Epilepsy & Behavior 48 (2015) 4–14

Contents lists available at ScienceDirect

Epilepsy & Behavior

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

Effect of GLT-1 modulator and P_{2X7} antagonists alone and in combination in the kindling model of epilepsy in rats

Neha Soni, Prashant Koushal, B.V.K. Reddy, Rahul Deshmukh, Puneet Kumar *

Department of Pharmacology, ISF College of Pharmacy, Moga 142001, Punjab, India

ARTICLE INFO

Article history:
Received 25 December 2014
Revised 21 April 2015
Accepted 22 April 2015
Available online 1 June 2015

Keywords:
GLT-1
P_{2X7}
Ceftriaxone
Brilliant Blue G
Pentylenetetrazole

ABSTRACT

Introduction: Multiple lines of investigation have explored the role of glutamatergic and purinergic systems in epilepsy, related cognitive impairment, and oxidative stress. Glutamate transporters, particularly GLT-1 expression, were found to be decreased, and purinergic receptor, P_{2X7} expression, was found to be increased in brain tissue associated with epilepsy. The present study was carried out to investigate the effect of ceftriaxone (GLT-1 upregulator) and Brilliant Blue G (P_{2X7} antagonist) against PTZ-induced kindling in rats. The study was further extended to elucidate the cross-link between glutamatergic and purinergic pathways in epilepsy.

Material and methods: Systemic administration of subconvulsant dose of PTZ (30 mg/kg) every other day for 27 days (14 injections) significantly increased the mean kindling, and developed generalized tonic–clonic seizures, and reduced motor co-ordination, cognitive skills, oxidative defense (increases lipid peroxidation, nitrite levels and decreases GSH level) and acetylcholinesterase enzyme activities in the cortex and subcortical region. Treatments with CEF (100 and 200 mg/kg) and BBG (15 and 30 mg/kg) alone and in combination (CEF 100 mg/kg and BBG 15 mg/kg) significantly decreased the mean kindling score and restored behavioral and oxidative defense activities compared with treatment with PTZ.

Conclusions: The combination of both the drugs was shown to have better effect in preventing kindled seizures and a significantly synergistic effect compared with their effect alone in PTZ-kindled rats. The present study elucidated the mechanistic role of GLT-1 modulator and selective P_{2X7} antagonist and their combination against PTZ-induced kindling. The study for the first time demonstrated the cross-link between glutamatergic and purinergic pathways in epilepsy treatment.

1. Introduction

Epilepsy a common neurological disorder characterized by episodes of neurologic dysfunction in which abnormal neuronal firing is manifest clinically by changes in motor control, sensory perception, behavior, and/or autonomic function that apparently result from complex processes involving several neurotransmitters [1,2]. Changes occurring during epileptogenesis contribute to network imbalances between excitation and inhibition which result in the enduring predisposition to the generation of epileptic seizures [3]. Excitation is modulated by excitatory neurotransmitters and mostly by glutamate [4]. It is generally accepted that the majority of glutamate transport in the CNS, particularly as related to excitatory transmission, is mediated by a group of high-affinity, sodium-dependent, glutamate transporters, thereby terminating the transmitter signal and protecting neurons from an excitotoxic action of glutamate and providing cells throughout the body with glutamate for metabolic purposes [5,6]. Approximately 80% of the glutamate transporters expressed in the hippocampus are GLT-1 [7]. Although expressed primarily by astrocytes, GLT-1 is also expressed on neuronal axon terminals. The expression of GLT-1a in axon terminals has potentially important implications for the physiology of excitatory synaptic transmission in regulating synaptic glutamate, maintaining glutamate stores in the presynaptic terminal, interacting with glutamate receptors, contributing a glutamate-regulated anionic conductance to the plasma membrane of the presynaptic bouton, and controlling crosstalk between excitatory synapses. GLT-1b is present to a lesser extent [8]. Relatively little is known about the mechanisms that regulate GLT-1 or the other Na^+-dependent glutamate transporters, but it suggests that the expression of GLT-1/EAAT-2 is regulated by transcriptional and/or posttranscriptional processes [6]. It was suggested that malfunctioning of glutamate transporters (GLTs) is one of the main causative factors of hyperexcitation, seizure spread, and neurotoxicity [6,9–11]. Some studies also reported that an increased GLT-1 expression can protect mice against status epilepticus (SE)-induced death, neuropathological changes, and chronic seizure development [12,13]. On the basis of previous reports, we can speculate that enhancing GLT-1/EAAT2 protein expression is a potential therapeutic approach to treat epilepsy.

The beta-lactam antibiotics, such as ceftriaxone (CEF), enhance the ex vivo expression of a neuroprotective protein GLT-1 in a concentration-dependent manner [14]. This CEF-induced GLT-1

* Corresponding author at: Pharmacology Division, ISF College of Pharmacy, Moga 142001, Punjab, India. Tel.: +91 1636 324200, 324201; fax: +91 1636 239515.
E-mail address: punnubansal79@gmail.com (P. Kumar).

http://dx.doi.org/10.1016/j.yebeh.2015.04.056
1525-5050/

upregulation blocks the metabotropic glutamate receptor (mGluR)-dependent long-term depression (LTD) at the mossy fiber (MF)–CA3 hippocampal synapse [15].

Brilliant Blue G (Coomassie Brilliant Blue G) (BBG) is a selective non-competitive antagonist of P_{2X7} type of purinergic receptors and is well tolerated by human beings [16,17]. The increased expression of P_{2X7} R is found in the hippocampus, mainly within mossy fibers and the dentate gyrus of the chronically epileptic rat [18]. There is a crosstalk between purinergic and glutamatergic pathways, as it has been demonstrated that the Na^+-influx triggered by the activation of P_{2X7} affects the activities of glutamate transporters via a reduction in the amplitude of transporter currents. Stimulation of P_{2X7} signaling is shown to downregulate the activity for glutamate transport through GLT-1 transporters by triggering posttranscriptional regulation of GLAST/GLT-1 expression via phosphoinositol 3-kinase cascade and production of oxidants [19,20].

Kindling is a model of epilepsy produced by repeated administration of an initially subconvulsive electrical or chemical stimulus that results in an increase in seizure activity, culminating in a generalized seizure [21]. The electrographic and behavioral components of kindled seizures are thought to be similar to human partial-onset seizures, as the focal component of the kindled seizure can progress into a generalized seizure. In this model, the effect of drugs on both focal and generalized seizure types can be assessed.

With this background, the present study was conducted to explore the effect of CEF (GLT-1 modulator) and BBG (P_{2X7} antagonist) administration on seizure development induced by repetitive administration of subconvulsant dose (30 mg/kg) of PTZ in rats. The effect of each drug alone and in combination on epilepsy-induced cognitive impairment and oxidative stress in PTZ-kindling model in rats was also assessed.

2. Material and methods

2.1. Experimental animals

Wistar rats, weighing 200–250 g, were used in the present study. Animals were obtained from the Central Animal House facility of ISF College of Pharmacy, Moga, and housed in a group of three in polypropylene cages with husk bedding under standard conditions of light and dark cycle with food and water ad libitum. Animals were acclimatized to laboratory conditions before behavior observations. All the behavioral assessments were carried out between 9:00 h and 15:00 h. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of ISF College of Pharmacy, Moga, Punjab, approval no. 163 dated 08 March 2014 and was carried out in accordance with the guidelines of the Indian National Science Academy (INSA) for the use and care of experimental animals.

2.2. Drugs, sources, and route of administration

The drugs used in the present study, PTZ and BBG, were obtained from Sigma (St Louis, MO, USA), and ceftriaxone (CEF) injection in powder form was a commercial preparation of Microlab, which comes under the brand name Gramocef. Pentylenetetrazol and ceftriaxone were dissolved in normal saline. Drugs were administered i.p. 30 min before the injection of PTZ. The route of administration and dose of drugs (CEF [14] and BBG [22]) were selected based on the previously published reports. All drugs were administered by i.p. route [21]. One specific group of rats was assigned to one specific drug treatment, and each group comprised a minimum of 6 animals.

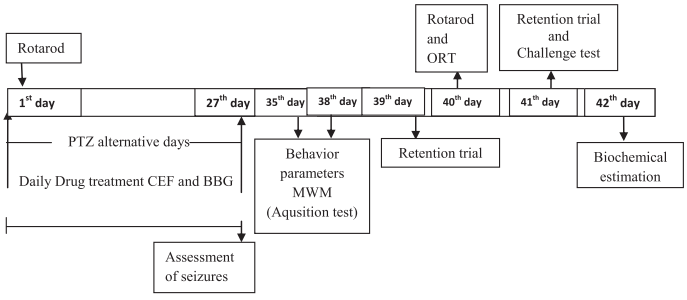
2.3. Experimental procedure and treatment schedule

We used a total of 8 groups in the study (n = 6). The 1st group was normal control, and the 2nd group was the PTZ-treated group, administered the subconvulsant dose of PTZ = 30 mg/kg, i.p, every other day for 27 days. The 3rd and 4th groups were ceftriaxone (100 and 200 mg/kg) with PTZ. The 5th and 6th groups were low-dose BBG (15 and 30 mg/kg) with PTZ; the 7th group was a combination of ceftriaxone (100 mg/kg) and BBG (15 mg/kg) along with PTZ; and the 8th group was standard drug, i.e., diazepam (3 mg/kg) with PTZ. Drug treatment was started on the 1st day with PTZ injection and continued to the 27th day until the development of the fully kindled stage. Seven days later, after the last injection of PTZ, i.e., on the 35th day, the rats were subjected to behavioral tests. Using the Morris water maze (MWM), from the 35th to the 38th day, the acquisition trial was performed, and on the 39th and the 41st day, the retention test was performed. Other behavioral tests, i.e., object recognition task (ORT) and rotarod, were performed on the 40th day. After the behavioral part of the experiment, the challenge test was performed, in which one additional injection of PTZ was given to rats on the last day of the retention test. This challenge test was performed in order to determine the occurrence of clonic seizures, and after 24 h of the challenge test, all the groups were sacrificed, brains were removed, and then cortical and subcortical regions were separated for biochemical analysis to estimate the levels of lipid per-oxidation, glutathione, nitrite, protein, and acetyl cholinesterase.

2.3.1. Experimental groups

Sr. no.	Groups (n = 6)
1	Normal
2	PTZ control (30 mg/kg) i.p.
3	PTZ (30 mg/kg) i.p. + CEF (100 mg/kg)
4	PTZ (30 mg/kg) i.p. + CEF (200 mg/kg)
5	PTZ (30 mg/kg) i.p. + BBG (15 mg/kg)
6	PTZ (30 mg/kg) i.p. + BBG (30 mg/kg)
7	PTZ (30 mg/kg) i.p. + CEF (100 mg/kg) + BBG (15 mg/kg)
8	PTZ (30 mg/kg) i.p. + diazepam (3 mg/kg)

2.3.2. Treatment schedule



2.3.3. PTZ-induced kindling in rats

Pentylenetetrazol was given in a subconvulsant dose of 30 mg/kg, i.p., on alternate days (days 1, 3, 5, 7, and 9) for 27 days (14 injections) according to the standardized procedure [21]. After each injection of PTZ, occurrence of CNS excitation was noted for a period of 30 min by observing the animals in a plexiglass chamber (30 × 24 × 22 cm) with partition in between. The intensity of behavioral seizure was evaluated using a scoring scale: Stage 0, no response; Stage 1, ear and facial twitching; Stage 2, myoclonic jerks without upright position; Stage 3, myoclonic jerks, upright position with bilateral forelimb clonus; Stage 4, tonic–clonic seizures; and Stage 5, generalized tonic–clonic seizures and loss of postural control. Animals were considered fully kindled by PTZ after two consecutive Stage 5 seizures; at that time, the administration of PTZ was discontinued. If an animal failed to kindle it was excluded from the study.