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Central and peripheral antinociceptive effects of ellagic acid in different animal models of pain

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ABSTRACT

The present study was conducted to evaluate the analgesic effects of p.o., i.p., or i.c.v. administration of ellagic acid (EA), and investigate the possible mechanisms underlying the systemic antinociceptive activities in different animal models of pain. Using radiant heat tail-flick test, EA (100–1000 μmol/kg, p.o.) only resulted in antinociception at 1000 μmol/kg. Also, EA (10–660 μmol/kg, i.p.) produced the antinociceptive effect in a dose-dependent manner with an ED_{50} of 122 μmol/kg. In addition, the i.c.v. administration of EA (0.1–2 μmol/rat) resulted in dose-dependent antinociception with an ED_{50} of 0.33 μmol/rat. EA induced antinociception (330 μmol/kg. i.p.) was reversed by naloxone (1 mg/kg, i.p.). Likewise, EA (1–33 μmol/kg, i.p.) produced significant dose-dependent antinociception when assessed using acetic acid-induced abdominal writhing test with an ED_{50} of 3.5 μmol/kg. It was also demonstrated that pre-treatment with L-arginine (100 mg/kg, i.p.), a nitric oxide (NO) precursor, and methylene blue (20 mg/kg, i.p.), a guanylate cyclase (GC) inhibitor, significantly enhanced antinociception produced by EA suggesting the involvement of L-arginine–NO–cGMP pathway. Additionally, administration of glibenclamide (10 mg/kg, i.p.), an ATP-sensitive K^+ channel blocker, significantly reversed antinociceptive activity induced by EA. Moreover, EA treatment had no effect on the motor activity of rats when tested in rota-rod task. The present results indicate that the dose-related antinociceptive action of EA has both peripheral and central components which involve mediation by opioidergic system and L-arginine–NO–cGMP–ATP sensitive K^+ channels pathway.

1. Introduction

Phenolic compounds are secondary metabolites widely found in fruits, mostly represented by flavonoids and phenolic acids. They are usually referred to a diverse group of naturally occurring compounds with phenolic structural features (Tsao, 2010). They have synthetic, medicinal and industrial value. Naturally occurring polyphenols and or flavonoids are known to have numerous biological activities. They are found to be potential candidates for use as drugs, for example, in diseases like AIDS, heart ailments, ulcer formation, bacterial infection, mutagenesis and neural disorders. In contrast, little is known about the effects of polyphenols on the modulation of pain transmission (Handique and Baruah, 2002).

Ellagic acid (EA, 2,3,7,8-tetrahydroxybenzopyrano[5,4,3-cde]benzopyran-5-10-dione) is an excretion product of many plant species of economic importance, particularly fruits and nuts (Clifford and Scalbert, 2000; Wada and Ou, 2002). It is found in strawberries, cranberries, walnuts, pecans, and red raspberry seeds (Whitley et al., 2003). Also, this compound is a major component of pomegranate juice, an increasingly popular dietary supplement used by the American adult population (Corbett et al., 2010). It has been reported to show different pharmacological effects including chemoprevention (Townsend and Tew, 2003), inhibition of tumorigenesis (Buniatian, 2003), anti-inflammation and antioxidant (Festa et al., 2001; Lei et al., 2003; Solon et al., 2000), neuroprotection against diabetic neuropathy (Liu et al., 2011), inhibition of anaphylactic reaction *in vivo* and *in vitro* (Choi and Yan, 2009), and also inhibition of lipopolysaccharide-induced prostaglandin E2 synthesis in human monocytes (Karlsson et al., 2010).

Few studies have investigated the antinociceptive effects of EA *in vivo*. Rogerio et al. (2006) examined the anti-inflammatory and antinociceptive effects of EA in animal models. Their findings

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showed that EA significantly decreased paw edema, as measured by calipers after an injection of 1% carrageenan, and the number of acid-induced writhing periods in mice. They suggested that the reduction in writhing periods may be *via* cyclooxygenase inhibition or another antinociceptive pathway. In 2008, Beltz et al. examined the antinociceptive effects of EA in the rat hot-plate model. Fischer et al. (2008) evaluated the antinociceptive potential of two derivatives of EA, 3,4,3′-trimethoxyflavellagic acid and 3,4,3′-trimethoxy flavellagic acid 4′-O-glucoside, in the formalin model of pain. They showed that these compounds inhibited both phases of formalin induced nociception. Additionally, Corbett et al. (2010) showed that EA also decreases paw edema induced by 3% carrageenan and may interact with known cyclooxygenase inhibitor, ketorolac.

However, research studies have not clearly demonstrated the underlying mechanisms involved in the antinociceptive effects of EA. So, the primary aim of this study is to investigate the peripheral and central antinociceptive effects of EA using tail-flick and writhing tests. The second one is to evaluate whether there are interactions among EA, the opioid receptors and also L-arginine–NO–cGMP–K^+ channel pathway in the EA-induced antinociception.

2. Materials and methods

2.1. Animals

All animal care and experimental procedures were in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. We followed the ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann, 1983), as well as our institutional guidelines for experiments with animals, designed to avoid suffering and limit the number of animals. The number of animals and intensities of noxious stimuli used were the minimum necessary to demonstrate consistent effects of drug treatments.

Experiments were conducted using adult male Wistar rats (220 ± 20 g) and also Swiss mice (25–30 g) purchased from the central animal house of the Jundishapur University of Medical Sciences (Ahvaz-Iran). They were housed at 22 ± 2 °C and 12 h light/dark cycles (light from 7:00 to 19:00 h) with free access to food and water *ad libitum*. All animals were randomly divided into groups of 8 in each, acclimatized and habituated to the laboratory environment for at least 1 week prior to the experiments and were used only once throughout the experiments. In all experiments, data were collected by a blinded, randomized and controlled design.

2.2. Drugs and chemicals

The following drugs were used: Ellagic acid (EA), Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME), L-arginine hydrochloride (L-arginine), methylene blue, and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Glibenclamide donated by Poursina Pharmaceutical Co. (Tehran, Iran). Naloxone hydrochloride and morphine sulfate ($5H_2O$) were donated by Temad Pharmaceutical Co. (Tehran, Iran). All drugs were dissolved in normal saline (0.9% NaCl) and buffered to a pH of 7, while EA was dissolved in 10% DMSO/normal saline. Respective controls received only solvent vehicle. Drug concentrations were freshly prepared in such a way that the necessary dose could be injected in a volume of 5 ml/kg by both p.o. and i.p. routes unless otherwise stated in the method. The vehicle alone had no effects per se on the nociceptive responses. Doses and drug administration schedules were selected based on previous reports (Beltz et al., 2008; Rogerio et al., 2006) and on pilot experiments in our laboratory.

For i.c.v. injections, a permanent guide cannula was implanted in the right ventricle of the brain as described by Stoppa et al. (2008). Briefly, each rat was anesthetized with a mixture of ketamine hydrochloride (50 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.) and a 23-gauge, 12-mm stainless steel guide cannula was stereotaxically placed (Stoelting Stereotaxic Apparatus, Wood Lane, IL, USA) in the right lateral ventricle of the brain. The stereotaxic coordinates, according to Paxinos and Watson (2006), were: 0.8 mm posterior to the bregma, 2 mm lateral to the midline and 4 mm below the top of the skull. The guide cannula was anchored with jewelers' screws and dental acrylic cement. A 12.5 mm stylet was inserted into the guide cannula to keep it patent prior to injection. All animals were allowed 7 days to recover from surgery. Drugs or saline were delivered in a volume of 5 μl/rat. To ascertain the exact site of i.c.v. injection, some rats were injected i.c.v. with 5 μl of 1:10 diluted Indian ink and their brains were examined macroscopically after sectioning.

The i.c.v. injections of the vehicle and EA (0.1–2 μM/rat) were performed using a 10-μl Hamilton syringe over a period of 30 s with a constant volume of 1 μl. After completion of each i.c.v. injection, the needle was left in place for further 30 s to facilitate infusion of the drug solution. The i.c.v. injections of the drugs were performed 10 min before the nociceptive test.

2.3. Antinociceptive analysis

2.3.1. Central nociceptive model induced by radiant heat stimulation in rats

The antinociceptive effects of EA and the reference drug (morphine), represented by the time required for rat tail-flick after exposure to a source of radiant heat, were evaluated according to the description of D'Amour and Smith (1941). Briefly, each animal was placed in a Plexiglas box that allowed its tail to be free, then the box was placed on the tail stimulator analgesia meter (IITC Inc., USA) with the tail occluding a slit over a photocell for radiant heat stimulation generated by a power lamp mounted in a reflector. The tail-flick response was elicited by applying radiant heat to the point 1/3 of length away from the tip of the tail. When the rat felt pain and flicked its tail, the light of the lamp fell on the photocell such that the timer was automatically stopped. The intensity of the heat stimulus in the tail-flick test was adjusted so that the rat flicked its tail within 3–6 s. A 10 s cut-off time was set in order to prevent tissue damage. Before experiment, the heat stimulation latency of all animals were tested, and those with response time of <2 or >6 s to heat stimulation were excluded. The tail-flick responses were measured before, and 15, 45, 90, 150 and 210 min after the administration of EA (100–1000 μmol/kg, p.o.; 10–660 μmol/kg, i.p.; and 0.1–2 μmol/rat, i.c.v.), morphine (13.2 μmol/kg, i.p.), or respective vehicles. Tail-flick latencies were converted to % MPE (maximal possible effect) as follows: % MPE = 100x(post drug latency-predrug latency)/(cut-off time-predrug latency). Each animal was used as its own control.

2.3.2. Visceral nociceptive model induced by acetic acid stimulation in mice

All animals were acclimatized to laboratory environment for at least 2 h before testing. The abdominal writhing test induced by acetic acid stimulation in mice as originally described by Siegmund et al. (1957). Briefly, EA (1–33 μmol/kg) or the vehicle were intraperitoneally administrated 30 min prior to acetic acid injection. Immediately after intraperitoneal injection of 0.1 ml/10 g acetic acid (0.6% v/v) in normal saline (0.9% w/v NaCl), animals were isolated for observation. The numbers of abdominal writhing