Antiepileptic effects of exenatide in penicillin induced acute epilepsy model in rats

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Glucagon-like peptide-1 receptors are widely expressed in the brain and its association with nitric oxide is suggestive of its role in epilepsy. In this study, we investigated the effects of exenatide, a glucagon-like peptide-1 receptor agonist, on the epileptiform activity induced by penicillin injection. The study used 72 male Wistar albino rats in 9 groups. All groups except the last group which received only exenatide, received intracortical penicillin injection to induce epileptiform activity. Exenatide was intraperitoneally injected in II-IV groups, at doses of 50, 100, 200 μg/kg, respectively. Sodium nitroprusside (SNP) and Nω-nitro-L-arginine methyl ester (L-NAME) were injected to the V-VIII groups either alone or with exenatide. Electrocorticography was recorded for 3 h. While administration of 200 μg/kg exenatide reduced the frequency of epileptiform activity, 50 and 100 μg/kg doses of exenatide were not effective. When the effective dose of exenatide and the SNP were injected together the spike frequency decreased significantly. When the effective dose of exenatide was given with L-NAME spike frequency significantly decreased only between 90 and 110 min. There was no statistically significant difference in terms of latency and amplitude between the experimental groups. Exenatide had an anticonvulsant effect in penicillin-induced acute epilepsy model which is possibly via nitric oxide and include another pathway since its effect was partially blocked by L-NAME and potentiated by SNP.

Keywords: Brain electrocorticography, Glucagon-like peptide (GLP), Nitric oxide

Epilepsy is one of the most common neurological disorders characterized by recurrent seizures caused by abnormal and synchronous neuronal discharges in the brain. According to the World Health Organization (WHO), approximately 50 million people worldwide, including 23 million from Asia, are affected by epilepsy. Besides, anti-epileptic drugs can reveal psychiatric comorbidities in epilepsy patients. Depression and cognitive impairment are the major secondary complications that are often associated with epileptic patients. Therefore, various experimental epilepsy models are used to illuminate the molecular mechanisms of epilepsy and to develop new drugs for resistant patients. Penicillin model epilepsy is one of the most frequently used focal epilepsy models. Penicillin applied locally to the cortex inhibits gamma-aminobutyric acid-A (GABA-A) receptors, increasing neuronal excitability, thereby causing epileptiform activity in the brain. Moreover, GABA is released in approximately 40% of brain synapses.

Glucagon-like peptide-1 (GLP-1), an incretin family hormone secreted from L cells of the small intestine in response to food intake, has a role in regulating glucose homeostasis and gastric emptying. The major function of GLP-1 is to stimulate glucose-dependent insulin release from the pancreatic islets. GLP-1 binds to a Glucagon-like peptide-1 receptor (GLP-1R) that signals by activating adenylyl cyclase via stimulatory Gα and subsequently producing cyclic adenosine monophosphate (cAMP). GLP-1 is predominantly produced in the gut and to a smaller extent in the brain while its receptors are widely expressed in central nervous system, mainly in large neurons such as pyramidal neurons in the cortex, Purkinje neurons in the cerebellum, as well as the nucleus tractus solitarius, the hypothalamus and brain stem. In addition to its effect on regulation of metabolic functions, GLP-1 enters the cerebrospinal fluid (CSF) and may have effects such as neuronal cell protection, proliferation and differentiation, as well as play antioxidant and anti-inflammatory roles. GLP-1 agonists have also been reported to increase nitric oxide (NO) synthesis in endothelial cells. This reported association between GLP-1 agonists and the NO system suggesting that exenatide may also affect epileptiform activity via the NO system. In recent years, the GLP-1/GLP-1R system in the central

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nervous system has attracted the attention of neuroscientists because of its promnesic and neuroprotective effects in different neurodegenerative disorders, including Alzheimer's disease and Parkinson's disease\textsuperscript{14}.

Nitric oxide plays a role in the development and pathogenesis of seizures and its production is induced by exenatide\textsuperscript{3}. NO pathway has been shown to influence regulation of various behavioural and emotional functions\textsuperscript{15}. In excitatory neurons, NMDA receptors and nNOS are interconnected by postsynaptic density protein 95 at glutamatergic synapses and activation of nNOS depends on calcium influx mediated by NMDA receptors\textsuperscript{16}. NO was studied in a variety of experimental epilepsy models and suggested to have a role in epilepsy\textsuperscript{17}. The only study about the GLP-1R and epilepsy we came across in the literature demonstrated that GLP-1R knockout mice displayed decreased latency to seizure onset, increased seizure severity in a kainic acid induced epilepsy model\textsuperscript{18}.

Since the available data in the literature link GLP-1R, NO and epilepsy the current paper investigates for the first time the role of exenatide, a GLP-1R agonist, on penicillin-induced epileptiform activity in rats. The interaction of GLP-1 receptors with NO system in epilepsy we came across in the literature demonstrated that GLP-1R knockout mice displayed decreased latency to seizure onset, increased seizure severity in a kainic acid induced epilepsy model\textsuperscript{18}.

Materials and Methods

Experimental animals

Seventy-two male Wistar albino rats weighing 180-240 g were used in the study. They were obtained from the University Animals Research Center. Experimental designs and procedures were approved by Ondokuz Mayıs University Animal Research Committee in accordance with the European Commission regulations (2016-HAYDEK-5). Rats were kept in a temperature controlled environment (22±1°C) on a 12 h light/dark cycle with free access to standard rat food and tap water.

Experimental Groups

The rats were randomly divided into nine experimental groups each with 8 animals as follows: Gr. I: 500 IU penicillin (Control group); Gr. II-IV: 50, 100 and 200 μg/kg exenatide + 500 IU penicillin, respectively; Gr. V: 100 μg/2 μL L-NAME + 200 μg/kg exenatide + 500 IU penicillin; Gr. VI: 50 μg/2 μL SNP + 200 μg/kg exenatide + 500 IU penicillin; Gr. VII: 100 μg/2 μL L-NAME + 500 IU penicillin; Gr. VIII: 50 μg/2 μL SNP + 500 IU penicillin; and Gr. IX: 200 μg/kg exenatide. Administration of all test compounds were done Intracortically (i.c.).

Chemicals

The epileptiform activity was induced by injecting 500 IU of penicillin G potassium salt into the cortex (i.c.) with a Hamilton microsyringe in a volume of 2.5 μL. Exenatide was administered intraperitoneally (i.p.) 30 min before penicillin injection or alone. L-NAME (100 μg/2 μL) and SNP (50 μg/2 μL) were given intracortically 30 min before penicillin injections. Penicillin G potassium salt, exenatide, SNP and L-NAME were dissolving in physiologic saline solution. Urethane was dissolved in saline and administered at a dose of 1.25 g/kg (i.p.). Dosages of SNP and L-NAME were determined according to Marangoz et al.,\textsuperscript{3} and penicillin G dosages were determined according to Arslan et al.\textsuperscript{19}. Exenatide was obtained from AstraZeneca (Turkey) and all other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, US).

Surgical procedures and electrophysiological recordings

After the animals were anesthetized with urethane, an incision was made in the scalp in the rostrocaudal direction. Two holes with a diameter of 0.2 mm were drilled using a stereotaxic device (the hole 2 mm lateral and 3 mm anterior to bregma is for the positive electrode, the hole 2 mm lateral and 3 mm posterior to bregma is for the negative electrode). Custom-made stainless-steel screws were placed in the holes. The common reference electrode was placed on the mosquito used for exclusion\textsuperscript{20}. Brain electrocorticography (ECoG) recordings taken via copper wires wrapped around the screws were amplified and continuously monitored and recorded on an eight-channel recorder (Powerlab, 4/SP, AD Instruments, Australia). Two more holes were drilled on the skull, one for penicillin injection (1 mm lateral and 1 mm posterior to bregma) and one for SNP and L-NAME injections (2 mm lateral and 1 mm posterior to bregma). The basal brain activity was recorded for 10 min at the beginning of experiments in each animal. ECoG recordings continued for 3 h from the onset of epileptiform activity. The frequency and amplitude of the ECoG activity were analyzed off-line.

Statistical analysis

The data were analyzed using SPSS Version 17.0 software (Inc. Chicago, USA) after the electrophysiological
recordings were converted into numerical values. The normality of the data was tested with the One-Sample Kolmogorov-Smirnov test. After verifying that the data from electrophysiological recordings were normally distributed, one-way analysis of variance and post hoc Bonferroni tests were performed for multiple comparisons. For all statistical tests, \( P < 0.05 \) was considered statistically significant. All results are presented as the means±standard error of the mean (SEM).

**Results**

In the presented study, three different doses of exenatide were first used to determine the effective dose of exenatide on epileptiform activity created by penicillin, after which the interaction between the effective dose and the nitrergic system was investigated. There was no difference between the basal activity of the animals in any group and no spontaneous epileptiform spike was observed during the basal activity recordings. The epileptiform activity started approximately 1-4 min after the penicillin injection. ECoG recordings lasted for 3 hours after the injections and epileptiform activity was evident with the presence of spikes and spike-wave complexes in the recordings (Fig. 1).

When only 200 \( \mu \text{g/kg/i.p.} \) exenatide was injected without penicillin injection, there was no change in basal ECoG activity and no spontaneous spike occurred (Fig. 1J). There was no statistically significant difference in terms of latency of epileptiform spikes and spike amplitude between the groups \( (P > 0.05) \).

Rats were administered 50, 100 or 200 \( \mu \text{g/kg} \) exenatide (i.p.) 30 min before penicillin injection (Fig. 1C, 1D, 1E and Fig. 2). The spike frequency of epileptiform activity was not affected by 50 and 100 \( \mu \text{g/kg} \) exenatide \( (P > 0.05) \). However, injection of 200 \( \mu \text{g/kg} \) exenatide significantly decreased the spike frequency between 10-20, 60-120, and 140-150 min compared to the control group \( (P < 0.05) \) (Fig. 2).

![Fig. 1 — (A) Basal brain activity; (B) Intracortical (i.c.) injection of penicillin (500 IU) [caused epileptiform activity on ECoG]; Intracortical administration of (C) 50; (D) 100; (E) 200 \( \mu \text{g/kg} \) exenatide [C & D: No change in the mean frequency and amplitude of epileptiform activity caused by penicillin, but (E) changed the average frequency and amplitude of epileptiform activity caused by penicillin]; (F) 200 \( \mu \text{g/kg} \) exenatide and L-NAME (100 \( \mu \text{g/2 \muL, i.c.} \) [reduced the frequency of epileptiform activity between 90 and 110 min after penicillin administration]; (G) L-NAME (100 \( \mu \text{g/2 \muL, i.c.} \) [no change in the average frequency of epileptiform activity]; (H) 200 \( \mu \text{g/kg} \) exenatide and SNP (50 \( \mu \text{g/2 \muL, i.c.} \) [reduced the frequency of epileptiform activity between 0 and 170 min; (I) SNP (50 \( \mu \text{g/2 \muL, i.c.} \) [reduced the average frequency of epileptiform activity between 10 and 40, 50 and 70, 160 and 170 min]; and (J) Basic ECoG activity in an animal with only 200 \( \mu \text{g/kg} \) exenatide. [Representative ECoGs are presented for 180 minutes after penicillin administration]
Injection of L-NAME (100 μg/2 μL) did not cause a significant change in spike frequency after penicillin injection (\(P > 0.05\)). SNP (50 μg/2 μL) significantly decreased the spike frequency between 10-40, 50-70, 160-170 min after penicillin injection (\(P < 0.05\)) (Fig. 3).

When the effective dose of exenatide (200 μg/kg) and L-NAME (100 μg/2 μL) were administered together, the spike frequency decreased significantly only between 90 and 110 min after the penicillin injection compared to the control group (\(P < 0.05\)). There was no significant change in spike frequency between 0-90 and 110-180 min (\(P > 0.05\)) (Fig. 3).

Administration of the effective dose of exenatide (200 μg/kg) and SNP (50 μg/2 μL) together significantly decreased the mean spike frequency between 0 and 170 min compared to the control group (\(P < 0.05\)) (Fig. 3).

**Discussion**

In the present study initially, the effects of the nitrergic system and exenatide on the epileptiform activity induced by penicillin were investigated separately, after which the interaction of both systems on epilepsy was studied. Exenatide with a dose of 200 μg/kg was found to have an anticonvulsant effect. Co-administration of SNP and exenatide was observed to decrease spike frequency more than exenatide alone. It was found that co-administration of L-NAME and exenatide did not completely block the effect of exenatide on spike frequency. There was no change in the saline-only group.

Intracortical penicillin administration triggers an epileptogenic focus formation similar to the focal seizures of epileptic patients. The penicillin G, which causes epileptic seizures by inhibition of the GABA system, is structurally similar to bicuculline, which is a GABA antagonist\(^{27,22}\).

The ease of establishing the experimental acute epilepsy model, similarity to human seizures, long duration of epileptic activity, knowing the mechanism of the epileptic model, and the formation of spontaneous, rapid, and effective seizures are the advantages of choosing the penicillin-induced model for the study. GABA and glutamate neurotransmitters can have a significant effect on the stimulating and inhibitory currents of the neurons. Besides, GABA and glutamate are the most basic and important neurotransmitters for epilepsy. It has been reported that GABA contributes to the prevention of the excessive excitability of the nervous system\(^{23}\).

GLP-1 receptor agonists and DPP-4 inhibitors, which are among the new drugs, are used in the treatment of type 2 diabetes. Immunological experiments showed the presence of release-regulatory presynaptic receptors for GLP-1, which is characterized as a neurohormone, in mouse glutamatergic and GABAergic nerve terminals\(^8\). Stimulation of presynaptic GLP-1Rs increases the release of glutamate and GABA from depolarized synaptosomes\(^5\). GLP-1 increases GABA signaling via pre- and postsynaptic pathways\(^24\). GLP-1 and exendin-4 increased the frequency of the hypocretin neurons in the mouse hypothalamic slice without increasing the amplitude of the naturally stimulating postsynaptic currents and suggests presynaptic modulation of glutamate release from the afferent fibers\(^25\).

There are very limited studies in the literature investigating the relationship between GLP-1 receptor agonists and epilepsy. In a pentylenetetrazole-induced experimental epilepsy model, 75 and 150 μg/kg/i.p. doses of liraglutide significantly prevented seizure severity, restored behavioral activity, prevented the
formation of oxidative defense enzymes and different neurochemicals in the mouse brain\textsuperscript{26}. GLP-1 analogues (liraglutide and lixisenatide) have shown protective effects in the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) rodent model of Parkinson's disease and have provided positive effect on the development of motor function\textsuperscript{27}. Exenatide can cross the blood-brain barrier and strengthen neurogenesis in the brain\textsuperscript{28}. Clinical studies with neuroprotective benefits have been conducted with exenatide in the treatment of several neurological disorders such as Alzheimer's, Parkinson's, brain injury from trauma, and paralysis\textsuperscript{29}.

The fact that multiple pathogenic mechanisms are involved in epilepsy pathogenesis suggests that the use of drugs targeting multiple pathogenic mechanisms should have greater efficacy in epilepsy treatment. Therefore, GLP-1 analogues present great promise as a novel treatment/adjunct treatment for epilepsy and associated complications like depression and cognitive impairment\textsuperscript{30}. Further studies are needed to better understand the neurotrophic and neuroprotective effects of GLP-1 in epileptic subjects.

Epileptic seizures can lead to the development of neurological diseases, such as cognitive impairment. Serious and prolonged seizures can even cause neuronal deaths\textsuperscript{31}. The pathologies arising from epileptic seizures have been resolved by various clinical applications including antibiotics and anticonvulsant drugs\textsuperscript{32}.

In the experimental rat model by penicillin-induced epileptiform activity, the NO donor SNP (50 µg) reduced spike frequency, which shows that an increase in the amount of NO reduces epileptiform activity\textsuperscript{33}. Studies have shown that GLP-1 stimulates NO production by increasing the expression of eNOS\textsuperscript{34,35}.

In our study, the results revealed that the interaction of exenatide and SNP significantly inhibited epileptiform spike frequency. The fact that liraglutide, one of the GLP-1 agonists, shows an important antiepileptic activity, possibly by increasing brain GABA and glutathione levels, supports our study\textsuperscript{36}. The glutamatergic and GLP-1R signal mechanisms interact in many brain regions, and the GLP-1R signal can alter excitatory synaptic transmission through cellular mechanisms involved in long-term synaptic plasticity\textsuperscript{37}.

Pentylenetetrazole (PTZ) kindling model is a chronic epilepsy model characterized by a continuous increase in seizure predisposition. PTZ kindling causes changes at the molecular and cellular levels, which are responsible for oxidative stress and neurodegenerative changes in the hippocampus\textsuperscript{38}. In the PTZ kindling model of epilepsy, a significantly higher nNOS expression and enzymatic activity have been reported in the hippocampus of mice\textsuperscript{39}. It has been shown that liraglutide, a GLP-1 agonist had antiepileptic effects, prevented secondary complications (such as cognitive impairment and depression), and the sudden changes of neurotransmitter amounts in the mouse brain caused in the PTZ kindling model\textsuperscript{18}.

In the present study, intraperitoneal injection of exenatide decreased epileptiform spike frequency induced by penicillin injected into the cortex. When exenatide and SNP were administered together a further reduction in spike frequency occurred, which showed that SNP potentiated the effects of exenatide. Administration of L-NAME together with the effective dose of exenatide partially eliminated the effect of exenatide on the spike frequency. The finding that the effect of exenatide is enhanced by SNP but not completely blocked by L-NAME indicates that exenatide can act on the NO pathway but can also use a different pathway to affect spike frequency. In light of these results, exenatide can be expected to be used for the treatment of epilepsy together with other antiepileptic drugs in the future. However, further study is needed to elucidate the mechanisms of the effects of exenatide other than the nitrergic system in epilepsy. The study also suggests that exenatide, which is used in diabetic patients to regulate blood glucose levels, can also be safely used in diabetic patients with epilepsy.

**Conclusion**

Exenatide significantly decreased the epileptiform activity in the brain cortex in the experimental epilepsy model induced by intracortical penicillin injection. Its effect was further increased when administered with SNP, which suggests that exenatide may exhibit its effects via the NO pathway. Exenatide, which is used to treat type 2 diabetes, can also boosts neurogenesis in the brain by crossing the blood-brain barrier, suggesting that it could be used to treat epilepsy along with other antiepileptic drugs. Further research that includes chronic experimental epilepsy models, behavioural epilepsy models and diabetic animals would be valuable to ascertain its antiepileptic effects.
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Conflict of Interest

Authors declare no competing interests.

References


