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The effect of aprepitant, a neurokinin-1 receptor antagonist, on epileptiform activity in the penicillin model of epilepsy

Selma Cirrik, Elif Dogan, Ali Aslan, Niyazi Tasci

Ordu University, Faculty of Medicine, Department of Physiology, Ordu, Türkiye

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Abstract

Substance P (SP) is a peptide neurotransmitter that plays a role in pain transmission, stress, inflammation, and emesis through neurokinin-1 receptor (NK-1R). Previous studies of NK-1R antagonists suggest that SP can have either convulsant or anticonvulsant effects. Aprepitant, an NK-1R antagonist, is clinically used to prevent nausea and vomiting in chemotherapy and post-operative patients. However, the effects of aprepitant on epilepsy have not been studied yet. Therefore, the objective of this study was to explore the influence of aprepitant on epileptiform activity in a rat model of epilepsy induced by penicillin. Male Wistar rats, aged 2.5 to 3 months, were divided into 5 groups (n=6): Control, Vehicle, Aprepitant, Substance P, and Aprepitant+Substance P. Aprepitant was administered intragastrically (30 mg/kg) 24 hours and 3 hours before surgery. Substance P (10 pmol) was administered intraventricularly during the surgical procedure. Induction of epileptiform activity was achieved by the injection of penicillin G (500 IU) into the cortex. Electrocorticography (ECoG) recording was conducted online using the PowerLab data acquisition system for over 3 hours. The disparity in spike frequency and amplitude among groups for each 10-minute interval was assessed through One-Way ANOVA and subsequent Tukey post-hoc tests, utilizing the SPSS 15.0 software package. Epileptiform activity was observed in all rats within 3 minutes after the injection of penicillin into the cortex. No significant difference was found between groups in terms of spike frequency and amplitude. When the percentage changes in mean spike frequency and amplitude values were calculated, no significant difference was shown between the groups. Using a penicillin-induced epilepsy model, the present study showed that aprepitant does not affect epileptiform activity in the presence or absence of exogenous SP. Further studies are needed to understand possible anticonvulsant or proconvulsant properties of aprepitant.

Keywords: Penicillin, epileptiform activity, substance P, aprepitant, electrocorticography

Introduction

Epilepsy is among the prevalent neurological disorders, characterized by increased excitability of neurons in the cortical and subcortical regions of the brain. Traumatic brain injuries, developmental abnormalities, inflammation, and hypoxia are defined as the main factors that can trigger epilepsy; however, the cause of epilepsy remains unknown in approximately half of cases. The initiation of seizures may induce alterations in consciousness and behavior, as well as motor activity [1-3]. During epileptic seizures, the balance between glutamate and gamma-aminobutyric acid (GABA) shifts towards the excitatory glutamate side, leading to an abnormal increase in electrical activity. However, ongoing research indicates the involvement of other neurotransmitters in epilepsy, such as dopamine, norepinephrine, serotonin, histamine, acetylcholine, melatonin, nitric oxide, and adenosine [1,2].

Substance P (SP) is a tachykinin family member neuropeptide with 11-amino acid derived from the preprotachykinin A (PPT-A) gene. It is extensively expressed in the central and peripheral nervous systems and has physiological effects such as pain transmission, inflammation, emesis, stress, anxiety, depression, and neurodegeneration through the neurokinin-1 receptor (NK-1R) [4]. Previous studies indicated that the SP/NK-1R system may also play a role in epilepsy [5]. For example, intrahippocampal injection of SP was shown to trigger seizures and neuronal damage in response to subconvulsive perforant

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Corresponding Author: Selma Cirrik, Ordu University, Faculty of Medicine, Department of Physiology, Ordu, Türkiye
Email: selmacrrk@yahoo.com

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path stimulation in rats [6]. In mice deficient in the PPT-A gene, a reduction in behavioral seizures in response to kainic acid or pentylenetetrazol injection was reported, along with decreased hippocampal cell death in kainite-induced epilepsy [7]. Furthermore, the antiseizure effects of NK-1R antagonists were shown in experimental epilepsy models induced by kainic acid [8], bicuculline, maximal electroshock [9], and sodium channel inhibitors [10]. Despite studies indicating the convulsant properties of SP, various reports showed that the speed of spontaneous epileptiform discharges induced by GABA-A receptor blockade in cortical slices slowed in the presence of SP. According to these results, it was suggested that SP increases the excitability of GABAergic interneurons in the entorhinal cortex and hippocampus; therefore, it can be considered as an anticonvulsant [11-13].

Although conflicting results exist, the majority of findings in the literature demonstrated that SP might act as a convulsant neurotransmitter, and NK-1R antagonists were suggested as potential anticonvulsants. Despite the discovery of more than three hundred NK-1R antagonists, only a limited few are currently clinically viable. One of them, aprepitant, can cross the blood-brain barrier and inhibit the emetic reflex by blocking NK-1R in the main emetic centers. It is specifically used to prevent nausea and vomiting induced by chemotherapy [5,14,15]. However, the effects of aprepitant on epilepsy have not been studied yet. As a clinically used NK-1R antagonist, it is important to reveal whether aprepitant has convulsant or anticonvulsant properties. Therefore, the objective of this study was to explore the impact of aprepitant on epileptiform activity in a rat model induced by penicillin.

Material and Methods

Animals and Grouping

In the present study, 30 male Wistar rats weighing between 200-300 g were used, and all the experimental procedures were approved by the local ethics committee for animal experiments (Approval number: 2021/02-10). The rats were randomly divided into 5 groups as Control, Vehicle, Aprepitant, Substance P, and Aprepitant+Substance P (n=6). A surgical procedure and intracortical penicillin injection were performed in all animals to create the penicillin-induced epilepsy model, as described below. In the Control group, rats underwent penicillin-induced epilepsy without any pre-treatment, and epileptiform activity was recorded. In the other groups, drug or vehicle applications were administered as described below.

Intragastric Drug or Vehicle Administration

In the aprepitant and aprepitant + substance P groups, aprepitant (30 mg/kg, prepared in a mixture of 10% ethanol:60% propylene glycol:30% water) was given intragastrically twice: 24 hours and 3 hours before surgery. For the Vehicle and Substance P groups, the aprepitant solvent (10% ethanol:60% propylene glycol:30% water) was administered in the same manner [16].

Intracerebroventricular (i.c.v) Drug or Vehicle Administration

In the Substance P and Aprepitant+Substance P groups, SP (10 pmol, 3 microliters), and in the Vehicle and Aprepitant groups, an equal volume of phosphate buffer (as the SP vehicle), were administered into the lateral ventricle using a Hamilton microsyringe, as defined below [17].

Surgical Procedure and Induction of Epilepsy

The animals were fasted overnight before surgery, but access to water was freely provided. Electrocorticography (ECoG) recordings were obtained from all animals following the same surgical procedure under urethane anesthesia (1.25 g/kg, intraperitoneal, ip). Briefly, the scalps of the animals were incised in a rostro-caudal direction after they were placed in the stereotaxic frame. After removing the soft tissue over the somatomotor cortex, the bregma was identified as the reference point. Using a stereotaxic device and referencing the bregma, two holes were drilled: one 3 mm lateral and 4 mm rostral from the bregma, and the other 3 mm lateral and 4 mm caudal from the bregma, each with a diameter of 0.2 mm. Stainless steel screws were placed into these holes (1 mm vertical depth), and the ground electrode was fixed on the pinna. Then electrodes were connected to the PowerLab data acquisition system via copper wires.

After observing baseline electrical recordings, an injection of penicillin G (500 IU; 2.5 microliters) was administered into the cortex to induce epileptiform activity. The intracortical penicillin injection was performed 1.6 mm lateral and 0.5 mm caudal to the bregma and reached a depth of 3.5 mm using a Hamilton microsyringe.

30 min after the penicillin G injection, substance P or phosphate buffer (as a vehicle for substance P) was injected into the lateral ventricle 2.2 mm lateral and 1.2 mm posterior to the bregma using a Hamilton microsyringe to a depth of 4.2 mm in all rats except the control group.

ECoG recording was performed online using the PowerLab data acquisition system for over 3 hours without the use of filters. Throughout this period, a temperature-controlled heating pad was used to maintain the body temperature of the animals.

The percentage values of the frequency and amplitude of the epileptiform activity were calculated according to the following formulas;

Frequency value %

$$= \frac{\text{The mean of spike frequency after substrate administration}}{\text{The mean of spike frequency before substrate administration}} \times 100$$

Amplitude value %

$$= \frac{\text{The mean of spike amplitude after substrate administration}}{\text{The mean of spike amplitude before substrate administration}} \times 100$$

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Statistical Analysis

All data were statistically evaluated using SPSS 15.0. The normality of the data was tested with the Kolmogorov-Smirnov test, revealing that all groups had normal distribution. Differences between groups in terms of spike frequency and amplitude for each period were analyzed using one-way ANOVA and post-hoc Tukey test. All results are presented as mean±standard error (SEM), and a significance level of $p<0.05$ was considered statistically significant.

Results

The baseline activity of each animal was recorded for 5 min and confirmed to be free of epileptic activity. Epileptiform activity was observed within 0-3 minutes following the intracortical injection of penicillin (500 IU) in all experimental groups.

In the control group, the mean spike frequency was 77.3±5.5 spikes per minute at the 90th minute. Administration of vehicle, aprepitant, or SP did not result in significant changes in the mean spike frequency ($p>0.05$). The values were not significantly different from the control group, with recorded spike frequencies of 71.3±5.8, 69±9.2, 65±7.8, and 68.8±8.4 spikes per minute in the Vehicle, Substance P, Aprepitant, and Aprepitant+Substance P groups, respectively (Figure 1 and Figure 2).

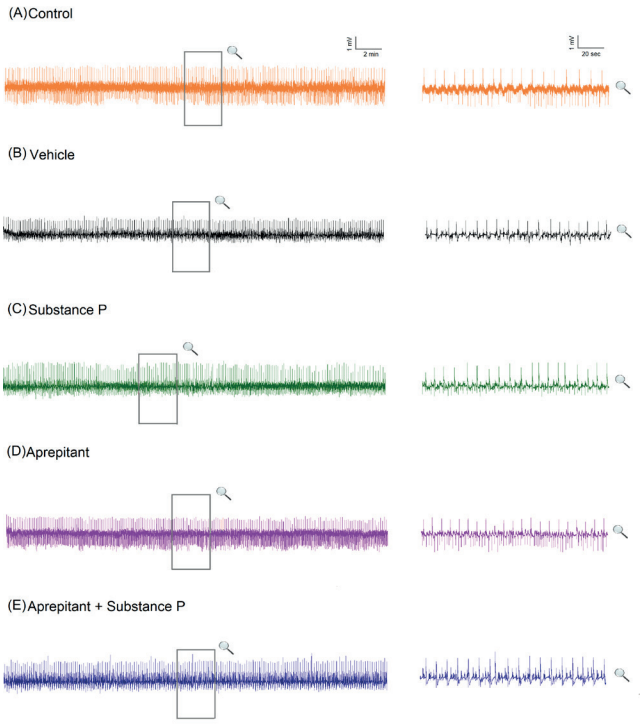

Figure 1. Representative ECoG recordings at 90th minute (**A**) Penicillin (500 IU, intracortical) induced epileptiform activity on ECoG (**B**) Vehicle injection did not affect the mean frequency and mean amplitude of penicillin-induced epileptiform activity on ECoG (**C**) Administration of Substance P did not affect the mean frequency and mean amplitude of penicillin-induced epileptiform activity on ECoG (**D**) Administration of aprepitant did not affect the mean frequency and mean amplitude of penicillin-induced epileptiform activity on ECoG (**E**) The combination of aprepitant and Substance P did not affect the mean frequency and mean amplitude of penicillin-induced epileptiform activity on ECoG

In the control rats, the mean spike amplitude was 1.4761±0.5 mV at the 90th minute. This value did not change in the treatment groups and was recorded as 1.4614±0.5 mV in the vehicle group, 1.3296±0.7 mV in the Substance P group, 1.6302±0.4 mV in the Aprepitant group, and 1.56235±0.5 mV in the Aprepitant+Substance P group (Figure 3).

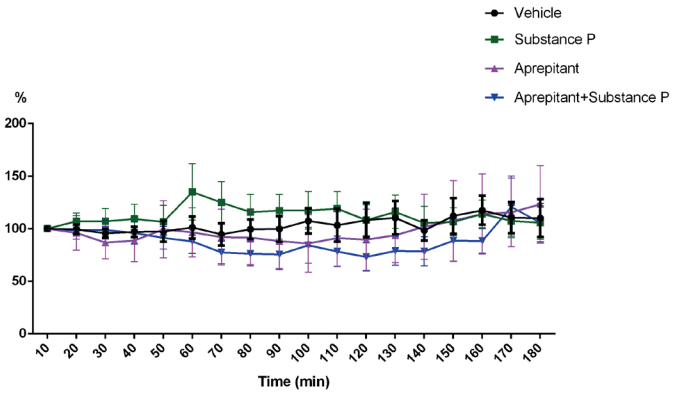

Figure 2. The effects of vehicle, Substance P, aprepitant and the combination of aprepitant+Substance P on the mean spike frequency of penicillin-induced epileptiform activity. Substance P, aprepitant and combined aprepitant+Substance P treatments did not affect the mean frequency of epileptiform activity. The percentage frequency value of epileptiform activity was calculated using the following formula; Frequency value %=(The mean of spike frequency after substrate administration)/(The mean of spike frequency before substrate administration)x100

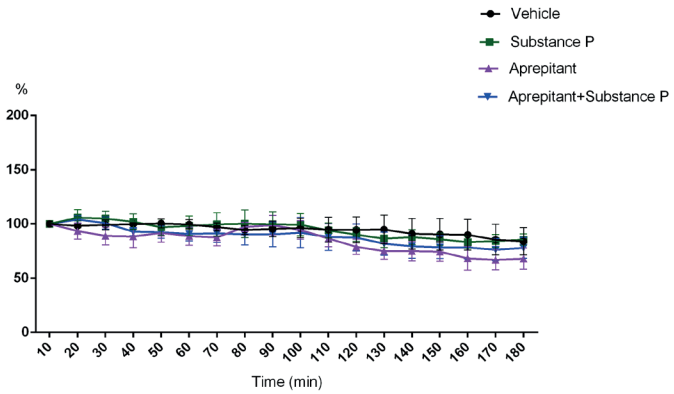

Figure 3. The effects of vehicle, Substance P, aprepitant and combination of aprepitant+Substance P on the mean spike amplitude of penicillin-induced epileptiform activity. Substance P, aprepitant and combined aprepitant+Substance P treatments did not change the mean amplitude of epileptiform activity. The percentage amplitude value of epileptiform activity was calculated using the following formula; Amplitude value %=(The mean of spike amplitude after substrate administration)/(The mean of spike amplitude before substrate administration)x100

Discussion

This study demonstrated that aprepitant, a clinically utilized NK-1R antagonist, has no effect on epileptiform activity induced by penicillin. It is suggested that the SP/NK-1R pathway may not be involved in penicillin-induced epileptogenesis in rats.

Based on the World Health Organization report stating that

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there are approximately 50 million epilepsy patients worldwide, epilepsy can be considered one of the most common neurological disorders. The underlying reasons for epilepsy cannot be elucidated in approximately half of patients. Furthermore, problems related to antiepileptic drugs, such as their focus on alleviating symptoms rather than dealing with the fundamental causes of epilepsy, and the presence of various side effects, including depression and cognitive impairment, persist. Therefore, both clinical and experimental studies continue extensively [18-21]. Animal models of epilepsy are useful in research aiming to understand the pathophysiology of epilepsy and develop treatment strategies. Different chemical convulsants such as kainic acid, pentylenetetrazol, bicuculline, and penicillin are used to induce epileptic seizures in experimental studies [22,23]. Penicillin epilepsy is one of the most commonly used experimental epilepsy models. Penicillin G, a beta-lactam antibiotic used in the present study, acts as a GABA-A receptor antagonist. Thus, intracortical injection of this antibiotic initiates abnormal electrical activity by suppressing GABAergic neurotransmission [22]. The increased neuronal excitability caused by penicillin leads to epileptic seizures that initiate focally and spread to become generalized [24-26]. Consistent with the literature, epileptiform ECoG activity was observed in all rats injected with intracortical penicillin G within 0-3 minutes in this study.

SP is a peptide neurotransmitter expressed both in the central and peripheral nervous systems. In addition to its role in nociception, depression and neurogenic inflammation, SP also plays a role in the regulation of emesis. Expression of NK-1R was demonstrated in brain regions associated with the emetic reflex, such as the nucleus solitary tract and area postrema. Since NK-1R activation leads to nausea and vomiting, its specific antagonists are utilized as antiemetic agents in practice [4,27]. Currently, the specific NK-1R antagonists allowed for use in clinical practice, generally needed for chemotherapy and post-operative patients, are aprepitant (administered orally) and fosaprepitant (an intravenous prodrug of aprepitant) [14]. Although epilepsy is a pathophysiological event involving SP and NK-1R, the effects of the clinically widely used NK-1R antagonist, aprepitant, on epilepsy are unknown. In the current study, the influence of aprepitant on epileptiform activity was examined in a model of epilepsy induced by penicillin. Our results indicated that pretreatment with aprepitant did not significantly alter epileptiform activity triggered by penicillin injection. Analysis of ECoG recordings showed that the changes in frequency and amplitude of the spikes in the Aprepitant group were not significantly different from the Control or Vehicle groups. The central efficacy of aprepitant at the dose we used, including changes in brain activity and feeding behavior, was demonstrated in previous studies [16]. Therefore, the lack of change in ECoG results might depend on the lack of involvement of NK-1R in penicillin-induced epilepsy, rather than the ineffective dose of aprepitant.

Previous studies showed that epilepsy-induced changes in SP expression may vary depending on the epilepsy model, brain region, and the time elapsed after the induction of epilepsy. For example, 30 minutes after perforant path stimulation, increased levels of hippocampal PPT-A mRNA and SP have been reported by Liu and colleagues [6]. However, unchanged SP levels were shown in the frontal cortex, striatum, and hippocampus 30 minutes after pentylenetetrazol and bicuculline applications [28], or 15 minutes after electroconvulsive stimulation [29]. Following kainic acid administration, examinations of PPT-A mRNA levels in the hippocampus, striatum, and frontal cortex on days 2, 10, and 30 revealed that PPT-A mRNA increased only in the hippocampus on day 2 [30]. In the present study, changes in SP expression levels were not investigated, which could be considered as a limitation of the study. Although plasticity in SP synthesis during 3-hour ECoG recording is unknown, the observation that the SP receptor antagonist aprepitant did not induce any changes in epileptiform activity suggests that intracortical penicillin injection may not change central SP synthesis.

The results of previous studies with NK-1R antagonists suggest that SP can be either a convulsant [8-10] or anticonvulsant neurotransmitter [11-13]. Different results may come from variations in the experimental epilepsy models or receptor antagonists. The effect of any NK-1R antagonist in an epilepsy model triggered by penicillin or the efficacy of aprepitant in any epilepsy model has not been previously investigated. In the present study, for the first time, we showed that aprepitant did not affect epileptiform activity in the penicillin-induced epilepsy model. Moreover, unchanged epileptiform activities were also observed in the presence of exogenous SP in the Substance P group and Aprepitant+Substance P group. Although the administration route is different, it was previously demonstrated that hippocampal injection of same dose of SP triggered epilepsy in response to subconvulsive perforant pathway stimulation [6]. The discrepancy in the effects of SP may be due to the experimental epilepsy model. Therefore, we suggest that the NK-1R pathway may not have been activated, at least during the first 3 hours of ECoG recording, and its activation with exogenous SP does not alter epileptiform activity in the penicillin model of epilepsy. However, to support this view, it is necessary to examine changes in the expression of SP and NK-1R, which are key components of the pathway, as well as the GABA-A receptor, which mediates the effect of penicillin.

Conclusion

In the current study, to evaluate the involvement of the SP/NK-1R pathway in the penicillin-induced epilepsy model, aprepitant was chosen as NK-1R antagonist. It is important to understand the anticonvulsant or proconvulsant properties of this clinically used drug to avoid or select its usage in patients diagnosed with epilepsy. Our results showed the aprepitant has no effect on epileptic seizures, at least in the penicillin-induced epilepsy

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model. But further studies are needed to understand its effect in different epilepsy models.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
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Ethical Approval
This study received ethical approval from the Local Ethics Committee of Experimental Animals at Ordu University (Approval number: 2021/02-10, date: March 30, 2021).

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