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Proconvulsant effect of bisphenol A in penicillin-induced epileptiform activity

Bisfenol A'nın penisilinle indüklenen epileptiform aktivitedeki prokonvülzan etkisi

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SUMMARY

Objective: Epilepsy is a neurological disease characterized by seizures that can affect all age groups. Experimental epilepsy models have been used in order to prevent and treat epileptic seizures. Bisphenol A (BPA) is found in the plastics that are used in our daily lives and causes harmful effects on the central nervous system. In this study, we aimed to investigate the effect of BPA in the penicillin-induced epileptiform activity.

Method: Male Wistar rats weighing 205 ± 220 grams were separated into 4 groups: Control (n=7), 125 µg/kg BPA (n=7), 250 µg/kg BPA (n=7) and 500 µg/kg BPA (n=7). Rats were anesthetized with urethane and were fixed to a stereotaxic device. With the stereotaxic guidance, an electrode was placed over the left somatomotor cortex and is connected to the recorder. 500 IU penicillin G was administered intracortically for induction of epileptiform activity. After 30 minutes from penicillin injection, the doses of BPA or olive oil were administered intraperitoneally, and electrocorticography recording continued for 180 minutes after drug injection.

Results: BPA, at a dose of 125 µg/kg, did not significantly change either the means of spike frequency or amplitude when compared to the control group.BPA, at the doses of 250 and 500 µg/kg, showed proconvulsant activity by increasing the mean spike frequency in the 50 and 40 minutes (respectively) until the end of the experiment without changing the amplitude.

Conclusions: The results of the present study provide electrophysiological evidence that BPA increases the epileptiform activity. Therefore, we suggest that epilepsy patients should avoid exposure to BPA.

Keywords: Epilepsy, Epileptiform activity, Penicillin, Seizure, Electrocorticography, ECoG, Bisphenol A

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ÖZET

Amaç: Epilepsi, tüm yaş gruplarını etkileyebilen nöbetlerle karakterize bir nörolojik hastalıktır. Epileptik nöbet oluşumundan korunmak ve nöbetleri tedavi etmek amacıyla deneysel epilepsi modelleri kullanılmaktadır. Günlük hayatımızda kullandığımız plastiklerde bulunan bisfenol A (BPA), santral sinir sisteminde zararlı etkilere yol açmaktadır. Bu çalışmada, bisfenol A'nın penisilinle oluşturulan epileptiform aktiviteye etkisini bulmayı amaçladık.

Yöntem: 205 ± 220 gram ağırlığındaki Wistar erkek sıçanlar kontrol (n=7), 125 µg/kg BPA (n=7), 250 µg/kg BPA (n=7) ve 500 µg/kg BPA (n=7) olmak üzere 4 gruba ayrıldı. Sıçanlar üretan ile anesteziye alındıktan sonra stereotaksik cihaza sabitlendi. Stereotaksi cihazı yardımıyla, sol somotomotor korteksin üzerine bir elektrot yerleştirildi ve bu elektrot kayıt cihazına bağlandı. Epileptiform aktivite oluşturmak için 500 IU penisilin G intrakortikal olarak uygulandı.

Penisilin enjeksiyonundan 30 dakika sonra, belirlenen dozlarda BPA veya zeytinyağı intraperitoneal olarak uygulandı ve elektrokortikografi kaydı, ilaç enjeksiyonundan itibaren 180 dakika daha sürdürüldü.
Bulgular: 125 µg/kg dozunda uygulanan BPA, kontrol grubuyla karşılaştırıldığında ortalama spike frekansı veya amplitüdünde anlamlı bir değişikliğe neden olmadı. 250 ve 500 µg/kg dozlarında uygulanan BPA, prokonvulzan aktivite göstererek ortalama spike frekansını sırasıyla 50. ve 40. dakikalardan itibaren deneyin sonuna kadar artırırken, amplitüdde bir değişiklik oluşturmadı.
Sonuç: Bu çalışmanın sonuçları BPA'nın epileptiform aktiviteyi arttırdığına dair elektrofizyolojik kanıtlar sunmaktadır. Bu nedenle epilepsi hastalarının BPA maruziyetinden kaçınması gerektiğini düşünmekteyiz.
Anahtar sözcükler: Epilepsi, Epileptiform aktivite, Penisilin, Nöbet, Electrokortikografi, ECoG, Bisfenol A

INTRODUCTION

Epilepsy is one of the most common neurological diseases characterized by recurrent seizures, which may cause mental and physical dysfunction by affecting the nervous system. Epileptic seizures are caused by disruption of the balance between excitatory and inhibitory systems in the central nervous system [1]. Experimental epilepsy models have been used in order to prevent seizures and develop more effective antiepileptic drugs [2]. One of these models is the penicillin model experimental epilepsy. Intracortical penicillin administration triggers an epileptogenic focus formation similar to focal seizures of epileptic patients [3]. Penicillin, structurally similar to GABA antagonist bicuculline which blocks the inhibitory GABA system and causes the formation of epileptic seizures [4].

We contact thousands of harmful chemicals every day, and many of them are risky for our health. One such compound is BPA that can leak from polycarbonate plastics, epoxy resins, and other products in contact with drinks and foods [5-7]. BPA, a synthetic chemical, readily penetrates into food and beverages at elevated temperatures or as a result of packaging damage. This compound enters into the body primarily through the digestive tract and through the respiratory system and skin [8-10]. Even at low levels, BPA can induce aggression, anxiety, hyperactivity, and learning-memory impairment [11-14]. Although a number of studies reported that BPA produces adverse effects on the nervous system, no data is available in the literature about the impact of BPA on epileptic seizures. In the present study, we aimed to investigate the effects of BPA in the penicillin-induced epileptiform activity.

MATERIAL AND METHODS

Animals

After the local ethics committee approved all experimental procedures, specific-pathogen-free male Wistar rats weighing 205 ± 220 grams were purchased from the Animal House of Ondokuz Mayis University. All animals were housed in a temperature controlled (23 ± 1 ^{O}C) environment on a 12 h light/dark cycle with free access to standard rat food and tap water.

Animals were separated into 4 groups: 1. Control (1 mL olive oil; i.p.), 2. 125 µg/kg BPA (1 mL; i.p.), 3. 250 µg/kg BPA (1 mL; i.p.) and 4. 500 µg/kg BPA (1 mL; i.p.). Each group composed of 7 rats.

Electrocorticography (ECoG) Recordings

Animals were starved for 24 hours before surgery. In the experiment day, rats were anesthetized with urethane (1.25 g/kg, i.p.) and placed in a stereotaxic device. With the stereotaxic guidance, two holes were drilled with a dental motor (OmniDrill35, WPI, Korea) and two screws were placed over the left somatomotor cortex and an earth lead was positioned over the left pinna [15, 16]. An electrode was wrapped around screws, and it was connected to the computerized electrocorticographic (ECoG) recording system by an isolated flexible cable and ECoG activity was continuously monitored on an eight-channel recorder (PowerLab, 8/SP, AD Instruments, Castle Hill, NSW, Australia). The frequency and amplitude of the ECoG activity were measured off-line.

Drugs

Olive oil, penicillin G potassium (I.E., Ulagay, Turkey) and bisphenol A (99% purity; Sigma Chemical Co., St. Louis, MO, USA) were used in experiments. All drugs were injected at an infusion rate of 0.5 µl/min using a Hamilton microsyringe (type 701N), and the needle stayed in place for a minute to prevent from backflow of the drug. A hole was drilled for intracortical (i.c.) penicillin G injection. Penicillin G was dissolved in normal saline and was injected through Hamilton microsyringe (type 701N) in a volume of 2.5 µl into the cerebral cortex using the stereotaxic device with reference to bregma (coordinates AP: −2.0 mm, LL: 2.0 mm, DV: −1 mm). Epileptiform activity was started after

approximately 2 minutes. After 30 minutes of recording, the doses of BPA (125, 250, 500) or olive oil (solvent of BPA) were administered intraperitoneally and recording continued for 180 minutes.

Statistical analysis

Statistical comparisons were performed using the GraphPad Instat (v3.06) software (GraphPad Software, San Diego, CA, USA). After verifying that the data from electrophysiological recordings were normally distributed, one-way analysis of variance (ANOVA) and Tukey–Kramer post hoc tests were performed for multiple comparisons. For all statistical tests, $p < 0.05$ was considered statistically significant.

RESULTS

A single intracortical injection of penicillin (500 units) induced epileptiform activity approximately within 2 minutes after injection; the activity reached a constant level by 30 minutes after penicillin administration. Signs of epileptiform activity persisted for approximately 3 hours. The means of the spike frequency and amplitude of the epileptiform activity in the 80 minutes were 42.7 ± 3.4 spikes/min and 805 ± 35 µV, respectively (Fig. 1A).

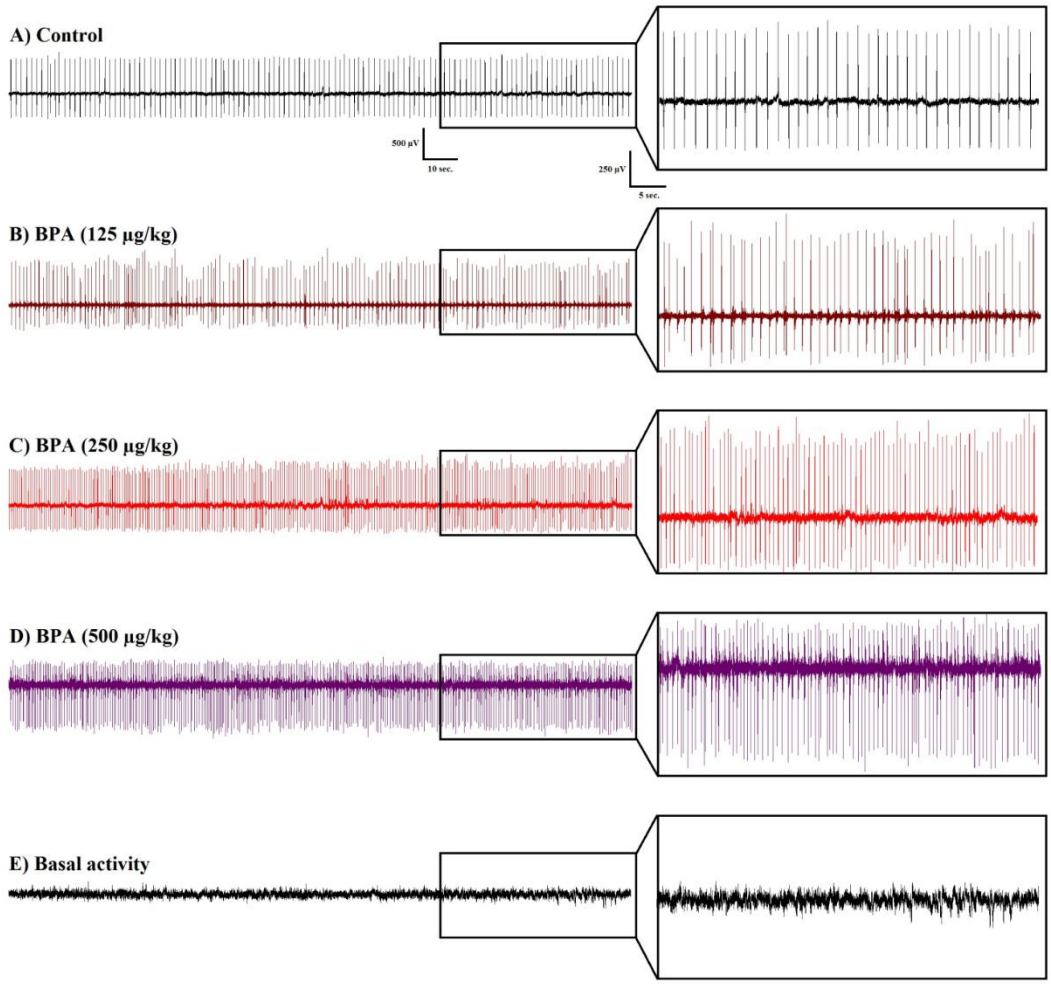

Figure 1: (A) The intracortical injection of penicillin (500 IU) induced epileptiform activity on ECoG. (B) The intraperitoneal administration of 125 µg/kg BPA did not influence either the mean frequency or amplitude of penicillin-induced epileptiform activity. (C) BPA, at a dose of 250 µg/kg (i.p.) significantly increased the mean of frequency of epileptiform activity in the 50 min after injection without changing the amplitude. (D) The administration of BPA, at the dose of 500 µg/kg, significantly increased the mean of frequency of epileptiform activity in the 40 min after injection without changing the amplitude. (E) Baseline ECoG activity before penicillin or the injection of other substances without penicillin. Representative ECoGs were taken 80 min after BPA or olive oil administration.

Three doses of BPA were used in this study. Administration of 125 µg/kg BPA (i.p.) did not significantly change either the means of frequency or amplitude when compared to the control group. The means of the spike frequency and amplitude of the epileptiform activity were 50.3 ± 7.4 spikes/min and 942 ± 71 µV, respectively (Fig. 1B). BPA, at the doses of 250 and 500 µg/kg, increased the mean frequency of the epileptiform activity in the 50 and 40 minutes without changing the amplitude ($p<0.01$ and $p<0.01$, respectively) (Fig. 2A-B). The means of spike frequency and amplitude in the 80 minutes were 67.3 ± 5.7 and 72.7 ± 7.5 spikes/min and 982 ± 21 µV and 1005 ± 76 µV at the doses of 250 and 500 µg/kg of BPA, respectively (Fig. 1C and 1D). No spike activity was observed before penicillin or after the injection of BPA alone (Fig. 1E).

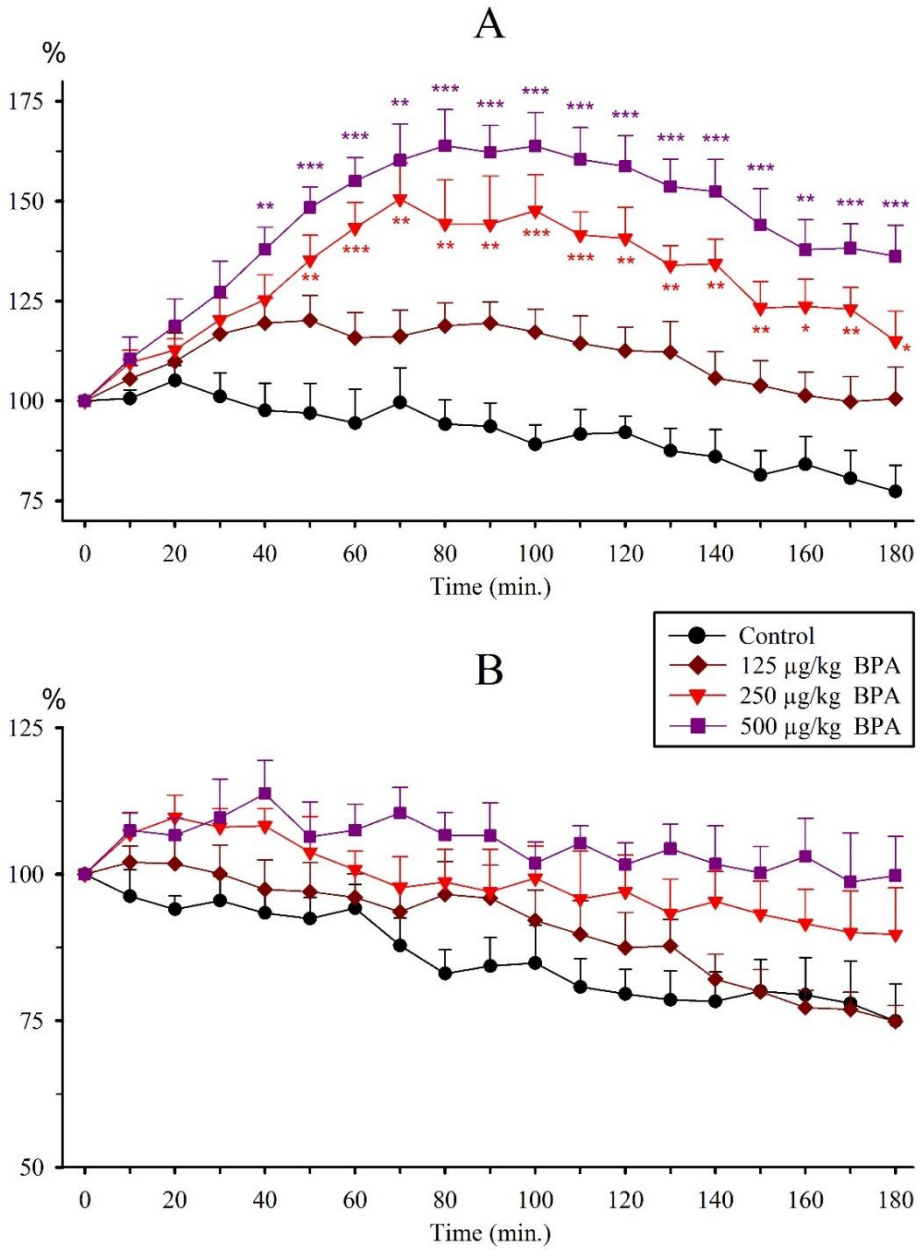

Figure 2: (A) The effects of intraperitoneal administration of BPA on the mean spike frequency of penicillin-induced epileptiform activity. (B) The effects of intraperitoneal administration of BPA on the mean spike amplitude of penicillin-induced epileptiform activity. (*$p<0.05$; **$p<0.01$; ***$p<0.001$). The percentage frequency of epileptiform ECoG activity value depends on both the frequency of epileptiform ECoG activity before and after the substance administered as it is defined as:

$$\text{Frequency Value \%} = \frac{\text{The mean of spike frequency after substance administered}}{\text{The mean of spike frequency before substance administered}} \times 100$$

DISCUSSION

The current study investigated the acute effects of BPA exposure in penicillin-induced epileptic seizure model. It has been shown for the first time that intraperitoneal administration of BPA, at the doses of 250 and 500 μg/kg, show proconvulsant activity without changing the spike amplitude.

Owing to an increase in the use of epoxy resins and polycarbonate plastics, exposure of BPA in several ways, especially foodstuffs have increased [17]. It has been suggested that BPA is a lipophilic substance that could easily accumulate in the brain and plays a role in neuronal functions [18]. The effects of BPA in the central nervous system have not fully elucidated yet. BPA is defined as estrogenic endocrine disrupter that can able to bind to estrogen receptors [19]. Published data suggested that BPA can link to both classical nuclear and non-classical estrogen receptors and influence on normal endogenous estrogenic actions [20, 21]. On the other hand, animal studies have noted that estrogens have exclusively proconvulsant properties. Topical application of estrogen has even been used as a model of focal epilepsy [22]. In addition, Ahmad and Vohora [23] reported that estriol, the third estrogen, has powerful proconvulsant effects in the mouse PTZ-kindling model. In this study, the proconvulsant effect of BPA may have been mediated by estrogen receptors.

On the other hand, some receptors or neurotransmitters are affected in the central nervous system by the exposure of BPA. Choi et al. [18] suggested that BPA affected synaptic $GABA_A$ receptor by decreasing the amplitude of GABAergic inhibitory postsynaptic currents in a concentration-dependent manner. The possible inhibition of GABAergic activity with the administration BPA disrupt the balance between excitatory and inhibitory systems [1]. For this reason, existing seizures may already increase in the central nervous system. Besides, BPA provoked BPA exposure causes a decrease in hippocampal and cerebral cortical [3H] glutamate uptake and in the levels of NMDA receptor subunits, R2A and R2B, in a sex-dependent manner [24]. Disruption of glutamate intake may lead to accumulation of glutamate in extracellular fluid, and in this way, seizures can be increased because of the actions of binding the glutamate to its own receptors.

CONCLUSION

To our knowledge, there have been no studies published showing the effects of BPA in epilepsy. In the present study, intraperitoneal administration of BPA showed proconvulsant activity in the penicillin-induced epileptiform activity. Due to this effect, it is recommended that epileptic patients should avoid exposure to BPA. Further studies are needed to clarify the pathways in which BPA increases epileptic seizures and how patients can prevent the effects of BPA.

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