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The effect of leptin on penicillin-induced epileptiform activity in rats

Mustafa Ayyildiz [a], Mehmet Yildirim [a], Erdal Agar [a,*], A. Kasım Baltaci [b]

[a] *Department of Physiology, Faculty of Medicine, University of Ondokuz Mayis, 55139 Samsun, Turkey*
[b] *Department of Physiology, Meram Medical School, University of Selçuk, Konya, Turkey*

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Abstract

Leptin is an adipose tissue-derived peptide hormone, which acts as a satiety factor to reduce appetite by interactions with hypothalamic neurons. The other possible physiological functions of leptin are still unclear. In this study, we have evaluated dose-dependent effect of leptin on penicillin-induced epileptiform activity, analyzed by electrocorticogram (ECoG). The epileptiform activity was induced by microinjection of penicillin into the left sensorymotor cortex. Thirty minutes after penicillin injection, 1, 2 or 10 μg of leptin was administrated intracerebroventricularly (i.c.v.). Leptin (1, 2 or 10 μg) alone did not significantly change the spike amplitudes in non-penicillin pretreated control animals. One or two micrograms of leptin significantly increased the frequency of epileptiform activity in the penicillin-pretreated animals. The high dose of leptin (10 μg) did not significantly change either amplitude or frequency of epileptiform activity. One microgram i.c.v. leptin was the most effective dose in changing of frequency on penicillin-induced epileptiform activity. The proconvulsant effects of leptin appeared 90 min after leptin (1 and 2 μg) injection. These data indicate that leptin increases the frequency of penicillin-induced epileptic activity. We speculate that this action of leptin might suggest that leptin may be a proconvulsant substance.

Keywords: Leptin; Penicillin; Epileptiform activity; ECoG

1. Introduction

Leptin, the obese (*ob*) gene product is a 16 kDa protein that is synthesized predominantly by white adipose tissue. Its receptors have been found in various regions of brain including cortex, cerebellum, brain stem, basal ganglia and hippocampus. Functionally, leptin regulates energy homeostasis mainly via interactions in the brain [8]. An understanding of biology of leptin offers significant insights into the complex interrelationships among adipose tissue, the nervous system and peripheral organs [1]. Leptin enters the brain via a saturable process, the exact structures responsible of leptin transport are unknown [3,4]. Based on experience with other polypeptide hormones, it had been suggested that leptin was transported by receptor-mediated transcytosis across the blood–brain barrier [13]. It was reported that leptin produces its effects on food intake mainly by reduction of neuropeptide Y levels and intracerebroventricularly (i.c.v.) leptin administration results in a more potent response compared with the response to systemic leptin administration, suggesting that the central nervous system is major site of its action [14].

Shanley et al. provided further evidence of a role for leptin in the central nervous system that is unrelated to hypothalamic control of energy balance [16,17]. They found that leptin inhibits hippocampal neurons via activation of large conductance Ca^{2+} activated K^+ channels, a process that may be important in regulating neuronal excitability [16]. Shanley et al. also reported that leptin, via Ca^{2+} activated K^+ channel stimulation, could modulate aberrant synaptic activity in hippocampal neurons by using hippocampal slices and cultured neurons [16,17]. They concluded that leptin inhibits epileptiform-like activity in rat hippocampal neurons via PI 3-kinase-driven activation of Ca^{2+} activated K^+ channels [16].

Our findings represent a first attempt at studying the role of leptin in the penicillin-induced epilepsy in the rats, in vivo. Since previous studies indicate that leptin may change the level of neuropeptide Y and nitric oxide in the central nervous system, which may contribute to the regulation of epileptiform activity [20,7]. In the light of literature, we evaluated the effects of intracerebroventricular administration of leptin on the penicillin-induced epileptiform activity in the rats.

* Corresponding author. Tel.: +90 362 457 6000x2223; fax: +90 362 457 6041.
E-mail address: eragar@omu.edu.tr (E. Agar).

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2. Materials and methods

All experimental procedures were conducted with government approval according to local guidelines for the care and use of laboratory animals. Forty-eight female Wistar rats, weighing 220 and 260 g were assigned to the following experiments and groups: intracerebroventricular delivery of (1) 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.); (2) 1 μg leptin (Calbiochem) dissolved in 1 μl aCSF (i.c.v.); (3) 2 μg leptin (i.c.v.); (4) 10 μg leptin (i.c.v.); (5) penicillin-pretreated + 1 μg leptin (i.c.v.); (6) penicillin-pretreated + 2 μg leptin (i.c.v.); (7) penicillin-pretreated + 10 μg leptin (i.c.v.); (8) penicillin + aCSF (i.c.v.). Each animal group was composed of six rats.

2.1. Induction of epileptiform activity

The animals were anesthetized with urethane (1.25 g / kg, i.p.) and placed in a stereotaxic frame. Rectal temperature was maintained between 36.5 and 37.0 °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 100 mmHg during the experiments (mean 120 ± 8 mmHg). All contact and incision points were infiltrated with procaine hydrochloride to minimize possible sources of pain.

The epileptic focus was produced by penicillin injection. For the penicillin injection, a bur hole was drilled into the pericranium overlying the left sensory-motor cortex (2 mm posterior to bregma and 3 mm lateral to sagittal stures) and 300 units penicillin G potassium was injected 1 mm beneath the brain surface by a Hamilton microsyringe (type 701N) (infusion rate 0.5 μl/min) [11].

2.2. Intracerebroventricular (i.c.v.) administrations of leptin or aCSF

For i.c.v. leptin injections, an additional bur hole was drilled into the pericranium overlying the left cerebral lateral ventricle (0.8 mm posterior to bregma and 1.5 mm lateral to sagittal suture). Thirty minutes after penicillin injection, a Hamilton microsyringe (type 701N) was inserted 2.5 mm beneath the brain surface and 1 μl of leptin or aCSF were injected stereotactically into the left lateral ventricle (infusion rate 0.5 μl/min). After infusion, the injection needle remained inside the brain for 2 min to prevent leptin or aCSF (Calbiochem) diffusion along the needle track.

2.3. Electrophysiological recordings

Two Ag–AgCl ball electrodes were placed over the left somatomotor cortex (electrode coordinates: first electrode, 2 mm lateral to sagittal suture and 1 mm anterior to bregma; second electrode, 2 mm lateral to sagittal suture 5 mm posterior to bregma). The common reference electrode was fixed on the pinna. The electrocorticogram (ECoG) activity was continuously monitored on a two-channel recorder (PowerLab, 4/SP). All recordings were stored on a computer. The frequency and amplitude of epileptic activity was analyzed off line.

2.4. Statistical analysis

All statistical procedures were performed using SPSS statistical software package. Data analysis was performed using Repeated Measures Analysis of Variance and Dunnett's *t*-test for comparisons. Data are expressed as the means ± S.E.M. Statistical significance was set at $p < 0.05$.

3. Results

Intracerebroventricular injection of different doses of leptin (1, 2 or 10 μg) did not cause any change of the frequency or amplitude of ECoG activity in respect to control base line in non-penicillin injected animals (Fig. 1(Ab, Bb and Cb)).

Intra-cortical injection of penicillin (300 units) induced an epileptiform ECoG activity characterized by bilateral spikes and spike-wave complexes (Fig. 1(Ac, Bc and Cc)). This ECoG activity began 5 min after penicillin application and lasted for 3–5 h. It reached a constant level as a frequency and amplitude in 30 min. The mean of spike frequency and amplitude were 25 ± 4 spike/min, 860 ± 96 μV, respectively. Leptin was administrated 30 min after penicillin injection. The doses of 1 or 2 μg leptin significantly increased the mean of frequency of epileptiform activity to 29 ± 2, 36 ± 3, 46 ± 4; 24 ± 3, 42 ± 4, 36 ± 4 spike/min in 50, 100, 150 min after leptin injection (i.c.v.), respectively (Fig. 2). The best effects appeared 90 min after leptin injection and lasted for 200 min. Ten micrograms leptin did not change the frequency of epileptiform activity during the experiments (Figs.1Cd and 2). The doses of 1, 2 or 10 μg leptin did not significantly change the amplitude of epileptiform activity in all group during experiments. The amplitudes of epileptiform activity were 817 ± 74, 633 ± 63 and 720 ± 86 μV after 100 min from 1, 2 and 10 μg leptin (i.c.v.) injection, respectively (Fig. 1(Ad, Bd and Cd)). There was no change in the mean of frequency and amplitude in aCSF injected animals.

4. Discussion

The regulatory mechanisms of neuropeptide-metobolizing enzymes often play critical role in the pathogenic mechanisms of (e.g.) hypoxi-ischemia, epilepsy and chronic neurodegenerative disorders [2,10]. Epilepsy is associated with paroxysmal discharge of cerebral neurons and is characterized by several symptoms including alteration of behaviors and consciousness [5,9,19].

Leptin is thought to play an important role in carbohydrate and lipid metabolism, gastrointestinal and cardiovascular function, inflammation, immune response and reproduction [12] and modulate synaptic plasticity [15]. However, there is growing evidence that leptin can have other functions. In this study, we provide further evidence of a role for leptin in the nervous system that is unrelated to control of energy balance.

Previous studies have indicated that dose dependent (with 1 μg being the lowest i.c.v. dose having measurable effect) and long lasting (inhibition of nNOS activity starts 30 min after a single i.c.v. leptin injection, and is still present after a 12 h) [6,7]. The results of present study confirm that 1 μg i.c.v. leptin is the most effective dose in changing the frequency of penicillin-induced epileptic activity in the rat.

Shanley et al. reported that leptin induces a small reduction in the amplitude of evoked EPSCs, an action that is independent of any change in input resistance [15]. In contrast, in the present study, leptin did not significantly affect the amplitude of epileptiform activity in all groups. We also show that leptin increased the frequency of penicillin-induced epileptic activity. It may suggest that leptin affects Ca^{2+} activated K^+ channel activity since postsynaptic Ca^{2+} activated K^+ channel activity is vital in regulating the level of neuronal excitability, which determines action potential firing rate and burst firing patterns [16,18]. However, the results of the present study do not provide a support for the role of leptin as an anticonvulsant in contrast to Shanley et al.