Neuroscience Letters 550 (2013) 195–199

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Anticonvulsant screening of luteolin in four mouse seizure models

Mohd. Farooq Shaikh [1], Kah Ni Tan, Karin Borges *

School of Biomedical Sciences, The University of Queensland, St. Lucia, QLD 4072, Australia

HIGHLIGHTS

- Luteolin may be anticonvulsant due to its anxiolytic, anti-oxidant and anti-inflammatory properties.
- The effects of various luteolin doses (i.p.) were tested in four mouse seizure models.
- Acute luteolin had no effects in the 6 Hz, pentylenetrazole and maximal electroshock tests.
- Chronic luteolin had no effect in the 6 Hz model or a second hit pentylenetetrazole model.
- No evidence of anticonvulsant effects of luteolin was found in the seizure models used.

ARTICLE INFO

Article history:
Received 27 May 2013
Received in revised form 21 June 2013
Accepted 27 June 2013

Keywords:
Flavonoid
Inflammation
Pentylenetetrazole
6 Hz
Pilocarpine
TLR4

ABSTRACT

Luteolin, a common plant polyphenolic flavonoid, has antioxidant, neuroprotective, anxiolytic and anti-inflammatory properties, which led us to hypothesize that luteolin is anticonvulsant. Here, we evaluated the effects of acute and chronic luteolin injection (i.p.) in four mouse seizure models, the 6 Hz model, maximal electroshock test (MEST), pentylenetetrazole (PTZ) and second hit PTZ test in the chronic stage of the pilocarpine model. Using real-time PCR mRNA levels of toll like receptor 4 (Tlr4), were quantified in the pilocarpine model, because luteolin has been shown to block the downstream signaling of TLR4. Luteolin did not exhibit any consistent anti- or pro-convulsant actions after single dosing in the 6 Hz (0.3–10 mg/kg), MEST (0.3–20 mg/kg) and PTZ (3 mg/kg) tests, nor after repeated daily dosing (10 mg/kg) in the 6 Hz model. Tlr4 mRNA levels were upregulated 3 days after pilocarpine-induced status epilepticus (SE), but unaltered at three weeks in the chronic stage of the model. At that time, there was no effect of repeated luteolin injections (10 mg/kg, i.p.) in the second hit PTZ test, indicating that TLR-4 signaling may be not one of the main players determining the seizure threshold in this seizure model. In summary, we found no indications that luteolin is pro- or anti-convulsant in one chronic and three acute mouse seizure models.

1. Introduction

Luteolin, 3′,4′,5,7-tetrahydroxyflavone, belongs to the flavone subclass of flavonoids. It is a common polyphenolic compound found in vegetables, spices and medicinal herbs and has antioxidant, anti-tumorigenic, anxiolytic, and anti-inflammatory properties. Several flavonoids cross the blood brain barrier [1] and based on its structural properties, low partition coefficient and molecular weight, luteolin is likely to cross the blood brain barrier. Extracts from *Eclipta alba* contain low levels of luteolin and are being used in Indian traditional medicine to treat epilepsy. Anticonvulsant actions of *E. alba* were recently described in a maximal electroshock rat model [2]. Similarly, the flavonoid quercetin was anticonvulsant after acute or chronic administration in mice with lowered thresholds to pentylenetetrazole due to ethanol withdrawal [3]. Acute luteolin also protects neuroblastoma cells against oxidative stress [4]. In a previous study, oral and intraperioteneal luteolin (1–50 mg/kg) showed anxiolytic-like effects in mice [5], indicating that luteolin enters the brain and may increase inhibitory signaling. However, in binding assays with labeled flunitrazepam only low affinity (K_i = 60 μM) for the benzodiazepine binding site of $GABA_A$ receptors was found [5], which does not explain fully the anxiolytic effects.

Inflammation is found in epilepsy patients and models [6–8], including microglial activation, production of interleukin 1β [9] and expression of toll-like receptor 4 (TLR4) [10]. Even in naïve rats, stimulation of TLR4 receptors by lipopolysaccharide produced epileptiform discharges, which could be blocked by an interleukin 1 receptor antagonist [11]. This indicates that both

* Corresponding author at: School of Biomedical Sciences, Skerman Building 65, The University of Queensland, St. Lucia, QLD 4072, Australia. Tel.: +61 7 3365 3113; fax: +61 7 3365 1766.
E-mail address: k.borges@uq.edu.au (K. Borges).
[1] New present address: Brain Research Institute, Jeffrey Cheah School of Medicine and Health Sciences, Monash University Sunway Campus, Petaling Jaya, Selangor 46150, Malaysia.

0304-3940/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2013.06.065

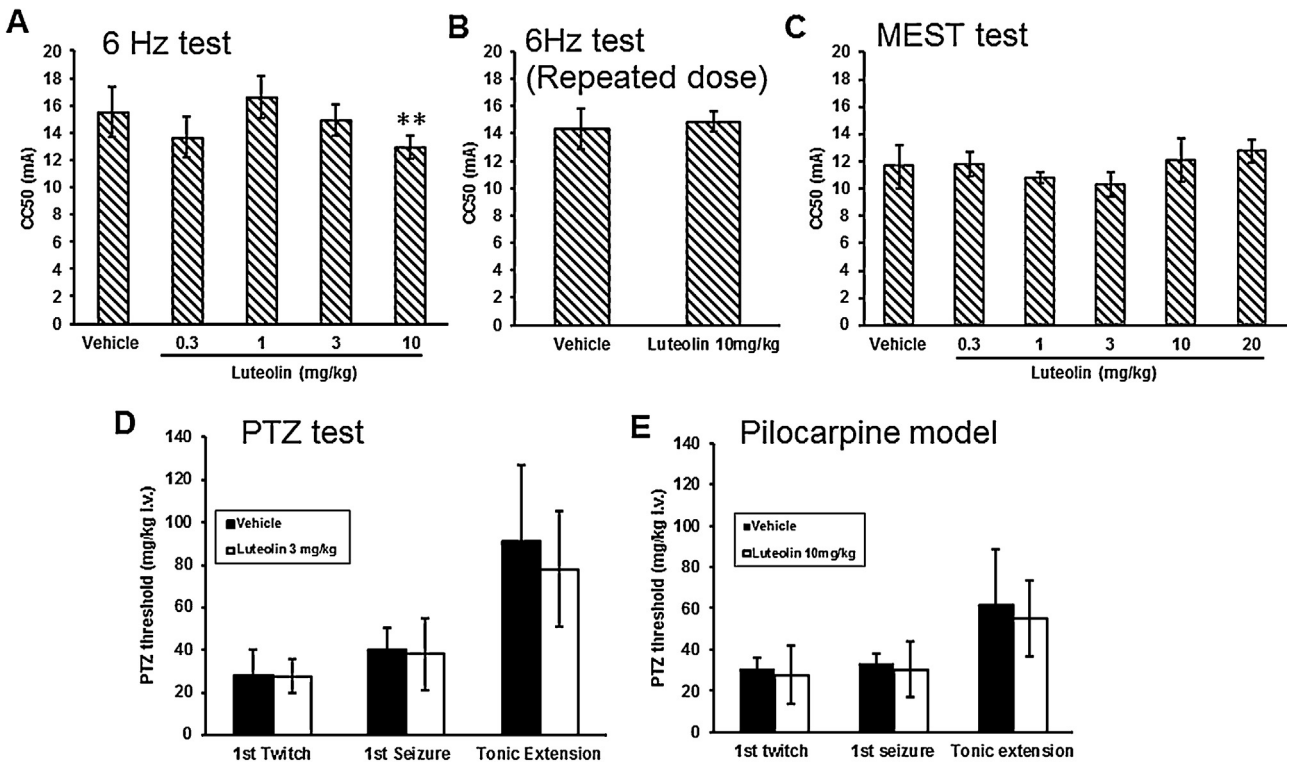

Fig. 1. Effects of luteolin in four seizure tests in CD1 mice. (A) Example of CC50s of luteolin measured in the 6 Hz test vs. vehicle ($n = 15$ mice per group, ANOVA $p = 0.0003$, no significant anticonvulsant effects in Dunnett's test), except $p < 0.01$ for 10 mg/kg luteolin, which seems to be an outlier in this experiment. (B) Lack of effect in 6 Hz test after repeated dosing for 4 days ($n = 15$ mice per group, $p = 0.42$, t-test). (C) Example of an MEST test showing lack of increase in the CC50 ($n = 15$ mice per group, $p = 0.005$ ANOVA, no significant anticonvulsant effects in Dunnett's test). (D) Lack of effect in PTZ seizure test ($n = 12$–15 mice per group, $p = 0.16$–89, separate t-tests for different seizure types). (E) Lack of effect in second hit PTZ seizure test in pilocarpine induced SE mice after 3 days repeated dosing ($n = 6$–8 mice per group, $p = 0.6$–0.9). All data are expressed as means ± SEM.

interleukin 1β and TLR4 may be involved in determining the seizure threshold in healthy animals. Luteolin seems to be an ideal compound to decrease these responses and the inflammation occurring with epilepsy. At micromolar concentrations luteolin inhibits cytokine expression by stimulated microglia, including interleukin 1β [12,13]. Also, luteolin interferes with TLR4 signaling [14] and pro-inflammatory transcription factors [12,15].

Based on all these findings mentioned above we hypothesized that luteolin is anticonvulsant and investigated its acute and chronic effects in four seizure models. We chose three standard acute seizure models without inflammation and the pilocarpine mouse model, which shows microglial activation and inflammation in the chronic stage [7,16]. We investigated the effects of luteolin on seizure thresholds, with luteolin being given 15 min before seizure induction. This time point was chosen, based on the finding that luteolin is metabolized rapidly with a half-life of about 5 h [17] and the plasma luteolin peak concentration was found at 30 min after luteolin (30 mg/kg) was given p.o. to rats [18]. A similar time point showed central effects of luteolin; when 1–10 mg/kg luteolin (i.p.) was injected 1 h before treatment with pentobarbital the latency to loss of the righting reflex was halved [5]. Unfortunately, the time points for the effects of luteolin in the forced swim test, the hole board test, the elevated plus maze test and the catalepsy test were not published [5].

2. Materials and methods

All experiments were approved by the Institutional Animal Care and Use Committee of the University of Queensland and conducted in accordance with their guidelines. Every effort was made to minimize animal suffering.

2.1. Mice and luteolin

Adult male CD1 mice (22–38 g, Animal Resources Center, Western Australia) were housed under a 12 h light–dark cycle with free access to food and water. Animals were injected (i.p.) with vehicle (0.5% Tween 80) or luteolin (AKScientific, Union City, CA), 15 min prior to seizure tests. Luteolin treatment (10 mg/kg, i.p.) was repeated daily for 3–4 days and 15 min prior to testing in the 6 Hz and second hit pentylenetetrazole (PTZ) seizure tests. According to the material safety data sheet the LD50 in animals is above 180 mg/kg (i.p.).

2.2. Animal seizure models

2.2.1. 6 Hz model

Fifteen minutes before stimulation with 0.2 ms-duration pulses at 6 Hz for 3 s (ECT Unit 57800, Ugo Basile), 0.5% tetracaine was applied to the corneas. Using the "up-and-down" method, electrical currents were applied to different mice with 2 mA intervals to determine the critical current intensity needed to induce seizures in 50% of CD1 mice (CC50) [19].

2.2.2. Maximal electroshock threshold (MEST) test

Corneal stimulations (50 Hz sine waves, 0.2 s duration starting at 11 mA) were generated by a Hugo Sachs Type 211 rodent shocker (Harvard Apparatus, Germany) with 1 mA increments to determine the MEST using the up down method [19,20].

2.2.3. PTZ model

10 mg/ml PTZ was infused at 150 μl/min into the tail vein of naïve mice or mice which had experienced pilocarpine induced