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Obestatin improves oxidative brain damage and memory dysfunction in rats induced with an epileptic seizure

Türkan Koyuncuoğlu[a], Caner Vızdıklar[a], Doğan Üren[a], Hakan Yılmaz[a], Çağan Yıldırım[a], Sefa Semih Atal[a], Dilek Akakın[b], Elif Kervancıoğlu Demirci[b], Meral Yüksel[c], Berrak Ç. Yeğen[a,*]

[a] Marmara University School of Medicine, Department of Physiology, Turkey
[b] Marmara University School of Medicine, Department of Histology and Embryology, Turkey
[c] Marmara University Vocational School of Health Related Professions, Istanbul, Turkey

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ABSTRACT

Obestatin was shown to alleviate renal, gastrointestinal and haemorrhage-induced brain injury in rats. In order to investigate the neuroprotective effects of obestatin on seizure-induced oxidative brain injury, an epileptic seizure was induced with a single intraperitoneal (i.p.) dose of pentylenetetrazole (PTZ, 45 mg/kg) in male Wistar rats. Thirty minutes before the PTZ injection, rats were treated with either saline or obestatin (1 μg/kg, i.p.). Seizure was video-taped and then evaluated by using Racine's scoring (0–5). For the assessment of memory function, passive-avoidance test was performed before seizure induction, which was repeated on the 3rd day of seizure. The rats were decapitated at the 24th or 72nd hour of seizures and brain tissues were obtained for histopathological examination and for measuring levels of malondialdehyde (MDA), glutathione (GSH), reactive oxygen radicals and myeloperoxidase (MPO) activity. Obestatin treatment reduced the average seizure score, decreased the occurrence and duration of generalized tonic-clonic seizures, presenting with a shorter latency to their onset. Increased lipid peroxidation and enhanced generation of oxygen-derived radicals detected at the post-seizure 72nd h were suppressed by the consecutive treatments of obestatin, but no changes were observed by the single obestatin treatment in the 24-h seizure group. Neuronal damage and increased GFAP immunoreactivity, observed in the hippocampal areas and cortex of PTZ-induced rats were alleviated in 3-day obestatin-treated PTZ group. PTZ-induced memory dysfunction was significantly improved in obestatin-treated PTZ group as compared to saline-treated rats. The present data indicate that obestatin ameliorated the severity of PTZ-induced seizures, improved memory dysfunction and reduced neuronal damage by limiting oxidative damage.

1. Introduction

Epilepsy is one of the most prevalent and serious neurologic conditions with high rates of disability and mortality [35]. Despite successive treatment regimens with various anti-epileptic drugs, more than 30% of adolescent and adult patients continue to have seizures [7] which are proven to occur due to an imbalance between the major excitatory (glutamate) and inhibitory (gamma-aminobutyric acid; GABA) inputs to neurons [21]. Since the current anti-epileptic drugs are not sufficient to create seizure-free patients, it was advised to search for novel agents that would interfere with the processes underlying post-injury epileptogenesis and recovery process [43]. Several studies have demonstrated that mitochondrial dysfunction along with oxidative and nitrosative stress in the hippocampus, which occurs upon the generation of the initial seizure is the key mechanism in epileptogenesis [9,31,59]. Thus, numerous antioxidants were investigated for their potential in controlling seizure-induced oxidative stress and in protecting against seizures and consecutive cognitive impairment [49,61]. Accordingly, several neuropeptides (e.g leptin, ghrelin, neuropeptide Y, galanin, ghrelin, somatostatin and dynorphin) some of which have antioxidant properties were also proven to be effective in modulating epileptic seizures [27,40].

* Corresponding author at: Marmara Universtiy School of Medicine, Basibüyük Mah. Maltepe Basibüyük Yolu No. 9/1, 34854 Maltepe, Istanbul, Turkey.
E-mail addresses: byegen@marmara.edu.tr, berrakyegen@gmail.com (B.Ç. Yeğen).

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Ghrelin pretreatment was shown to delay the onset of pentylenetetrazol (PTZ)-induced seizures and to reduce oxidative stress by increasing antioxidant enzyme activities [37,38]. It was demonstrated that plasma ghrelin levels were decreased following a PTZ-induced seizure [3], while exogenous administration of ghrelin had significantly improved memory that was impaired by PTZ [5]. Similary, in humans decreased ghrelin levels were measured in serum and saliva of epileptic patients during the first hours after a seizure [4], but the levels were significantly elevated during the period with anti-epileptic treatment [18]. Despite significant increases in serum, urine and saliva levels of ghrelin in patients under the anti-epileptic treatment [55], levels of obestatin, another peptide hormone encoded from the same gene with ghrelin [65], were not changed, suggesting that endogenous obestatin had no impact in the pathophysiologic mechanism of epilepsy. On the other hand, intracerebroventricular administration of obestatin in rats has been reported to have sleep-promoting [52], thirst-inhibiting [48], anxiety-suppressing effects along with improvement in memory performance [8]. We have previously demonstrated that obestatin administration has alleviated renal [26], gastrointestinal [41,53] and subarachnoidal haemorrhage-induced oxidative injury in rats [13]. However, to the best of the authors' knowledge, effect of obestatin on seizure-induced oxidative injury of the brain was not studied yet. In the light of the aforementioned studies, it can be hypothesized that modulation of oxidative stress by exogenous obestatin may improve memory and attenuate oxidative brain injury due to an epileptic seizure. Accordingly, in the present study we investigated the neuroprotective effects of obestatin on oxidative brain damage following a single dose PTZ-induced seizure.

2. Materials and methods

2.1. Animals and drugs

Wistar Albino male rats (n = 60, 250–400 g) were used in the study and housed in an air-conditioned room with 12-h light and dark cycles, where the temperature (22 ± 2 °C) and relative humidity (65–70%) were kept constant. Rats were fed with standard laboratory chow with free access to water. All experimental protocols were approved by the Marmara University Animal Care and Use Committee (76.2014.mar). Obestatin was purchased from Phoenix Pharmaceuticals, Inc. (Burlingame, CA, USA) and pentylenetetrazole (PTZ) was obtained from Sigma-Aldrich, (St. Louis, MO, USA).

2.2. Experimental design

Rats were randomly divided into five groups (n = 12 in each group; Fig. 1). In control groups, rats were injected intraperitoneally (ip) either with only saline or obestatin (1 μg/kg/day, ip) and were decapitated after 72 h. The remaining four groups were injected with pentylenetetrazole (PTZ, 45 mg/kg, ip) to induce seizures. Thirty minutes before the PTZ injection, rats were treated with either saline or obestatin (1 μg/kg; ip). Half of the rats in both saline- or obestatin-treated PTZ rats was decapitated at the 24th h of seizure induction, while the other half was continued to be treated daily with saline or obestatin (1 μg/kg/day, ip) until they were decapitated at the 72nd hour. The rationale in choosing the dose of obestatin was based on our previous study that has reported its neuroprotective and antioxidant effects in haemorrhage-induced brain injury [13]. For histopathological examination, 4 rats in each group were decapitated at the 24th or 72nd hour of seizures following transcardial paraformaldehyde perfusion, while the brain tissues of the non-perfused 8 rats in each group were used for biochemical measurements. Brain tissues obtained for biochemical measurements were stored at −20 °C for measuring malondialdehyde (MDA), glutathione (GSH) levels and myeleoperoxidase (MPO) activities, as well as the levels of oxygen radicals.

2.3. Induction and scoring of seizures

In order to induce an epileptic seizure, rats were injected with a single dose of PTZ (45 mg/kg ip; in saline), which was confirmed in preliminary experiments as the intermediate convulsive dose [50]. The rat was placed in a plexiglas observation box (38 × 30 × 25 cm) and PTZ-induced seizure was video-taped for the following 30 min, and then the recorded 30-min period was evaluated by using Racine's scoring (0–5) [45], where 0: no behavioral changes; 1: facial movements with twitching of ears and whiskers; 2: myoclonic jerks without rearing; 3: myoclonic jerks with rearing; 4: clonic convulsions accompanied with posture loss; 5: generalized tonic-clonic seizures.

2.4. Assessment of memory function

Passive avoidance test was performed using a box with two compartments (each 20 × 20 × 20 cm) separated by a guillotine gate (Northel Passive Avoidance System, Istanbul) [14]. For the acquisition trial, rat was placed in the illuminated (with a 100 W bulb) chamber on the first day of the experiment before any injections were made. Due to their innate tendencies for preference of dark areas, rats are expected to enter the dark chamber when put in the illuminated part. Immediately after the rat has entered the dark compartment, the door between two chambers was automatically closed and an electrical foot shock (0.3–0.6 mA) was given for 5 s, and those that did not enter the dark compartment were excluded from the study. After shock, the door was opened and then rat was placed back in the compartment where no shock was delivered. On the 71st h of PTZ or saline (control group) injection, the rats were again placed in the illuminated compartment to evaluate the passive avoidance response. Based on the previous reports [22], the rats that have avoided entering the dark chamber with a latency over 300 s were considered to have normal learning and memory performance, while a shorter latency to enter the dark chamber was regarded as memory dysfunction.

2.5. Malondialdehyde and glutathione assays in the brain tissues

Brain tissues were homogenized with ice-cold trichloroacetic acid (10% per 1 g tissue) with Ultra Turrax tissue homogenizer. Malondialdehyde (MDA) levels were measured for products of lipid peroxidation by observing the formation of thiobarbituric acid reactive substances. Lipid peroxidation was described as MDA equivalents using a coefficient of $1.56 \times 105\ M^{-1}\ cm^{-1}$. Results are expressed as nmol MDA/g tissue. Glutathione (GSH) levels were measured by using modified Ellman procedure [57]. After centrifugation at 10,000 rpm for 10 min at 4 °C, 0.5 ml of supernatant was added to 2 ml of 0.3 mol/l $Na_2HPO_4 \cdot 2H_2O$ solution. A 0.2 ml solution of dithiobisnitrobenzoate (DTNB, 0.4 mg/ml of 1% sodium citrate) was added and the absorbance was read at 412 nm. GSH levels were calculated using a coefficient of $1.36 \times 105\ M^{-1}\ cm^{-1}$ and results are given as GSH/g tissue.

2.6. Measurement of myeloperoxidase activity in the brain tissues

Myeleoperoxidase (MPO), which is an enzyme mostly located in azurophilic granules of polymorphonuclear leukocytes, is commonly used to demonstrate accumulation of neutrophils in tissues [57]. Tissue MPO activity is evaluated by measuring the hydrogen peroxide dependent oxidation of o-dianizidine