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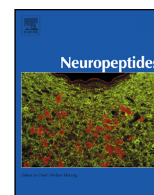

The interaction between ghrelin and cannabinoid systems in penicillin-induced epileptiform activity in rats

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A B S T R A C T

The majority of experimental and clinical studies show that ghrelin and cannabinoids are potent inhibitors of epileptic activity in various models of epilepsy. A number of studies have attempted to understand the connection between ghrelin and cannabinoid signalling in the regulation of food intake. Since no data show a functional interaction between ghrelin and cannabinoids in epilepsy, we examined the relationship between these systems via penicillin-induced epileptiform activity in rats. Doses of the CB1 receptor agonist arachidonyl-2-chloroethylamide (ACEA) (2.5 and 7.5 μg), the CB1 receptor antagonist N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3 carboxamide (AM-251) (0.25 and 0.5 μg) and ghrelin (0.5 and 1 μg) were administered intracerebroventricularly (i.c.v.) 30 minutes after the intracortical (i.c.) application of penicillin. In the interaction groups, the animals received either an effective dose of ACEA (7.5 μg, i.c.v.) or a non-effective dose of ACEA (2.5 μg, i.c.v.) or effective doses of AM-251 (0.25, 0.5 μg, i.c.v.) 10 minutes after ghrelin application. A 1 μg dose of ghrelin suppressed penicillin-induced epileptiform activity. The administration of a 0.25 μg dose of AM-251 increased the frequency of penicillin-induced epileptiform activity by producing status epilepticus-like activity. A 7.5 μg dose of ACEA decreased the frequency of epileptiform activity, whereas a non-effective dose of ACEA (2.5 μg) did not change it. Effective doses of AM-251 (0.25, 0.5 μg) reversed the ghrelin's anticonvulsant activity. The application of non-effective doses of ACEA (2.5 μg) together with ghrelin (0.5 μg) within 10 minutes caused anticonvulsant activity, which was reversed by the administration of AM-251 (0.25 μg). The electrophysiological evidence from this study suggests a possible interaction between ghrelin and cannabinoid CB1 receptors in the experimental model of epilepsy.

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1. Introduction

Epilepsies are the most prevalent neurological disorder; they are characterized by recurrent, unprovoked seizures. Epilepsies are complex syndromes due to their multi-factorial origins and manifestations; researchers have proposed several mechanisms to explain why the excitability of neurons increases in epilepsy (De Curtis et al., 2012). However, certain mechanisms in epilepsy are still unknown. Some studies have suggested a relationship between epilepsy and neuropeptides, such as neuropeptide Y (NPY), leptin and ghrelin (Cowley et al., 2003; Kovac and Walker, 2013).

Ghrelin is an endogenous ligand of the growth hormone (GH) secretagogue receptor 1a (GHSR1a) (Kojima and Kangawa, 2005); it is mainly produced by neurons in the hypothalamus and A-like cells of the oxyntic stomach mucosa (Camina et al., 2004). Ghrelin has a significant role in many physiological processes, such as appetite regulation, cardiac and gastrointestinal function, carbohydrate metabolism, adipose and reproductive tissue, cell proliferation and behavioural effects (Kojima and Kangawa, 2005; Korbonits et al., 2004). Ghrelin has the therapeutic potential to suppress kainic acid-induced excitotoxicity in mice hippocampi (Lee et al., 2010). In addition, a number of clinical and experimental studies have attempted to find the link between ghrelin and epilepsy (Aslan et al., 2009; Lee et al., 2010; Obay et al., 2007; Xu et al., 2009). Ghrelin has been suggested to possess anticonvulsive properties in three different experimental paradigms, including the kainate model (Aslan et al., 2009; Lee et al., 2010; Obay et al., 2007). Ghrelin also has a neuroprotective role against the pilocarpine model of status epilepticus (Xu et al., 2009). It is important to note that ghrelin did not completely prevent seizures in these models. However, Lucchi et al. (2013) suggested that ghrelin's neuroprotective role was completely independent from its anticonvulsant effect in the pilocarpine model of epilepsy.

Cannabinoid receptors are located in several adult brain regions: the pyramidal cell layers of the hippocampus, the dentate gyrus, layers I and IV of the cortex, the basal ganglia, the molecular layer of the cerebellum, the amygdala, the nucleus accumbens, the

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prefrontal cortex, the cingulate, the periaqueductal gray, the dorsal horn and lamina X in the spinal cord (Herkenham et al., 1991). The CB1 receptor is one of the most abundant G-protein-coupled receptors within the brain, and it is responsible for a wide variety of peripheral and central processes such as the retrograde inhibition of neurotransmitter releases and control of neuronal excitability (Herkenham et al., 1991). Conversely, cannabinoids exhibited anticonvulsive effects through the activation of cannabinoid CB1 receptors in various models of experimental epilepsy, such as the maximal electroshock model for grand-mal seizures (Wallace et al., 2001), the rat pilocarpine model of acquired epilepsy (Falenski et al., 2007), the *in vitro* hippocampal neuronal culture models of acquired epilepsy and status epilepticus (Blair et al., 2006), the pentylenetetrazole (PTZ) model of myoclonic seizures in mice (Gholizadeh et al., 2007) and the penicillin-induced model of epileptiform activity in rats (Arslan et al., 2013; Kozan et al., 2009).

Little evidence is available to show the interaction between ghrelin and cannabinoid systems in the regions associated with food intake and metabolism. Tucci et al. (2004) provided the initial evidence of a functional relationship between hypothalamic ghrelin and endocannabinoid systems in relation to appetite in male rats. The hypothalamic neurophysiological effects of ghrelin require the presence of CB1 receptors, and the endogenous cannabinoid system is necessary for the mediation of the orexigenic and central AMP-activated protein kinase (AMPK) stimulatory effects of ghrelin in mice (Kola et al., 2005, 2008). Ghrelin requires GHS-R1a to affect AMPK activity in mice's hypothalamic, liver and adipose tissue (Lim et al., 2013). Lim et al. (2013) suggested that an intact ghrelin signalling pathway is necessary for these effects of cannabinoids on AMPK activity. Moreover, endocannabinoids inhibited ghrelin-induced cAMP response-element binding protein (CREB) phosphorylation by negatively interacting with the NMDA receptor and decreasing the number of phosphorylated NR1 subunits (Cuellar and Isokawa, 2011). The possible relationship between ghrelin and cannabinoid systems in the experimental model of epilepsy has not been studied. Therefore, this study evaluates the role of intracerebroventricular (i.c.v.) injections of the CB1 agonist ACEA and the CB1 antagonist AM-251 in relation to ghrelin's effect on penicillin-induced epileptiform activity in rats.

2. Materials and methods

2.1. Animals

Specific-pathogen-free animals were purchased from the Animal House of Ondokuz Mayis University. The rat breeders were obtained from DETAM (Istanbul, Turkey), and the animals used were bred at the University of Ondokuz Mayis Experimental Research Centre. The local ethics committee approved all experimental procedures (2009/42). Experiments were carried out on 112 adult male Wistar rats weighing 220–260 g. All animals were given free access to standard rat food and tap water ad libitum before the experiments. The rats were kept in a temperature-controlled environment (22 ± 1 °C) on a 12-hour light/dark cycle. The rats were divided into the following experimental groups:

1. Normal saline (2.5 µl, i.c)
2. 500 units penicillin (2.5 µl, i.c.)
3. 500 units penicillin (2.5 µl, i.c.) + dimethylsulfoxide (DMSO) (2.5 µl, i.c.v.)
4. 500 units penicillin (2.5 µl, i.c.) + normal saline (2.5 µl, i.c.v.)
5. 500 units penicillin (2.5 µl, i.c.) + 1 µg ghrelin (i.c.v.)
6. 500 units penicillin (2.5 µl, i.c.) + 0.5 µg ghrelin (i.c.v.)
7. 500 units penicillin (2.5 µl, i.c.) + 0.25 µg AM-251 (i.c.v.)
8. 500 units penicillin (2.5 µl, i.c.) + 0.5 µg AM-251 (i.c.v.)
9. 500 units penicillin (2.5 µl, i.c.) + 2.5 µg ACEA (i.c.v.)
10. 500 units penicillin (2.5 µl, i.c.) + 7.5 µg ACEA (i.c.v.)
11. 500 units penicillin (2.5 µl, i.c.) + 1 µg ghrelin (i.c.v.) + 0.25 µg AM-251 (i.c.v.)
12. 500 units penicillin (2.5 µl, i.c.) + 1 µg ghrelin (i.c.v.) + 0.5 µg AM-251 (i.c.v.)
13. 500 units penicillin (2.5 µl, i.c.) + 1 µg ghrelin (i.c.v.) + 2.5 µg ACEA (i.c.v.)
14. 500 units penicillin (2.5 µl, i.c.) + 1 µg ghrelin (i.c.v.) + 7.5 µg ACEA (i.c.v.)
15. 500 units penicillin (2.5 µl, i.c.) + 0.5 µg ghrelin (i.c.v.) + 2.5 µg ACEA (i.c.v.)
16. 500 units penicillin (2.5 µl, i.c.) + 0.5 µg ghrelin (i.c.v.) + 2.5 µg ACEA (i.c.v.) + 0.25 µg AM-251 (i.c.v.)

Each animal group was composed of seven rats.

2.2. Drugs and drug administration

AM-251 (N-(piperidine-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide) and ACEA (arachidonyl-2-chloroethylamide) (Sigma Chemical Co., St. Louis, MO, USA) were used in the experiments. AM-251 and ACEA were dissolved in dimethylsulfoxide (DMSO) with sterile physiological saline (final solution DMSO/saline 3:7 volume/volume, respectively), and the requisite doses were administered intracerebroventricularly in 1 µl volumes. The drug dosages were determined according to Aslan et al. (2009) and Kozan et al. (2009).

The ICV injections were performed according to the method reported by Arslan et al. (2013). Briefly, a microsyringe was inserted into the left lateral ventricle of each rat through a stereotaxic apparatus. The drug solution was injected at an infusion rate of 0.5 µl/min using a Hamilton microsyringe (type 701N), and the needle remained in place for an additional minute to prevent backflow of the drug. The epileptic focus was produced with an injection of 500 units of penicillin G potassium 1 mm beneath the brain surface.

In the first set of experiments, doses of ACEA (2.5 and 7.5 µg, i.c.v.), AM-251 (0.25 and 0.5 µg) or ghrelin (1 µg) were administered 30 minutes after the i.c. application of penicillin. In the second set of experiments, the animals received either an effective dose of ACEA (7.5 µg, i.c.v.), a non-effective dose of ACEA (2.5 µg, i.c.v.) or effective doses of AM-251 (0.25 and 0.5 µg, i.c.v.) 10 minutes after the administration of ghrelin (1 µg, i.c.v.).

2.3. Placement of electrodes for electrocorticography (ECoG) recordings

The animals were anaesthetized with urethane (1.25 g/kg, i.p.) and placed in a rat stereotaxic apparatus. With stereotaxic guidance, two screw electrodes were placed over the left somatomotor cortex and a ground lead was positioned over the nasal sinus. Bipolar two Ag–AgCl ball electrodes were placed over the somatomotor cortex of the left hemisphere (Kozan et al., 2007; Yildirim et al., 2010). The electrocorticographic (ECoG) activity was continuously monitored on an eight-channel recorder (PowerLab, 4/SP, AD Instruments, Castle Hill, NSW, Australia). The frequency and amplitude of the epileptiform ECoG activity were analysed off-line.

2.4. Statistical analysis

All results are presented as the means ± standard error of the mean (SEM). Statistical comparisons were made using GraphPad Instat (v3.06) software (GraphPad Software, San Diego, CA, USA). The normality of the data was tested with the Shapiro–Wilk test before analyses. After verifying that data from electrophysiological recordings were normally distributed, one-way analysis of variance (ANOVA) and Tukey–Kramer post hoc tests for multiple

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