before the injection of abscisic acid. In formalin test, 50 μl of formalin solution (2.5%) in saline was injected in the dorsal surface of the left hind paw using a tuberculin syringe (Formalin group). In addition, a group of rats received sodium salicylate (300 mg/kg, i.p.) 30 min before formalin injection as positive control group.

2.6. Formalin test

Formalin-test was used as a model for induction of biphasic pain including neurogenic phase which immediately started after subcutaneous formalin injection and followed with an inflammatory phase which lasted for 40–50 min (Dubuisson and Dennis, 1977). Seven days after recovery this test was performed as follows. The Rats received different doses of abscisic acid 30 min prior to formalin injection. In antagonist groups, the animals were pretreated with GSK0660 or naloxone 15 min prior to abscisic acid injection. Each rat was immediately put back into a 30 × 30 × 30 cm Plexiglas box equipped with a mirror placed below the floor at a 45°angle. Recording biting and licking behavior continued for 60 min in 5-min blocks. In this study, total time spent for biting and licking behavior was between 0 and 5 min and 15–60 min was calculated separately as early neurogenic (first) and late inflammatory (second) phases.

2.7. Tail flick test

Tail flick analgesiometer apparatus is commonly utilized for evaluating the antinociceptive effects of drugs in spinal and supraspinal levels. Here, abscisic acid-induced antinociception was assessed by the tail-flick test (D'amour and Smith, 1941). The tail-flick latency for each rat was determined three times and the mean was designated as baseline latency before drug injection. The intensity of the beam was adjusted to produce mean control reaction time between 4 and 6 s. The cut-off time was fixed at 15 s to avoid any damage to the tail. After assessing the baseline threshold, the rats received the mentioned drugs, and the reaction latency was determined in different times after injection. The tail-flick latencies were converted to the percentage of antinociception (maximum possible effect, MPE) according to the following formula:

$$\%MPE = (\text{reaction time of test} - \text{basal reaction time})/(\text{cut} - \text{off time} - \text{basal reaction time})*100$$

2.8. Hot plate test

The hot plate test is one of the most commonly used tests to evaluate antinociceptive effects of drugs (Carter, 1991) in supraspinal levels. Before drug treatment and for calculating baseline latency, the rats were retained on the hot plate heated surface (52 ± 2 °C) which was equipped with digital display. To prevent tissue damage 30 s cut-off time was considered. Behaviors related to licking or lifting hind paws (whenever started faster) were considered as latency response at 30, 60, 90 min after the treatment of drugs. The following formula was used to calculate the antinociception: %MPE = (latency after drug