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Original article

The possible mechanisms of protocatechuic acid-induced central analgesia

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ABSTRACT

It is aimed to investigate the central antinociceptive effect of protocatechuic acid and the involvement of stimulation of opioidergic, serotonin 5-$HT_{2A/2C}$, α2-adrenergic and muscarinic receptors in protocatechuic acid-induced central analgesia in mice. Time-dependent antinociceptive effects of protocatechuic acid at the oral doses of 75, 150 and 300 mg/kg were tested in hot-plate (integrated supraspinal response) and tail-immersion (spinal reflex) tests in mice. To investigate the mechanisms of action; the mice administered 300 mg/kg protocatechuic acid (p.o.) were pre-treated with non-specific opioid antagonist naloxone (5 mg/kg, i.p.), serotonin 5-$HT_{2A/2C}$ receptor antagonist ketanserin (1 mg/kg, i.p.), α2-adrenoceptor antagonist yohimbine (1 mg/kg, i.p.) and non-specific muscarinic antagonist atropine (5 mg/kg, i.p.), respectively. The antinociceptive effect of protocatechuic acid was observed at the doses of 75, 150 and 300 mg/kg in tail-immersion test, at the doses of 150 and 300 mg/kg in hot-plate test at different time interval. The enhancement in the latency of protocatechuic acid-induced response to thermal stimuli was antagonized by yohimbine, naloxone and atropine in tail-immersion test, while it was antagonized only by yohimbine and naloxone pretreatments in hot-plate test. These results indicated that protocatechuic acid has the central antinociceptive action that is probably organized by spinal mediated cholinergic and opiodiergic, also spinal and supraspinal mediated noradrenergic modulation. However, further studies are required to understand how protocatechuic acid organizes the interactions of these modulatory systems. As a whole, these findings reinforce that protocatechuic acid is a potential agent that might be used for pain relief. Additionally, the clarification of the effect and mechanisms of action of protocatechuic acid will contribute to new therapeutic approaches and provide guidance for new drug development studies.

1. Introduction

Phenolic compounds have gained attention since they are bioactive compounds. Protocatechuic acid (3,4-dihydroxybenzoic acid), an antioxidant phenolic acid, can reach tissues in amounts which can exert biological effects on health (Semaming et al., 2015). It has been reported that protocatechuic acid shows antioxidant, antihyperglycemic and neuroprotective effects (Masella et al., 2012). Because Protocatechuic acid can easily crosses the blood brain barrier, it gains attention in the inhibition of neurodegenerative progress based on existing data (Krzysztoforska et al., 2017). Protocatechuic acid also possesses anti-inflammatory and antinociceptive effects in different animal models (Lende et al., 2011). More recently, Dhanshree et al., (2017) showed that the treatment with protocatechuic acid for 21 days increased the pain threshold in diabetic neuropathic pain model. Although the antinociceptive effect of protocatechuic acid was shown in a few studies (Lende et al., 2011), the mechanisms of antinociceptive action have not been clarified yet. Revealing the mechanism of action of drugs is highly important to identify the effect profiles of drugs and rational drug use. Pain perception and control process

Abbreviations: ATR, atropine; CTRL, control group; HT, serotonin; i.p., intraperitoneal; KTS, ketanserin; MPE, maximal possible effect; NLX, naloxone; p.o., per oral; PCA, protocatechuic acid; YOH, yohimbine; δ, delta; κ, kappa.

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from the periphery to the central nervous system are mediated through ascending and descending networks that include endogenous opioid-, monoamine-, and acetylcholine-mediated mechanisms via their own receptors (de Freitas et al., 2004; Kirkpatrick et al., 2015). This study aimed to investigate the time-dependent central antinociceptive effects of protocatechuic acid at the per oral doses of 75, 150 and 300 mg/kg, and the investigation of spinal and supraspinal organization of its antinociceptive effect by pre-treatment with non-specific opioid antagonist naloxone (5 mg/kg, i.p.), serotonin 5-$HT_{2A/2C}$ receptor antagonist ketanserin (1 mg/kg, i.p.), α_2-adrenoceptor antagonist yohimbine (1 mg/kg, i.p.) and non-specific muscarinic antagonist atropine (5 mg/kg, i.p.) before the administration of 300 mg/kg (p.o.) protocatechuic acid in hot-plate (integrated supraspinal response) and tail-immersion (spinal reflex) tests in mice.

2. Materials and methods

2.1. Animals

Adult Balb-c male mice was used in experimental studies. The mice were maintained at constant room temperature (22 ± 2 °C) under a 12 h light–dark cycle with free access to standard food and water *ad libitum*. The animals received only water during the six hours preceding the experiments to avoid possible food interaction with protocatechuic acid. Analgesia tests were performed between 11:00 and 17:00 h. All experimental protocols were performed according to the principles and guidelines adopted by the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised in 1985) and approved by the Local Ethics Committee of Anadolu University and Osmangazi University, Eskisehir, Turkey.

2.2. Drugs and treatments

Protocatechuic acid (≥97% pure), α_2-adrenoceptor antagonist yohimbine (≥98% pure), serotonin 5-$HT_{2A/2C}$ receptor antagonist ketanserin (>97% pure), opioid antagonist naloxone (≥98% pure) and muscarinic receptor antagonist atropine (≥99% pure) (Sigma, St. Louis, MO, USA) were used in this study. All animals were randomly divided into groups of six animals each and baseline values of pain thresholds measured by hot-plate and tail-immersion tests. The first group was designated as control group that saline administered (p.o.) only (solvent vehicle). To the other three groups were orally treated with 75, 150 and 300 mg/kg protocatechuic acid by a gavage needle (18 G × 3 in. × 2.25 mm) which corresponds to an esophageal cannula. The antinociceptive effect of protocatechuic acid was tested time-dependently at 30, 45, 60, 90 and 120th minutes. Treatment schedule for mechanism of action studies as follows: pre-treatment with 5 mg/kg atropine 15 min before the 300 mg/kg protocatechuic acid administration, pre-treatment with 1 mg/kg ketanserin 30 min before the 300 mg/kg protocatechuic acid administration, pre-treatment with 1 mg/kg yohimbine 30 min before the 300 mg/kg protocatechuic acid administration, pre-treatment with 5 mg/kg naloxone 15 min before the 300 mg/kg protocatechuic acid administration, separately. All pre-treatments were performed as intraperitoneal (i.p.) route. The analgesia test procedures performed for mechanism of action studies were applied 45 min after 300 mg/kg protocatechuic acid administration since protocatechuic acid showed most significant antinociception at the dose of 300 mg/kg at 45 min.

2.3. Analgesia test procedures

2.3.1. Hot-plate test

The pain reflexes in response to ~~a~~ thermal stimulus were measured by using a Hot-Plate Analgesia Meter (No. 7280, Ugo Basile Instruments, Comerio, Italy) (Eddy and Leimbach, 1953). The mice were gently put on the surface of the hot plate, set to 55 ± 0.5 °C. The latency of hind paw licking, hind paw flicking, or jumping was measured as reaction time. The cut-off time was taken as 20 s to minimize hind paw damage.

2.3.2. Tail-immersion test

The painful thermal stimuli was induced by dipping the tail tips of mice into a hot water bath (Heto, Allerod, Denmark) at 52.5 ± 0.2 °C (Schmauss and Yaksh, 1984). The withdrawal latency of the tail from the hot water was noted as reaction time. The maximum cut-off time was 15 s to avoid the injury of tissues of the tail.

The results of the analgesia tests were expressed as a percentage of the maximal possible effect (MPE%) which was calculated over the latencies of response against thermal stimuli (Coelho et al., 2005):

$$\text{MPE\%} = [(\text{Postdrug latency}) - (\text{Predrug latency})]/[(\text{Cutoff time})(\text{Predrug latency})] \times 100$$

2.4. Data analyses

Statistical differences were analyzed using two-way ANOVA followed by Bonferroni method in antinociceptive action studies and one-way analysis of variance (ANOVA) followed by Newman-Keuls post-hoc tests in mechanism of action studies. The statistical analyses were carried out using GraphPad Prism version 5.0. The results were expressed as the mean ± standard error of the mean to show variation in groups. Differences were considered significant when $P \leq 0.05$.

3. Results

3.1. Central antinociceptive effect of protocatechuic acid

The MPE% values that describes the antinociceptive effect of protocatechuic acid in the hot-plate and tail-immersion tests are shown in Fig. 1 A and B, respectively. Protocatechuic acid at the doses of 150 and 300 mg/kg enhanced the response latency against thermal stimulus significantly ($P < 0.05$ and $P < 0.001$ for 150 mg/kg at 45 and 60th min, respectively; $P < 0.001$, $P < 0.001$ and $P < 0.01$ for 300 mg/kg at 45, 60 and 90th min, respectively) compared with the control group in the hot-plate test. 75, 150 and 300 mg/kg protocatechuic acid succeeded in increasing threshold significantly ($P < 0.01$ for 75 mg/kg at 60th min; $P < 0.05$, $P < 0.001$ and $P < 0.001$ for 150 mg/kg at 30, 45 and 60th min, respectively; $P < 0.001$ for 300 mg/kg at 30–60 min time interval) compared with the control group in the tail-immersion test.

3.2. Mechanism of action studies

Figs. 2 and 3 show that how pre-treatment of ketanserin (A), yohimbine (B), atropine (C) and naloxone (D) affect the MPE% values that describes the antinociceptive effect of 300 mg/kg protocatechuic acid in hot-plate and tail-immersion tests, respectively. The enhancement in the latency of 300 mg/kg protocatechuic acid-induced response to thermal stimuli was reversed significantly by yohimbine ($P < 0.01$) pre-treatment in hot-plate test, while it was reversed significantly by yohimbine ($P < 0.001$), naloxone ($P < 0.001$) and also atropine ($P < 0.01$) in tail-immersion test. The significant ($P < 0.001$) antinociception in 300 mg/kg protocatechuic acid-only group weakened in ketanserin and atropine pre-treated group ($P < 0.05$) in hot-plate test. The observed antinociception disappeared in naloxone pre-treated group although significant antagonism was not observed in hot-

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