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Original research article

Epileptogenic effects of G protein-coupled estrogen receptor 1 in the rat pentylenetetrazole kindling model of epilepsy

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ABSTRACT

Background: G protein-coupled estrogen receptor 1 (GPER-1) has been demonstrated in several parts of the brain and may play an important role in estrogen downstream signaling pathway. However, the effects of this receptor on epileptic seizure are not clearly known. Therefore, the effects of GPER-1 agonist, G-1, GPER-1 antagonist, G-15 and the main estrogenic hormone, 17β-estradiol were investigated on seizures and brain tissue oxidative damages induced by pentylenetetrazole (PTZ) in rats.

Methods: In this study, 30 adult male Wistar albino rats were used. Due to intraperitoneal (*ip*) injections of a subconvulsant dose of PTZ (35 mg/kg) which was repeated 12 times every 48 h, chemical kindling occurred and kindling seizure was recorded for 30 min. The rats were injected with 17β-estradiol (5 μg/kg, *ip*) or G-1 (5 μg/kg, *ip*), G-15 (5 μg/kg, *ip*), Saline, Ethanol and Dimethyl sulfoxide (DMSO) 30 min before each dose of PTZ. Observed seizures were classified between the phase 0–5. Thirty minutes later when the last 12. PTZ administration, all rats were sacrificed and the brain cortex, hippocampus sections were removed and the tissue superoxide dismutase (SOD), malondialdehyde (MDA) and nitric oxide (NO) levels on these tissues were studied.

Results: GPER1 agonist, G-1 and estrogenic hormone, 17β-estradiol significantly increased the development of PTZ kindling the seizures. However, GPER1 antagonist, G-15 did not change the development of PTZ kindling the seizures. In the cortex and hippocampus homogenates, the NO levels after G-1 administration had significantly increased ($p < 0.05$) compared to the PTZ groups but SOD activities and MDA levels demonstrated no difference between the groups.

Conclusions: This is the first study that explores that GPER-1 receptors have epileptogenic effect on PTZ-induced kindling rat. GPER1 may mediate the epileptogenic effect of estrogens by changing the oxidative or anti-oxidative parameters in the brain.

Introduction

Epilepsy is a heterogeneous syndrome characterized by recurrent and spontaneous seizures. Approximately 1% of the population in the world suffers from epilepsy. However, 20–30% of the patients are refractory to therapies using currently available antiepileptic drugs [1–3]. Epileptogenesis involves histological, biochemical and physiological alterations that, over time, alter the balance between excitatory and inhibitory neurotransmission in multiple brain structures [4].

The epileptic seizure are related to a complex but still not defined interaction between the brain levels of neurosteroids, neuropeptides, GABA metabolism and estrogen receptors [5]. Several pathological states and even personal habits influence the relationship of estrogens to epilepsy. Brain injury, sometimes followed by epileptic seizure, is shown to be associated with the activation of estradiol [6]. Sleep deprivation and photic activation which are used in provoking seizure for diagnostic purposes are both modulated by estrogens [7]. Even low alcohol consumption increases the serum estradiol levels and stimulates estrogen receptors that might lead to the epileptic seizure [8].

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For estrogens, both of the nuclear receptor subtypes (ERα and ERβ) can also localize to the plasma membrane, where they can activate a number of signal transduction cascades. In addition, estradiol can also activate a G-protein coupled membrane estrogen receptor (GPER-1) with actions on adenylate cyclase, as well as other signaling mechanisms [9]. GPER1 can be present in the membrane, nucleus, endoplasmic reticulum, mitochondria, and Golgi apparatus, and its effects may vary by the cellular structures in which it is present [10,11]. GPER1 was shown to express in the brain, liver, heart, lungs, blood vessels, bones, ovaries, and breast tissues in the human body [12–14]. Investigations into the localization of this receptor within the brain have shown expression in several areas, including the cortex, hippocampus, and hypothalamus [15,16]. In pyramidal neurons, GPER1 has been shown to be expressed at the plasma membrane and in the cytoplasm, as well as along the dendritic processes [12,16]. In hippocampal neurons, GPER1 has been shown to localize to synaptic and extrasynaptic regions within dendritic spines [17]. Furthermore, GPER1 was shown to interact with PSD-95, suggesting that the receptor may couple to singling pathways within dendritic spines through its interaction with this scaffold protein [17,18]. G1 (1-[4-(6-bromobenzo[1,3]dioxol-5yl)-3a,4,5,9b-t-tetrahydro-3H-cyclopenta [c]quinolin-8-yl]-ethanone) is suggested to be the specific agonist of GPER1 [19]. It was shown that anxiety increased and such effect was mediated by GPER1 in G1-administered mice [20].

The presence of GPER1 has been demonstrated in several parts of the brain [15,16], however, the effects of this new receptor on pentylenetetrazole-induced epileptic seizure are not clearly known. The effects of GPER-1 agonist, G-1, GPER-1 antagonist, G-15 and the main estrogenic hormone,17β-estradiol, on pentylenetetrazole (PTZ) induced seizures and brain tissues oxidative damages were investigated in rats.

Materials and methods

Animals and laboratory

All animal procedures were approved by the local ethics committee of Kahramanmaraş Sutcu Imam University Medical Faculty. All efforts were made to minimize animal suffering and to reduce the number of animals used. The experimental subjects were adult male Wistar rats (300–350 g weight at the beginning of the experiments). They were housed in a quiet and temperature and humidity-controlled room (22 ± 3 °C and 65 ± 5%, respectively) in which a 12-h light/dark cycle was maintained (07:00–19:00 h light) throughout the course of the study, and *ad libitum* provision of feed.

PTZ kindling experiments

Rats were injected with a subconvulsive dose of PTZ (35 mg/kg, *ip*) with a total of 12 injections each week (on Monday, Tuesdays and Wednesdays) in order to achieve PTZ kindling. After PTZ injection, rats (n = 30) were divided into six groups: 17β-estradiol group, G-1 group, G-15 group, Saline (vehicle), Ethanol group (vehicle) and DMSO group (vehicle). Following every PTZ injection, the rats were observed for 30 min, convulsive activity was recorded and classified with the help of Fischer and Kittner scale as described below [21]. After the 30 min observation phase, the rats were injected with 17β-estradiol (5 μg/kg, *ip*) or G-1 (5 μg/kg, *ip*), G-15 (5 μg/kg, *ip*) and finally were put back into their cages. 17β-Estradiol and G-1, G-15 were used at a systemically effective dose [22–24]. The doses of the administered drugs were chosen in accordance with our previous studies and several pilot studies. Saline, Ethanol and DMSO treated animals are control groups for PTZ, 17β-estradiol and G-1, G-15, respectively.

An animal undergoing a stage 5 convulsion was considered to be fully kindled and was not further tested. The seizure stage rating scale was as follows:

Stage 0: no evidence of convulsive activity;
Stage 1: ear and facial twitching, head nodding;
Stage 2: myoclonic jerks;
Stage 3: forelimb clonuses with full rearing;
Stage 4: generalized clonic convulsions with loss of righting reflex, rearing, jumping and falling down; and
Stage 5: clonic-tonic convulsions with tonic hindlimb extensions.

All behavioral experiments involved in PTZ kindling were carried out in separate and isolated laboratories, which had the same environmental conditions as the colony room. PTZ and 17β-estradiol were purchased from Sigma Chemicals (St. Louis, MO, USA) and G-1, G-15 were purchased from Calbiochem (Darmsdat, Germany).

Biochemical measurements

Thirty minutes later when the last 12. PTZ administration, control and kindled rat were killed by cervical dislocation. The brain cortex and hippocampus tissues were isolated. Cortex and hippocampus brain samples were kept in a deep freezer at −20 °C. The frozen tissue samples of the brain cortex and hippocampus were thawed, weighed, and homogenized (2 ml Tris–HCl buffer) in 50 mM Tris–HCl buffer (pH: 7.5) while holding in an ice bath. Superoxide dismuthase (SOD), malondialdehyde (MDA), and nitric oxide (NO) levels were measured from the homogenates. Then, homogenates were centrifuged in +4 °C cold-centrifuge at 5000 cycle/min for 30 min, and supernatants were obtained. The SOD activity was carried out according to Fridovich's method [25]. This method utilizes xanthine and xanthine oxidase to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitrophonol-s-phenyl tetrazolium chloride) and form a red formazon dye. The SOD activity was measured according to the inhibition degree of the reaction. Lipid peroxidation levels in cortex and hippocampus brain samples were determined by estimating MDA using the thiobarbituric acid test. The level of MDA was determined by measuring the color intensity of the complex formed between MDA and thiobarbituric acid at 532 nm (Shimatzu-UV 1201 Spectrometer) according to Ohkawa et al. method [26]. Nitrite, and the stable output of NO radicals are commonly used as a measure of NO production [27]. NO measurement was carried out with the Griess method in order to detect nitrite levels [28].

Statistical analysis

Data were considered as means ± SE. A one-way ANOVA test subsequently a *post hoc* least significant difference (LSD) test was employed to analyze the data. A repeated measure of ANOVA was conducted to analyze the PTZ-kindling data. Significance level was accepted as $p < 0.05$. All statistical analyses were conducted by using GraphPad Prism software (GraphPad Software, Version 5.0, GraphPad Software Inc., San Diego, CA, USA).

Results

The effects of repeated administration of 17β-estradiol, G-1 and G-15 on the development of PTZ kindling

According to the results of the repeated administration of 17β-estradiol and G-1, 17β- and G-1 was observed to fail in preventing