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TRPV1 mediates the anticonvulsant effects of acetaminophen in mice

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ABSTRACT

Objective: Acetaminophen is one of the most commonly used analgesic and antipyretic drugs. It has been reported that acetaminophen has anticonvulsant effects in several animal models of seizure. An active metabolite of acetaminophen, AM404, inhibits the uptake of the endocannabinoid anandamide. However, the mechanism of the anticonvulsant effect of acetaminophen is unknown.

Methods: This study was performed to examine whether or not acetaminophen can protect against pentylenetetrazol-induced kindling in mice and to investigate the precise mechanisms of the anticonvulsant effect of acetaminophen using the fully kindled mouse models.

Results: Repeated administration of acetaminophen significantly delayed the progression of seizure severity induced by pentylenetetrazol. Additionally, acetaminophen showed a dose-dependent anticonvulsant activity against fully pentylenetetrazol-kindled seizures. AM404 also exhibited a dose-dependent anticonvulsant activity in fully kindled animals. The anticonvulsant activity of acetaminophen was antagonized by capsazepine and AMG9810, two transient receptor potential vanilloid-1 (TRPV1) antagonists. However, the transient receptor potential ankyrin 1 (TRPA1) antagonist HC030031 and CB1 receptor antagonist AM251 had no effect.

Conclusion: These findings suggest that acetaminophen has an anticonvulsant effect in pentylenetetrazol-kindled mouse models and TRPV1 mediates the anticonvulsant action.

1. Introduction

Acetaminophen is one of the most popular and widely used drugs for the treatment of pain and fever. Several mechanisms of acetaminophen action have been described that involve modulation of the endogenous cannabinoid system. Endocannabinoids are lipid mediators that act as endogenous agonists for type-1 and type-2 cannabinoid (CB1 and CB2, respectively) receptors, and anandamide and 2-arachidonoylglycerol (2-AG) are the main endogenous agonists. Additionally, anandamide is a potent activator of transient receptor potential vanilloid 1 (TRPV1), a member of the family of transient receptor potential (TRP) channels (De Petrocellis et al., 2000).

Previous studies have shown that the cerebrospinal fluid levels of anandamide are reduced in patients with untreated newly diagnosed temporal lobe epilepsy (Romigi et al., 2010), and mRNA levels of the CB1 receptor are decreased in the hippocampal tissue of patients with intractable temporal lobe epilepsy (Ludányi et al., 2008). Recent clinical trials for drug-resistant seizures showed that cannabidiol, the main nonpsychotomimetic compound from *Cannabis sativa*, reduced seizures (Devinsky et al., 2017). These findings suggest that the endocannabinoid system plays a role in the inhibition of seizures in humans with epilepsy.

Recent studies have revealed that active metabolites of acetaminophen are important for its mechanism of action. *N*-acetyl-p-benzoquinoneimine (NAPQI) is a toxic metabolite of acetaminophen formed in the spinal cord, liver and kidneys. NAPQI activates the potent transient receptor potential ankyrin 1 (TRPA1), and mediates the antinociceptive and hypothermic actions of acetaminophen (Andersson et al., 2011; Gentry et al., 2015). Acetaminophen can also be metabolized by fatty acid amide hydrolase (FAAH) to an arachidonic acid-conjugated metabolite of acetaminophen (AM404) in the brain and spinal cord (Högestätt et al., 2005). AM404 is known to inhibit the uptake of endocannabinoid anandamide into presynaptic neurons and activate cannabinoid and TRPV1 receptors (Beltramo et al., 1997; De Petrocellis et al., 2000). Accordingly, acetaminophen is considered to bring about its pharmacological actions by activation of cannabinoid receptors and/or the TRP channel system in the central nervous system.

The major cannabinoid receptors in the central nervous system are

Abbreviations: AM404, *N*-(4-hydroxyphenyl-5Z,8Z,11Z,14Z-eicosatetraenamide); GABA, γ-aminobutyric acid; PG, prostaglandin; TRPV1, transient receptor potential vanilloid-1; TRPA1, transient receptor potential ankyrin 1

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CB1 receptors, and their activation reduces seizure severity in pentylenetetrazol-kindled mouse models (Bahremand et al., 2009). Endocannabinoid anandamide was reported to induce significant anticonvulsant effects in several seizure animal models (Wallace et al., 2002; Manna and Umathe, 2012; Bhaskaran and Smith, 2010). We recently found that acetaminophen has a significant anticonvulsant effect against fully pentylenetetrazol-kindled seizures (Suemaru et al., 2018). However, the relationship between acetaminophen and epileptogenesis remains unknown. In this study, we examined the repeat administration of acetaminophen on the development of pentylenetetrazol kindling in mice. Moreover, we investigated the mechanisms of acetaminophen action using fully pentylenetetrazol-kindled mouse models and compared them with that of the typical antiepileptic drug, sodium valproate.

2. Methods and methods

2.1. Animals

All animal care and experimental procedures were in accordance with the Guiding Principles for the Care and Use of Laboratory Animals adopted by the Japanese Pharmacological Society and approved by the Ethical Committee for Animal Experimentation committee of Shujitsu University (approval code 025-002).

Male ICR mice were purchased from Shimizu Laboratory Supplies Co., Ltd. (Kyoto, Japan). Animals were maintained in an air-conditioned room with controlled temperature (22 ± 2 °C) under a 12/12 h light/dark cycle with lights on at 08:00 h. Mice were housed from 4 weeks of age in standard size plastic cages (32 × 18 × 24 cm) with paper bedding (4–5 mice per cage). The mice were allowed free access to food and water except during experiments.

2.2. Drugs

Acetaminophen (Sigma-Aldrich, St. Louis, MO, USA), AM404 (Sigma-Aldrich), and AM251 (Sigma-Aldrich) were dissolved in polyoxyethylene castor oil (cremophor®) vehicle (18:1:1, saline: cremophor®: ethanol). WIN55212-2 (Sigma-Aldrich), capsazepine (Sigma-Aldrich) and AMG9810 (Tocris Bioscience, Bristol, UK) were emulsified in 1% Tween 80. HC030031 (Tocris Bioscience) was emulsified in 0.5% methylcellulose. Pentylenetetrazol (Sigma-Aldrich) and sodium valproate (Research Chemicals Inc., Toronto, Ontario, Canada) were dissolved in saline. Drugs were administered at a volume of 0.1–0.2 mL/10 g of body weight.

2.3. Acute pentylenetetrazol seizures

To set the test dose of acetaminophen for pentylenetetrazol kindling, we first determined the anticonvulsive action against maximal pentylenetetrazol seizures using naive mice (8 weeks of age, weight 32–37 g). Pentylenetetrazol at a dose of 80 mg/kg was injected intraperitoneally (i.p.) 30 min after the administration of acetaminophen. The animals were observed for 20 min after injection, and clonic and tonic seizures were monitored by an observer who was blind to the treatment.

2.4. Pentylenetetrazol kindling

Kindling is a phenomenon in which repeated application of initially subconvulsive stimulation leads to seizures and increased seizure susceptibility persists over long periods of time. Mice at 5 weeks of age weighing 25–28 g were used for the pentylenetetrazol kindling. Kindling was induced by daily i.p. administration of 40 mg/kg of pentylenetetrazol, 5 days per week for 12 days. The seizure score was determined using the Racine scale (stages 1–5). The seizure intensity was scored as follows: stage 0, no response; stage 1, ear and facial twitching; stage 2, myoclonic body jerks; stage 3, forelimb clonus, rearing; stage 4, clonic seizures, turn onto the side; and stage 5, generalized clonic seizures, turn onto the back (Racine, 1972). For the evaluation of epileptogenesis activity, acetaminophen at doses that negligibly affects acute pentylenetetrazol seizures (100 and 300 mg/kg, i.p.) was repeatedly administered to the animals 30 min before pentylenetetrazol injection for 12 days.

We also determined the anticonvulsive actions of acetaminophen, AM404 and WIN55212-2 (non-selective CB1 and CB2 receptor agonists) using fully pentylenetetrazol-kindled mice (8–10 weeks of age). Fully kindled was defined as the occurrence of three consecutive stage 4 or 5 seizures after administration of pentylenetetrazol (40 mg/kg) for 12 days. The kindled mice were injected intraperitoneally with acetaminophen, AM404, WIN55212-2 or valproate 30 min before the pentylenetetrazol (40 mg/kg, i.p.) test. Control groups received vehicle in the same manner. All behavioral seizures were observed for 20 min after injection of the test compound and recorded by an observer who was blind to the treatment.

To investigate the mechanism of the anticonvulsive action of acetaminophen, the effects of AM251 (CB1 antagonist/inverse agonist), capsazepine and AMG9810 (TRPV1 receptor antagonists) and HC030031 (TRPA1 receptor antagonist) were determined using fully pentylenetetrazol-kindled mice (Fig. 1). These drugs were injected 45 min before the test. HC030031 was orally administered and the other drugs were intraperitoneally administered. Acetaminophen or valproate was injected 30 min before the test.

2.5. Statistical analysis

All data contributing to seizure severity scores are expressed as the mean ± standard error of the mean (SEM). Development of pentylenetetrazol kindling was analyzed by repeated measures two-way ANOVA with treatment as a between-subjects factor and with day as a within-subject factor. Data of fully pentylenetetrazol-kindled seizures were analyzed by the Mann-Whitney test or Kruskal-Wallis test followed by Steel's test. The chi-square test was used to compare the incidence of seizures. P-values < 0.05 were considered significant.

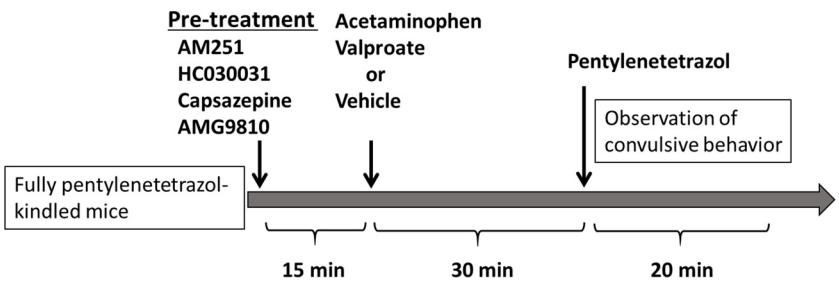

Fig. 1. Scheme of the experimental protocol for the fully pentylenetetrazol-kindled seizures test.