Neuropeptides xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Neuropeptides

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

The effect of leptin, ghrelin, and neuropeptide-Y on serum Tnf-A, Il-1β, Il-6, Fgf-2, galanin levels and oxidative stress in an experimental generalized convulsive seizure model

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ARTICLE INFO

Article history:
Received 18 July 2016
Accepted 4 August 2016
Available online xxxx

Keywords:
Leptin
Ghrelin
Neuropeptide-Y
Tnf-A
Il-1β
Il-6
Fgf-2
Galanin
Oxidative stress
Convulsive seizure model

ABSTRACT

The objective of this study is to examine the effects of the endogenous ligands leptin, ghrelin, and neuropeptide Y (NPY) on seizure generation, the oxidant/antioxidant balance, and cytokine levels, which are a result of immune response in a convulsive seizure model. With this goal, Wistar rats were divided into 5 groups—Group 1: Saline, Group 2: Saline + PTZ (65 mg/kg), Group 3: leptin (4 mg/kg) + PTZ, Group 4: ghrelin (80 μg/kg) + PTZ, and Group 5: NPY (60 μg/kg) + PTZ. All injections were delivered intraperitoneally, and simultaneous electroencephalography (EEG) records were obtained. Seizure activity was scored by observing seizure behavior, and the onset time, latency, and seizure duration were determined according to the EEG records. At the end of the experiments, blood samples were obtained in all groups to assess the serum TNF-α, IL-1β, IL-6, FGF-2, galanin, nitric oxide (NO), malondialdehyde (MDA), and glutathione (GSH) levels. The electrophysiological and biochemical findings ($p < 0.05$) of this study show that all three peptides have anticonvulsant effects in the pentylenetetrazol (PTZ)-induced generalized tonic-clonic convulsive seizure model. The reduction of the levels of the pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 caused by leptin, ghrelin, and NPY shows that these peptides may have anti-inflammatory effects in epileptic seizures. Also, leptin significantly increases the serum levels of the endogenous anticonvulsive agent galanin. The fact that each one of these endogenous peptides reduces the levels of MDA and increases the serum levels of GSH leads to the belief that they may have protective effects against oxidative damage that is thought to play a role in the pathogenesis of epilepsy. Our study contributes to the clarification of the role of these peptides in the brain in seizure-induced oxidative stress and immune system physiology and also presents new approaches to the etiology and treatment of tendency to epileptic seizures.

1. Introduction

Epilepsy is a group of disorders characterized by seizures and excessive neuronal activity in the brain. All epileptic attacks develop through different mechanisms; however, they all share common features, including increased neuronal excitability and synchronicity. In general, the disruption of the balance between the excitatory and inhibitory systems is held responsible for the generation of seizures. It is also known that genetic mutations that result in abnormal ionic channel functions play a role in the generation of seizures (Stafstrom, 2006). Some recent studies have suggested that the neuropeptides leptin, ghrelin, and neuropeptide Y (NPY) play an important role in the modulation of epilepsy and that they may directly or indirectly affect epileptic seizures (Kovac and Walker, 2013).

Endogenous peptides play an important role in the pathogenesis of epilepsy, and they may also have important interactions with some pro-inflammatory cytokines and oxidative stress (Kovac and Walker, 2013; Li et al., 2011; Aguiar et al., 2012; Kir et al., 2013). Recent studies on epileptic patients and animal models have shown that there is a complex association between immune system abnormalities, epilepsy, and cytokines (Li et al., 2011). The immune system and associated inflammatory reactions could also play an important role in epileptogenesis (Mehler and Kessler, 1997; Jankowsky and Patterson, 1999; Schneider et al., 1998). During seizures cytokines, within the blood passing into the brain, these cytokines are found in the brain at low concentrations, and studies suggest that this passage of cytokines plays an important role in the generation of seizures and epileptogenesis (Li et al., 2011).

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http://dx.doi.org/10.1016/j.npep.2016.08.002
0143-4179/

Excessive free radical production plays a role in the pathogenesis of many diseases (e.g., atherosclerosis, diabetes mellitus, stroke, inflammatory diseases, cancer) including epilepsy. Free radicals damage DNA structure, destroy cells, lead to the loss of neurons, and become involved in the pathogenesis of these diseases due to the collapse of the immune system (Vila et al., 2000; Hans et al., 1999; Kossmann et al., 1995; Peltola et al., 1998; Peltola et al., 2000). The prolonged presence of high levels of reactive oxygen radicals in tissues increases the risk for neurodegeneration. Studies have shown that oxidative stress caused by free radical damage may have an important role in the mechanisms of epileptic seizure generation and progression. Therefore, the use of therapies directed at reducing oxidative stress to treat epilepsy has increased (Shin et al., 2011).

In light of this information, using a convulsive seizure model, we examined the effects of the endogenous ligands ghrelin, leptin, and neuropeptide Y on the generation of seizures, their antioxidant efficacy, their role in managing oxidative stress, and their effect on the cytokines that are the result of immune response.

2. Materials and methods

2.1. Animals

In our study, the convulsive seizure model contained Wistar-Albino rats, weighing 250–300 g. Animals were housed in cages at room temperature (22 ± 2 °C) and maintained under standard conditions with 12-h light/dark cycles. They were fed with a standard pellet diet and tap water ad libitum throughout the study. The study protocol was approved by the Institutional Animal Care and Ethical Committee of the University of Kocaeli (Approval Number: 3/3-2011).

2.2. Experimental protocol

The Wistar rats were divided into 5 groups. To examine seizure activity using EEG, two weeks before the study, recording electrodes were placed in all groups using a stereotactic device. Leptin, ghrelin, and NPY were delivered intraperitoneally to the rats in Group 1 (Saline), Group 2 (Saline + PTZ), Group 3 (Leptin + PTZ), Group 4 (Ghrelin + PTZ), and Group 5(NPY + PTZ) 30 min before the pentylenetetrazol (PTZ) injection dose of 65 mg/kg (Sigma Co.). Rat Leptin (PeproTech, USA), rat Ghrelin (Phoenix Pharmaceuticals, USA), and rat NPY (Bachem, Switzerland) were dissolved in normal saline. The Wistar rats were administered 4 mg/kg doses of leptin, 80 μg/kg doses of ghrelin, and 60 μg/kg doses of NPY intraperitoneally (Obeid et al., 2010; Obay et al., 2008; Gelfo et al., 2011).

2.3. EEG monitoring

Simultaneous EEG records were obtained in all groups. After the PTZ injection, epileptic seizure activity, the EEG records, and behavioral changes were monitored, and the onset, severity, and duration of seizures were evaluated (Ates et al., 2005; Velisek et al., 1992).

2.4. Biochemical studies

Leptin, ghrelin, and NPY are considered to be important factors involved in epileptogenesis. To evaluate their effects on certain biochemical markers, the TNF-α [(eBioscience Rat TNF-α Platinum ELISA kit (Austria)], IL-1β [(eBioscience Rat IL-1β Platinum ELISA kit (Austria)], IL-6 [eBioscience Rat IL-6 Platinum ELISA kit (Austria)], FGF-2 [UscnRat Fibroblast Growth Factor-2, Basic (FGF-2) ELISA kit (China)], and galanin [TSZ ELISA Rat Galanin (GAL) ELISA kit (USA)] levels were detected using a Dynex-DSX micro-ELISA device. GSH assays were performed by the Ellman method (Ellman, 1959), MDA was assessed by the Buege and Aust, 1978) method, and NO was assessed using the Griess reactive (Cortas and Wakid, 1990).

2.5. Statistical analysis

All of the study data are presented as mean ± SEM (standard error of mean), and statistical significance was set at $p < 0.05$. The statistical analyses of the study groups were performed after the data was tested for normality. Groups of two were compared using the unpaired *t*-test (two-tailed) or the Mann Whitney test (two-tailed). The ANOVA tests, the one-way variance analysis, or Kruskal-Wallis test were used to compare multiple groups. The statistics software GraphPad Prism 3.0 was also used.

3. Results

3.1.1. Effects of leptin, ghrelin, and NPY on convulsive seizures

Generalized tonic-clonic seizure activity was observed in all the animals in Group 2 that were administered PTZ injections at doses of 65 mg/kg. Generalized seizures started after clonic jerks that accompanied clonuses of the facial and fore-limb muscles (St 1, Onset); this was followed by clonic activity involving head, neck, and tail extension (St 2–3), the loss of the righting reflex that comprises tonic-clonic responses (St 4, Generalized major seizure), and ended with tonic flexion-extension with following prolonged tonic-clonic clonuses (St 5). The tonic-clonic seizure behavior was accompanied by an EEG with typical short-lasting, generalized, bilateral, synchronized, irregular, spike-and-wave activity. In various frequencies accompanying the tonic-clonic seizures, multiple spike-and-wave activities emerged, and following this intense activity, a low-voltage isoelectric line was observed on the cortical EEG (Fig. 1).

When the seizure severity in Group 2 that was delivered S + PTZ injections was examined, it was seen that generalized major seizures occurred in all of the animals in this group. The incidence of tonic-clonic seizures was identified as 100% (Table 1).

When Group 3, which was delivered leptin (4 mg/kg i.p.) + PTZ injections, was compared with Group 2, a statistically significant delay was identified in seizure onset ($p < 0.003$), minimal seizure onset ($p < 0.0005$), and generalized major seizure onset ($p < 0.006$), and seizure severity was reduced ($p < 0.003$) (Table 1).

When Group 4, which was delivered ghrelin (80 μg/kg i.p.) + PTZ, and Group 5, which was delivered NPY (60 μg/kg i.p.), were compared with Group 2, a statistically significant delay was detected in seizure onset ($p < 0.004$ and $p < 0.0003$, respectively) and minimal seizure onset ($p < 0.0001$ and $p < 0.004$, respectively), and the seizure severity decreased ($p < 0.0001$ and $p < 0.0001$, respectively). The tonic-clonic seizure incidence of ghrelin and NPY was identified as 28.57% in both groups. According to our findings, ghrelin administered at doses of 80 μg/kg and NPY at doses of 60 μg/kg decreased the onset, spread, and severity of convulsive seizures (Table 1).

3.1.2. Effects of leptin, ghrelin, and NPY on serum cytokines and galanin

The effects of leptin, ghrelin, and NPY on serum TNF-α, IL-1β, IL-6, FGF-2 and galanin levels in the convulsive seizure model are shown in Table 2.

The comparison of Group 1 (SF + SF) and Group 2 (SF + PTZ) revealed a statistically significant decrease of TNF-α, IL-1β, IL-6 levels ($p < 0.01$), and no statistically significant difference was observed in the FGF-2 or galanin levels.

When Group 3, which was delivered leptin, and Group 1 were compared, a statistical increase of ghrelin levels was identified ($p < 0.01$), and no significant difference was identified in the TNF-α, IL-1β, IL-6, FGF-2 levels. The comparison of Group 3 and Group 2 revealed a statistically significant increase ($p < 0.05$) of galanin and no significant difference in the TNF-α, IL-1β, IL-6, FGF-2 levels.

No statistically significant difference was identified between the TNF-α, IL-1β, IL-6, FGF-2 and galanin levels of Group 4 and Group 1.

Please cite this article as: Oztas, B., et al., The effect of leptin, ghrelin, and neuropeptide-Y on serum Tnf-A, Il-1β, Il-6, Fgf-2, galanin levels and oxidative stress in an ..., Neuropeptides (2016), http://dx.doi.org/10.1016/j.npep.2016.08.002