Neuropeptides 43 (2009) 295–302

Contents lists available at ScienceDirect

Neuropeptides

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

The role of nitric oxide in the inhibitory effect of ghrelin against penicillin-induced epileptiform activity in rat

Ali Aslan [a], Mehmet Yildirim [a], Mustafa Ayyildiz [a], Ayla Güven [b], Erdal Agar [a,*]

[a] Department of Physiology, Faculty of Medicine, University of Ondokuz Mayis, 55139 Samsun, Turkey
[b] Pediatric Endocrinology, Faculty of Medicine, University of Ondokuz Mayis, Samsun, Turkey

ARTICLE INFO

Article history:
Received 9 January 2009
Accepted 23 May 2009
Available online 23 June 2009

Keywords:
Ghrelin
Epileptiform activity
L-Arginine
L-NAME
7-NI
NOS

ABSTRACT

Ghrelin, a gastric peptide with key action on food intake, has been recently recognized as a potential anti-epileptic agent. In the present study, we investigated the involvement of nitric oxide in the effect of ghrelin on penicillin-induced epileptiform activity in rat. Thirty minutes after penicillin injection, ghrelin, at doses of 0.5, 1, 2 μg, was administered intracerebroventricularly (*i.c.v.*). Ghrelin, at a dose of 1 μg, significantly decreased the mean frequency of epileptiform activity without changing the amplitude whereas other doses of ghrelin (0.5 and 2 μg) did not alter either the mean of frequency or amplitude of epileptiform activity. The effects of systemic administration of nitric oxide synthase (NOS) inhibitors, non-selective N^G-nitro-L-arginine methyl ester (L-NAME), selective neuronal NOS inhibitor, 7-nitroindazole (7-NI) and NO substrate, L-arginine on the anticonvulsive effects of ghrelin were investigated. The administration of L-NAME (60 mg/kg, *i.p.*), 15 min before ghrelin (1 μg) application, reversed the anti-epileptiform effects of ghrelin whereas 7-NI (40 mg/kg, *i.p.*) did not influence it. The present study provides electrophysiological evidence that the intracerebroventricular injection of ghrelin has an inhibitory effect against epileptiform activity in the penicillin model of epilepsy. The anti-epileptiform activity of ghrelin was reversed by nonspecific nitric oxide synthase inhibitor L-NAME, but not selective neuronal nitric oxide synthase inhibitor 7-NI, indicating that ghrelin requires activation of endothelial-NOS/NO route in the brain.

1. Introduction

Ghrelin, a 28 amino acid acylated peptide hormone, was recently isolated from stomach, hypothalamus and the other tissues of mammals (Kojima et al., 1999; Kojima and Kangawa, 2005). It is becoming clear that ghrelin has effect on the pituitary hormone axis, appetite regulation, cardiac and gastrointestinal function, carbohydrate metabolism, adipose and reproductive tissue, cell proliferation and behavioral effects (Korbonits et al., 2004; Kojima and Kangawa, 2005). Cowley et al. (2003) discovered expression of ghrelin in a group of neurons adjacent to the third ventricule between the dorsal, ventral, paraventricular, and arcuate hypothalamic nuclei. They also found that ghrelin binding is present in extra hypothalamic sites, including the cerebral cortex, which raising possibility that peripherally circulating ghrelin can cross the blood–brain barrier to reach these cortical sites (Cowley et al., 2003; Hou et al., 2006). The efferents of hypothalamic ghrelin neurons make synaptic contacts appropriate to enable them to alter the activity of GABAergic neuropeptide Y (NPY)-containing nerve terminals in arcuate hypothalamic nuclei (Cowley et al., 2003; Kojima and Kangawa, 2005). Electrophysiological and biochemical evidence showed that NPY interact functionally with glutamate in the central nervous system (CNS) (Vezzani et al., 2000). It is known that a change in glutamatergic function is involved in both seizure initiation and propagation in the CNS (Schwarcz and Meldrum, 1985). Moreover, the administration of NPY reduced both primary and secondary epileptiform afterdischarges and suppresses seizures in a variety of animal seizure models (Bijak and Smialowska, 1995; Woldbye et al., 1996, 1997; Reibel et al., 2003; Woldbye and Kokaia, 2004). On the other hand, a few studies suggested that ghrelin can be considered as an antiepileptic agent without studying a possible mechanism (Obay et al., 2007, 2008). They noted that intraperitoneal injections of ghrelin, at doses of 20, 40, 60, 80 μg/kg, prolonged the onset time of the first myoclonic jerk, generalized clonic seizure and tonic generalized extension of pentylenetetrazole (PTZ)-induced seizures in rats. The maximal effect of ghrelin, was observed at a dose of 80 μg/kg, in the PTZ-induced epilepsy model of rat (Obay et al., 2007). In addition, levels of ghrelin significantly increased in the prepubertal children treated with valproic acid (Gungor et al., 2007).

The role of NO has been investigated in several experimental models of physiological, biochemical processes and neurological disease such as epilepsy (Smith et al., 1996; Gaskin et al., 2003; Korbonits et al., 2004; Bosnak et al., 2007). Korbonits et al. (2004) reported that ghrelin uses NO pathway on several of its

* Corresponding author. Tel.: +90 362 312 1919x2223; fax: +90 362 457 6041.
E-mail address: eragar@omu.edu.tr (E. Agar).

0143-4179/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.npep.2009.05.005

effects by stimulating NO synthesis. It was demonstrated that the stimulatory effect of ghrelin on growth hormone (GH) secretion is exerted through the activation of the NO intracellular pathway in the cultured porcine somatotropes (Rodríguez-Pacheco et al., 2005). It has also been demonstrated that the administration of ghrelin increased the level of nitric oxide synthase (NOS) in the hypothalamus (Gaskin et al., 2003). However, Riediger et al. (2006) have reported that nitric oxide directly inhibits ghrelin-activated neurons of the arcuate nucleus in rats. NO also strongly influences the excitability status of neurons, either in basal conditions or during paroxysmal activity (Ferraro and Sardo, 2004). To the best of our knowledge, there have been no reports regarding the effect of ghrelin in the penicillin model of epilepsy as well as the role of NO on the effect of ghrelin to date. Therefore, we decided for the first time, to investigate the effects of intracerebrovetricular (*i.c.v.*) injection of ghrelin on penicillin-induced epileptiform activity in rat. A dose–response curve was constructed for the ghrelin to determine the optimal dose for testing involvement of with nitric oxide pathway. To further investigate this mechanism, we used nonspecific NOS inhibitor, L-NAME and selective neuronal NOS inhibitor, 7-NI as well as NO precursor, L-arginine.

2. Materials and methods

2.1. Animals

Adult male Wistar rats weighing 195–220 g (Ondokuz Mayis University of Turkey) were used throughout this study after at least one week of acclimatization. All described procedures were approved by the local ethical committee. Animals were housed in groups of 3–4 and were allowed free access to the food and water except for the short time that the animals were removed from their cages for the experimenting. All animals were kept in a temperature controlled (22 ± 1 °C) environment on a 12 h light/dark cycle. Rats were assigned to the following experiments and groups: intracortical (*i.c.*) delivery of (1) 2.5 μl artificial cerebrospinal fluid [aCSF containing (mM): NaCl, 124; KCl, 5; KH_2PO_4, 1.2; $CaCl_2$, 2.4; $MgSO_4$, 1.3; $NaHCO_3$, 26; glucose, 10; HEPES, 10; pH 7.4 when saturated with 95% O_2 and 5% CO_2] (*i.c.*); (2) 500 units penicillin (2.5 μl, *i.c.*); (3) 500 units penicillin (2.5 μl, *i.c.*) + 0.5 μg ghrelin (*i.c.v.*); (4) 500 units penicillin (2.5 μl, *i.c.*) + 1 μg ghrelin (*i.c.v.*); (5) 500 units penicillin (2.5 μl, *i.c.*) + 2 μg ghrelin (*i.c.v.*); (6) 500 units penicillin (2.5 μl, *i.c.*) + 1000 mg/kg L-arginine (*i.p.*) + 1 μg ghrelin (*i.c.v.*) (7) 500 units penicillin (2.5 μl, *i.c.*) + 60 mg/kg L-NAME (*i.p.*) + 1 μg ghrelin (*i.c.v.*) (8) 500 units penicillin (2.5 μl, *i.c.*) + 40 mg/kg 7-NI (*i.p*) + 1 μg ghrelin (*i.c.v.*) (9) physiological saline/DMSO (7:3 v/v, 2.5 μl, *i.c.v.*). Each animal group was composed of six rats.

2.2. Induction of epileptiform activity

The animals were anesthetized with urethane (1.25 g kg^{-1}, *i.p.*) and placed in a stereotaxic frame. Rectal temperature was maintained between 36.0 and 37.5 °C using a feedback-controlled heating system. A polyethylene cannula was introduced into the right femoral artery to monitor blood pressure, which was kept above 110 mm Hg during the experiments (mean 118 ± 7 mm Hg). All contact and incision points were infiltrated with procaine hydrochloride to minimize possible sources of pain.

The left cerebral cortex was exposed by craniotomy (5 mm posterior to bregma and 3 mm lateral to sagittal sutures). The epileptic focus was produced by 500 units penicillin G potassium injection (1 mm beneath the brain surface by a Hamilton microsyringe type 701 N, (22s ga, bevel tip); infusion rate 0.5 μl/min) (Ayyildiz et al., 2007).

2.3. Drug and drug administration

Rat ghrelin (Sigma G-8903) and the other chemicals used in this study were purchased from Sigma Chemical Co. Ghrelin was dissolved in the sterile physiological saline that the requisite doses were administered intracerebroventricularly in a volume of 1 μg in 1 μl (Kamegai et al., 2001; Lawrence et al., 2002). Intracerebroventricular injections were administered into the left lateral ventricle of each rat through a stereotaxic apparatus with the coordinates of 0.8 mm posterior to the bregma, 2.0 mm lateral to the midline, and 4.2 mm ventral to the surface of the skull based on the atlas of the rat brain (Paxinos and Watson, 1986). Penicillin was prepared in sterile apyrogen distilled water and administered intracortically in a volume of 2.5 μl. L-Arginine and L-NAME were dissolved in sterile physiological saline solution to such concentrations that requisite doses were administered intraperitoneally in a volume of 7 ml/kg. 7-NI was dissolved initially in dimethylsulfoxide (DMSO) to which was added sterile physiological saline (final solution DMSO/saline 3:7 volume/volume, respectively) and administered intraperitoneally in a volume of 7 ml/kg body weight. In the first set of experiments, ghrelin, at the doses of 0.5, 1 and 2 μg (*i.c.v.*), was administered 30 min after penicillin (*i.c.*) application. In the second set of experiments, animals received L-arginine (1000 mg/kg, *i.p.*), L-NAME (60 mg/kg, *i.p.*) and 7-NI (40 mg/kg, *i.p.*) 15 min before an effective dose of ghrelin (1 μg, *i.c.v.*) (Bosnak et al., 2007).

2.4. Electrocorticographical recordings

Two Ag–AgCl ball electrodes were placed over the left neocortex (electrode coordinates: first electrode; 2 mm lateral to sagittal suture and 1 mm anterior to bregma; (primary motor cortex, M1), second electrode; 2 mm lateral to sagittal suture 5 mm posterior to bregma (secondary visual cortex mediomedial area, V2MM) (Paxinos and Watson, 1986). The common reference electrode was fixed on the right pinna. The ECoG activity was continuously monitored on a four-channel recorder (PowerLab, 4/SP, AD Instruments, Castle Hill, Australia). All recordings were made under anesthesia and stored on a computer. The frequency and amplitude of epileptiform ECoG activity was analyzed off line.

2.5. Data analysis

The results are given as the mean ± SEM. Statistical comparisons were made using SPSS software (SPSS Science, V12.0, Chicago, IL). Data analysis was performed using one-way ANOVA and Bonferroni-adjusted post-hoc *t*-tests for comparisons. For all statistical tests, $p < 0.05$ was considered significant.

3. Results

3.1. The effects of ghrelin on penicillin-induced epileptiform activity

Intracortical injection of penicillin (500 units) induced epileptiform activity. Epileptiform activity began within 2–4 min. It reached a constant level as to frequency and amplitude in the 30 min and lasted for 3–5 h. The mean of spike frequency and amplitude were 28.20 ± 1.55 spikes/min, 900 ± 177 μV, respectively (Fig. 1A).

Ghrelin was administered 30 min after penicillin injection. Intracerebroventricular ghrelin, at a dose of 1 μg, significantly decreased the mean frequency of epileptiform activity to 12.8 ± 1.8, 8.3 ± 2.1, 7.5 ± 1.8, 6.8 ± 1.6 spikes/min in the 90, 120, 150 and 180 min after ghrelin injection (*i.c.v.*) compared with penicillin-injected group, respectively (Fig. 2) without changing the amplitude.