Pharmacology, Biochemistry and Behavior 120 (2014) 57–64

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Understanding the anti-kindling role and its mechanism of Resveratrol in Pentylenetetrazole induced-kindling in a rat model

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ARTICLE INFO

Article history:
Received 8 November 2013
Received in revised form 18 January 2014
Accepted 22 January 2014
Available online 30 January 2014

Keywords:
Pentylenetetrazole
Resveratrol
Epileptogenesis
Kindling
Rat
Antioxidant

ABSTRACT

Background: Resveratrol is a polyphone chemical found in a number of plant species, including peanuts and grapes, but with significant amounts in red wine. In normal plant physiology, Resveratrol is produced as a defensive response to injury or parasitic attacks. Resveratrol has diverse biological properties and actions with potential clinical applications, including anti-inflammatory, antioxidant, anti proliferative, and neuroprotective effects.
Aim: The aim of the present study was to explore the effect and mechanism of Resveratrol in Pentylenetetrazole (PTZ) induced kindling in rats.
Materials and methods: In a PTZ kindled Wistar rat model, different doses of Resveratrol (25 mg/kg, 50 mg/kg and 75 mg/kg) were administered orally 30 min before the PTZ injection. The PTZ injection was given on alternate day till the animal became fully kindled or till 10 weeks. The following parameters were compared between control and various experimental groups: the course of kindling, stages of seizures, histopathological scoring of hippocampus, antioxidant parameters, DNA fragmentation and caspase-3 expression in the hippocampus, and neuron-specific enolase in the blood. One way ANOVA followed by Bonferroni post hoc analysis and Fischer's Exact test were used for statistical analyses.
The results: In the present study, Resveratrol showed dose-dependent anti-seizure effect. Resveratrol (75 mg/kg) significantly increased the latency to myoclonic jerks, clinic seizures as well as generalized tonic-clinic seizures, improved the seizure score and decreased the number of myoclonic jerks. PTZ induced kindling caused a significant neuronal injury, oxidative stress and apoptosis which were reversed by pretreatment with Resveratrol in a dose-dependent manner.
Conclusion: Our study suggests that Resveratrol has a potential antiepileptogenic effect on PTZ-induced kindling in rats. The possible underlying mechanisms of Resveratrol as an antiepileptic agent may be due to its antioxidative property and neuroprotective effect.

1. Introduction

About a dozen of new drugs have become available for epilepsy over the last 15–20 years. Current medications primarily act to symptomatically suppress seizure; however, there is minimal clinical evidence that they correct the underlying brain abnormalities causing epilepsy (epileptogenesis) or alter its natural history and long-term prognosis (Temkin, 2001). Thus, there is a need to identify drugs (antiepileptogenic drugs) which modify the disease as well as can inhibit the progression of epilepsy or completely prevent its development. However, at this point, no proven antiepileptogenic therapies have been developed for clinical use. Many antiepileptic medications were identified through screening assays that assessed efficacy against acutely provoked seizures in nonepileptic animals. As a result, they inhibit seizures through mechanisms that directly decrease neuronal excitability, such as by modulating neurotransmitter receptors and ion channels. A better strategy for developing antiepileptogenic therapies might be to interrupt the initial mechanistic events that trigger downstream cellular and molecular changes in the brain that lead to seizures. This approach is particularly plausible and clinically relevant for acquiring epilepsies that are caused by a remote brain injury (e.g., head trauma, stroke), with seizures starting after a prolonged period, from months to years later. During the latent period of epileptogenesis, histopathological and molecular changes (e.g., neuronal death, synaptic reorganization) that promote epileptogenesis occur and could be targeted for correction by an antiepileptogenic therapy. Epileptogenesis can be studied in numerous rodent models of symptomatic epilepsy, including kindling, post-status epilepticus models of Temporal Lobe Epilepsy, Traumatic Brain Injury, and stroke models, and models of febrile seizures (Walker et al., 2002; Stables et al., 2003; Pitkanen et al., 2007). There are two types of experimental protocol to evaluate drug effects on kindling acquisition: 1) drug is administered before each stimulation and the effects on kindling acquisition are determined relative to vehicle controls; 2) anticonvulsant drug effects study in fully kindled rats (Loscher and Brandt, 2010). In the present study we have followed the first protocol and demonstrated the effect on kindling acquisition.

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0091-3057/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.pbb.2014.01.010

After the realization of reduced cardiac risk by red wine popularly referred as French paradox, much interest has emerged in Resveratrol, which is the active constituent of red wine. Resveratrol (RESV; 3, 5, 4′-tri-hydroxy stilbene) is a type of polyphenol and an antimicrobial substance synthesized de novo by plants (a phytoalexin). RESV is found in the skin of red grapes and is a component of red wine (Fremont, 2000; Orallo, 2008). The other sources of RESV include raspberries, mulberries, plums, peanuts, bilberries, blueberries, cranberries, Scots pine, and Japanese knotweed. RESV is synthesized instinctively by the above plants as a protection to counter the bacterial and fungal infections, stress and injury (Balestrazzi et al., 2009; Maddox et al., 2010).

Studies in animal models imply a number of other beneficial health effects of RESV, which comprise anti-ischemic, antiviral, antioxidant and anti-inflammatory properties (Belguendouz et al., 1997; Jang et al., 1999; Manna et al., 2000; Sato et al., 2000; Kraft et al., 2009; Campagna and Rivas, 2010; Robich et al., 2010; Sun et al., 2010). Pertaining to the central nervous system, multiple cell culture investigations and in vivo studies in animal models of neurodegenerative diseases/brain injury point out that RESV is a potent neuroprotective compound (Sun et al., 1997; Bastianetto et al., 2000; Jang and Surh, 2003; Han et al., 2004; Sinha et al., 2002; Wang et al., 2002; Baur and Sinclair, 2006; Sakata et al., 2010; Liu et al., 2010; Shindler et al., 2010; Singleton et al., 2010).

Previous studies by various researchers demonstrated that RESV protects against neuronal death and acute seizures induced by the ionotropic glutamate receptor agonist, kainate and $FeCl_3$ (Gupta et al., 2001, 2002; Virgili and Contestabile, 2000). Studies have also explored the potential mechanism of RESV mediated neuroprotection against acute seizures (Shetty, 2011; Wang et al., 2004; Gao and Hu, 2005; Li et al., 2005). Study by Wu et al. demonstrated the effect of RESV on SE-induced epileptogenesis and chronic epilepsy induced by kainite (Wu et al., 2009). With this background the present study was planned to explore the effect of RESV on the kindling acquisition by Pentylenetetrazole (PTZ) in rats and its mechanism.

2. Materials and methods

2.1. Experimental animals

15–20 weeks old male Wistar rats weighing between 200 to 250 g were used for the present study. The animals were obtained from the maintained inbred colony of the institute central animal house and maintained at 23 ± 2 °C with a relative humidity of 65 ± 5% in 12 h light/dark cycle. Animals had free access to standard pellet chow diet and tap water ad libitum. Animals were acclimatized to laboratory conditions for at least 7 days prior to experimentation. Institutional Animal Ethics Committee (IAEC) approval (No.55/IAEC/267 dated 27.07.2011) was obtained before the start of the study and the study was carried out according to the National Institutes of Health Guide for Care and Use of Laboratory Animals guidelines.

2.2. Treatment scheduled

A total of 83 rats were divided into six groups: Group I: Control (physiological saline) (n = 8); Group II: PTZ (physiological saline + PTZ 30 mg/kg) (n = 15); Group III: VPA 200 (Sodium Valproate 200 mg/kg + PTZ 30 mg/kg) (n = 15); Group IV: RESV 25 (Resveratrol 25 mg/kg + PTZ 30 mg/kg) (n = 15); Group V: RESV 50 (Resveratrol 50 mg/kg + PTZ 30 mg/kg) (n = 15); Group VI: RESV 75 (Resveratrol 75 mg/kg + PTZ 30 mg/kg) (n = 15).

2.3. Drug preparation and dosing schedule

PTZ was dissolved in 0.9% physiological saline and injected intraperitoneally (i.p.) in a volume not exceeding 10 ml/kg, at a sub convulsive dose of 30 mg/kg every alternate day until the animal developed kindling or up to 10 weeks. Resveratrol (25, 50, 75 mg/kg) was given orally 30 mins before PTZ injection till the animal developed kindling or up to 10 weeks. Sodium Valporate (200 mg/kg) was dissolved in 0.9% physiological saline and administered by intraperitoneal injection 30 min before PTZ injection till the animal develops kindling or up to 10 weeks. Resveratrol, sodium valproate and Pentylenetetrazole was purchased from Sigma-Aldrich, USA.

2.4. Pentylenetetrazole (PTZ) induced kindling in rats

PTZ was injected i.p. as mentioned above. After each injection of PTZ, the rats were placed singly in isolated transparent Plexiglas cages and were observed for 2 hrs. The intensity of convulsions was rated according to the Racine scale (Racine, 1972) as follows: 0 — no response; 1 — ear and facial twitching; 2 — myoclonic jerks without rearing; 3 — myoclonic jerks with rearing; 4 — turn over into side position, clonic–tonic seizures; and 5 — turn over into back position, generalized tonic–clonic convulsions. an animal was considered fully kindled when it exhibits stage 4 or 5 of seizure score on three consecutive trials. there was no mortality or morbidity in any of the animals in any group.

2.5. Studies with Hippocampus

When the animal became fully kindled (exhibits stage 4 or 5 of seizure score on three consecutive trials), on the next day, it was sacrificed by decapitation under the overdose of intraperitoneal pentobarbitone anesthesia. The hippocampus was carefully dissected out of the brain and the following parameters were evaluated:

2.5.1. Histopathology of the hippocampus

One half of each brain was fixed in 10% formalin and stored for histopathological studies using hemotoxylin and eosin (H&E) stain. Thereafter, tissue was sliced, routinely processed, and embedded in paraffin wax. 5 μm coronal paraffin sections were cut, mounted and stained by haematoxylin and eosin. The acidophilic neuron, identified by intense cytoplasmic eosinophilia accompanied by chromatin dispersion with loss of nuclear membrane integrity (Fujikawa et al., 2000), was perceived as the marker for irreversible neuronal damage at the cellular level. The numbers of acidophilic neurons in different regions of the hippocampus were estimated on a 0–3 grading scale, 0 = none, 0.5 = slight (<10%), 1.0 = mild (10–25%), 1.5 = mild-to-moderate (26–45%), 2.0 = moderate (46–54%), 2.5 = moderate-to-severe (55–75%), and 3.0 = severe (>75%), as previously published (Fujikawa et al., 2000). The boundaries of each region were shown in Fig. 1. Sections were examined by a blinded investigator without knowledge of any other data on that animal.

2.5.2. Hippocampal DNA fragmentation study

DNA was isolated from hippocampal brain specimens using DNA isolation kits and stored for Agarose gel electrophoresis.

2.5.3. Hippocampal oxidative stress studies

Once the animal became fully kindled, on the next day, it was sacrificed. Brain was perfused with ice cold 0.9% physiological saline through cardiac puncture before decapitation. The hippocampus was promptly excised after decapitation, weighed and chilled in ice cold 0.9% physiological saline and the oxidative stress parameters were measured immediately.

2.5.3.1. Estimations of thiobarbituric acid-reactive substance (TBARS). The extent of lipid peroxidation was estimated according to the method of Ohokawa et al. in tissue homogenate (Ohokawa et al., 1979). Tissue homogenate was prepared in a ratio of 1 g of wet tissue to 9 ml of phosphate buffer (pH 7.2) using a homogenizer. To 0.1 ml of the homogenate, 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 20% acetic acid solution, 1.5 ml of a 0.8 % aqueous solution of thiobarbituric acid were