Araştırma Makalesi (Research Article)

Hidrojen Sülfür, Karbon Monoksit ve Nitrik Oksidin Sıçanlarda Pentilentetrazol İndüklü Nöbetler Üzerindeki Etkileri

The Effects of Hydrogen Sulfide, Carbon Monoxide and Nitric Oxide on Pentylenetetrazole-Induced Seizures in Rats

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Özet

Amaç: Sıçanlarda pentilentetrazol (PTZ) ile tetiklenen konvülsiyonlarda biyoaktif gaz mediyatörler hidrojen sülfür (H_2S), karbon monoksit (CO) ve nitrik oksit (NO)'in antikonvülzan etkilerinin araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Seksen erkek Wistar-Albino sıçan rastgele on gruba ayrılmıştır. Konvülsiyonlar, intraperitoneal olarak verilen 60 mg/kg PTZ ile tetiklendi. PTZ'den 30 dakika önce diazepam (1 ve 2 mg/kg), sodyum hidrosülfit (NaHS, 5 ve 10 mg/kg), CO salan molekül-2 (CORM-2, 5 ve 10 mg/kg), L-arginin (LARG, 30 ve 100 mg / kg) ve L-N^G-Nitro arjinin metil ester (L-NAME, 30 mg/kg) uygulandı. Nöbet latensi, nöbet süresi ve nöbet skoru kör iki gözlemci tarafından değerlendirildi. Veriler Kruskal Wallis testi ve ardından Tukey ile analiz edildi. P değeri <0.05 olması istatistiksel olarak anlamlı kabul edildi.

Bulgular: NaHS (10 mg/kg) nöbet süresini ve skorunu diazepam (2 mg/kg) ile benzer şekilde PTZ'ye göre anlamlı ölçüde azaltırken nöbet latensinde anlamlı bir değişikliğe neden olmadı. Diğer ajanlar, nöbet latensi, nöbet süresi ve nöbet skorunu PTZ'ye göre anlamlı şekilde değiştirmedi.

Sonuç: NaHS (10 mg/kg) PTZ'ye bağlı nöbetlerde koruyucu olabilir. Bu etkinin altında yatan mekanizmaların araştırılması için daha fazla çalışmaya ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Epilepsi, Hidrojen Sülfür, Karbon monoksit, Nitrik oksit, Nöbetler

Abstract

Objective: The present study aimed to investigate the anticonvulsant effects of bioactive gas mediator's hydrogen sulfide (H2S), carbon monoxide (CO) and nitric oxide (NO) in pentylenetetrazole (PTZ)-induced convulsions in rats.

Material and Methods: Eighty male Wistar-Albino rats randomly divided into ten groups. Convulsions were induced by administering 60 mg/kg PTZ intraperitonally (i.p). Diazepam (1 and 2 mg/kg), sodium hydrosulfide (NaHS, 5 and 10 mg/kg), CO releasing molecule-2 (CORM-2, 5 and 10 mg/kg), L-arginine (LARG, 30 and 100 mg/kg) and L-NG-Nitro arginine methyl ester (L-NAME, 30 mg/kg) were administered 30 minutes after PTZ. Seizure latency, seizure duration and seizure score were evaluated. Data were analyzed by the Kruskal Wallis test followed by posthoc analyses with Tukey. Any p value <0.05 was considered statistically significant.

Results: NaHS (10 mg/kg) significantly reduced seizure duration and seizure in comparison with PTZ similar to diazepam (2 mg/kg), but it did not cause any significant change in seizure latency. Other agents did not significantly change seizure latency, seizure duration, and seizure score compared to PTZ.

Conclusion: NaHS may be protective in PTZ-induced seizures. More research is needed to investigate the mechanisms underlying this effect.

Keywords: Carbon monoxide, Epilepsy, Hydrogen Sulfide, Nitric Oxide, Seizures

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INTRODUCTION

Epilepsy is a chronic disease characterized by recurrent seizures over a long period of time and loss of consciousness that often occurs during these seizures (1). It is one of the most common neurological disorders affecting 70 million people worldwide (2). Epilepsy causes clinical symptoms such as memory impairment, distraction and other cognitive disorders in 30-40% of patients (3). The primary aims of epilepsy treatment are to prevent seizures, to avoid adverse effects, and to facilitate patients to have active lives (4). Despite the variety of these drugs available for the treatment of epilepsy, pharmacological approaches cannot control seizures in all cases (5). However, many antiepileptic drugs used in the treatment may have interactions with some important drugs or drug groups such as antibiotics, anticoagulants, oral contraceptives, salicylates, cimetidine and isoniazid (6). Therefore, all these conditions make it necessary to develop more rational methods in the treatment of epilepsy.

Animal models of seizures and epilepsy are crucial to increase our understanding of the underlying mechanisms of epileptogenesis and the clinical effects of novel antiepileptic drugs (7). Pentylentetrazole (PTZ), bicyclic tetrazole derivative, is characterized by high bioavailability and rapid distribution after intraperitoneal (i.p.) injection and blocks γ-aminobutyric acid (GABA)-mediated transmission (8). PTZ has been used comprehensively to induce seizures at higher doses in animal models (9).

Hydrogen sulfide (H_2S), carbon monoxide (CO) and nitric oxide (NO) are characterized as bioactive gas mediators that have been investigated during the past 20 years for their roles in human physiology (10). These gasotransmitters have different protective effects against oxidative stress which are related to their neuropsychiatric functions (11, 12). It was shown that the endogenous CO system is protective against brain damage induced by recurrent febrile seizures (13). In addition it was indicated that the pathways for hydrogen sulfide biosynthesis may be targets for antiepileptic strategies in poststroke epilepsy treatment (14). There are some controversial results about NO effects on convulsions (15-17). Thus, the aim of this study was to investigate the possible anti-convulsant effects of H_2S, CO and NO in PTZ-induced epilepsy model.

MATERIALS AND METHODS

Eighty adult male Wistar rats (8-10 weeks old, weighing 180-220 g) were used for the current experiments. They were housed in cages in a controlled room with 12/12 light dark cycle, temperature of 20-22°C and relative humidity of 65%-70%. The study was performed in accordance with the guidelines for the care and use of laboratory animals approved by the Local Ethical Committee of Eskisehir Osmangazi University (25.06.2019/745). PTZ, diazepam, NaHS, CORM-2, LARG and L-NAME were dissolved in saline and PTZ injected 30 min after the last treatment with all agents according to de Oliveira et al. (18). A known anxiolytic drug, diazepam, was used as a positive control. Rats were randomly assigned into ten groups (n=8): PTZ group (60 mg/kg, i.p.), diazepam (1 mg/kg, i.p.)+PTZ group, diazepam (2 mg/kg, i.p.)+PTZ group, NaHS (5 mg/kg, i.p.)+PTZ group, NaHS (10 mg/kg, i.p.)+PTZ group, CORM (5 mg/kg, i.p.)+PTZ group, CORM (10 mg/kg, i.p.) and PTZ group, LARG (30 mg/kg, i.p.) and PTZ group, LARG (100 mg/kg, i.p.)+PTZ group, L-NAME (30 mg/kg, i.p.)+PTZ group. After PTZ injection, all rats were placed in a glass observation box and PTZ-induced seizure score, seizure latency, seizure duration were recorded for 30 min through direct observation by two blind investigators unaware of the groups. Behavioral seizures were rated according to the following Racine's scale (19) as described previously with the following stages: *(0) no abnormality; (1) Mouth and facial movements; (2) Head nodding; (3) Forelimb clonus; (4) Rearing; (5) Rearing and falling.*

All data were expressed as mean±SEM. Statistical analysis was performed using IBM SPSS software version 21.0. The Shapiro-Wilk normality test was performed to verify the distribution of values in the Gaussian curve. Normality was not verified for the variables and thus, the nonparametric Kruskal-Wallis test followed by posthoc analyses with Tukey test used. $P < 0.05$ was considered statistically significant.

RESULTS

It was shown that all treatment agents did not increase seizure latency significantly **(Figure 1)**.

It was found that NaHS (10 mg/kg) considerably lowered the seizure duration considerably. On the other hand, the other substances were ineffective in terms of lowering seizure duration **(Figure 2)**.

The seizure score was also found significantly lower in NaHS (10 mg/kg) than in PTZ groups. NaHS (5 mg/kg), CORM-2 (5 and 10 mg/kg), LARG (30 and 100 mg/kg) and L-NAME (30 mg/kg) had no effect **(Figure 3)**.

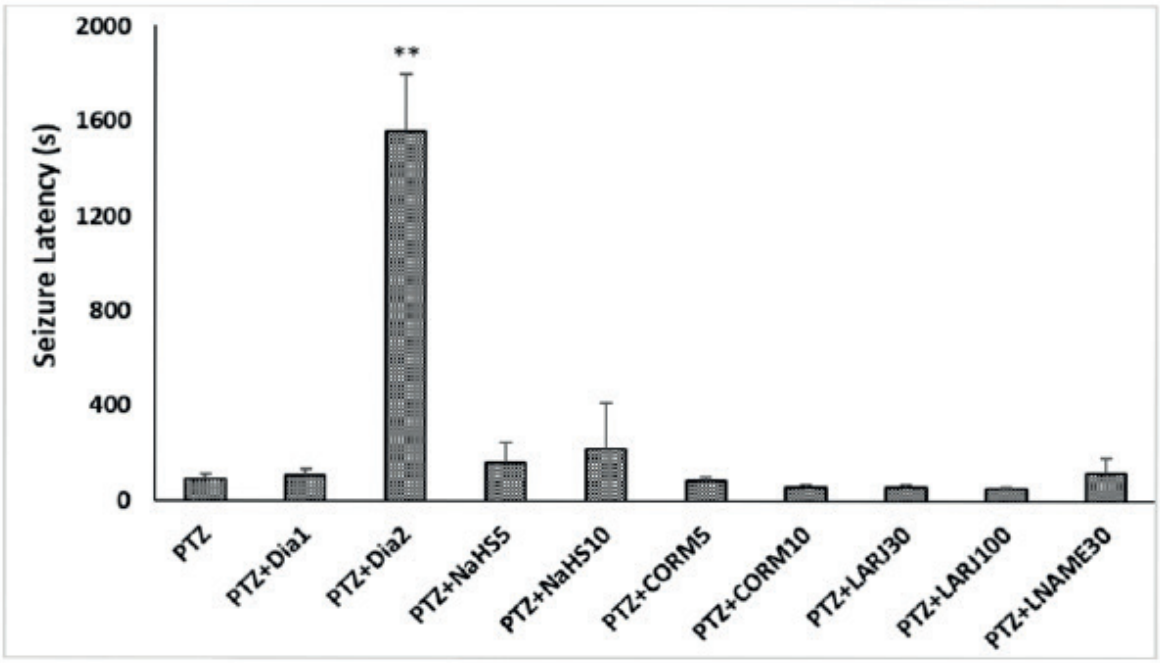

Figure 1. Comparison of the study groups regarding seizure latency. The effects of diazepam and other groups on the seizure latency were compared. PTZ: Pentylenetetrazole, Dia1: Diazepam 1 mg/kg, Dia2:Diazepam 2 mg/kg, NaHS5: Hydrogen sulfide donor 5 mg/kg, NaHS10: Hydrogen sulfide donor 10 mg/kg, CORM5: CO donor 5 mg/kg, CORM10: CO donor 10 mg/kg, LARG30: L-arginine 30 mg/kg, LARG100: L-arginine 100 mg/kg, LNAME30; L-nitroarginine methy ester 30 mg/kg. (**:p <0.01 vs. PTZ)

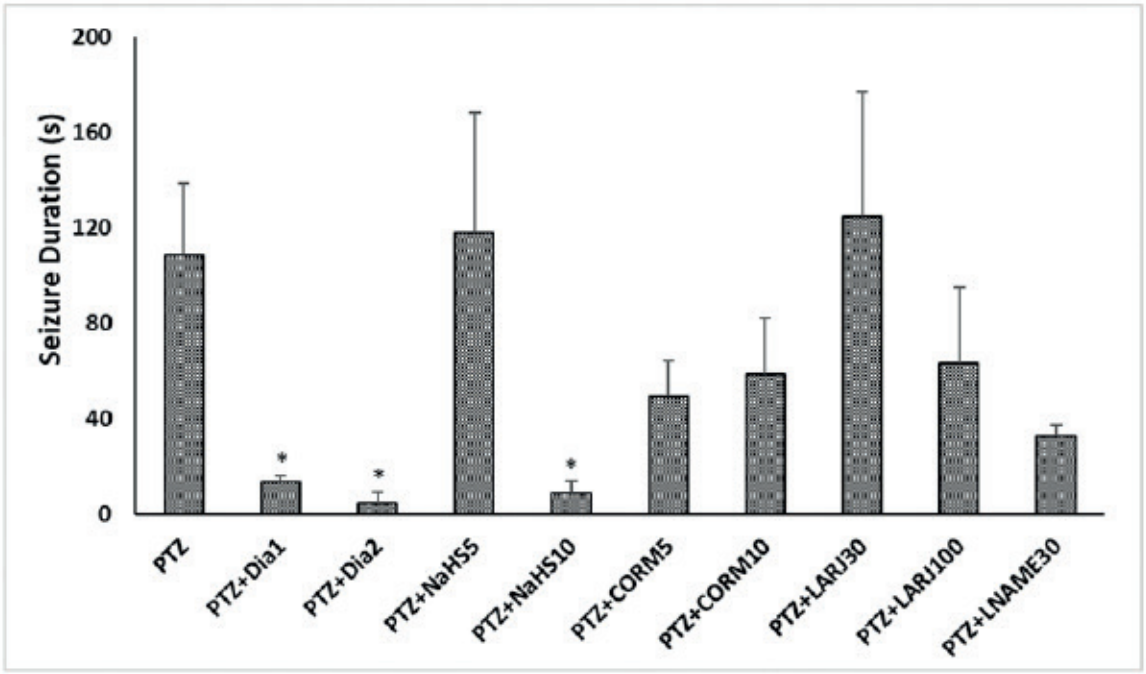

Figure 2. Comparison of the study groups regarding seizure duration. The effects of diazepam and other groups on seizure duration were compared. PTZ: Pentylenetetrazole, Dia1: Diazepam 1 mg/kg, Dia2:Diazepam 2 mg/kg, NaHS5: Hydrogen sulfide donor 5 mg/kg, NaHS10: Hydrogen sulfide donor 10 mg/kg, CORM5: CO donor 5 mg/kg, CORM10: CO donor 10 mg/kg, LARG30: L-arginine 30 mg/kg, LARG100: L-arginine 100 mg/kg, LNAME30; L-nitroarginine methy ester 30 mg/kg. (*:p<0.05 vs. PTZ)

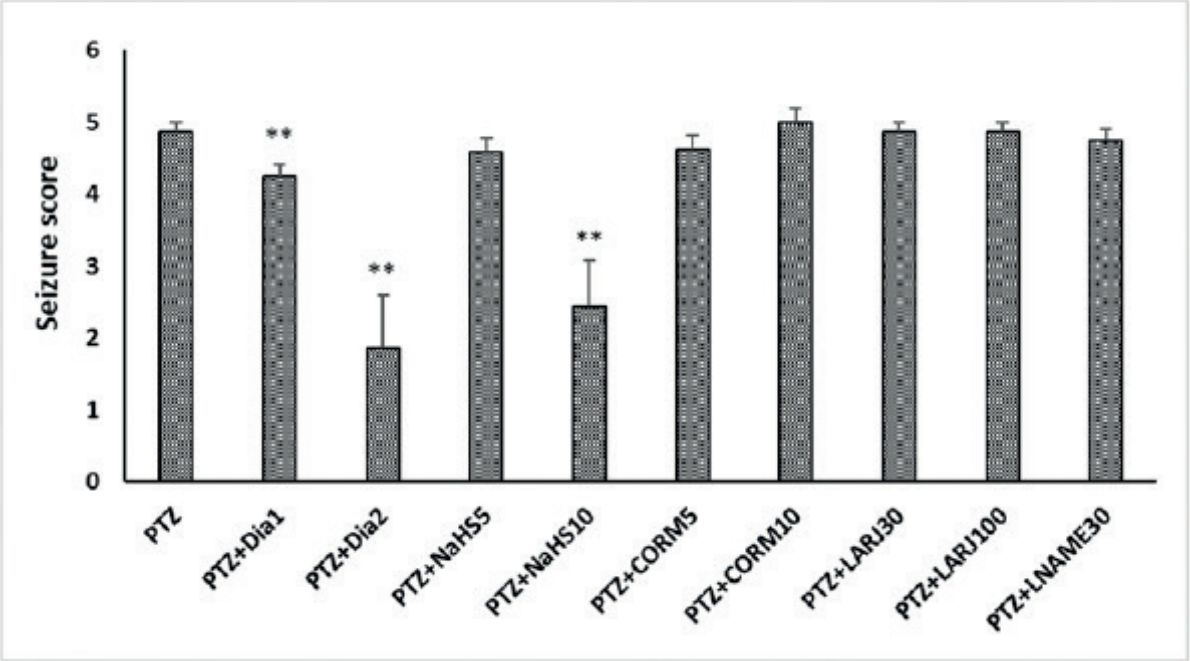

Figure 3. Comparison of the study groups regarding seizure score. The effects of diazepam and other groups on seizure score were compared. PTZ: Pentylenetetrazole, Dia1: Diazepam 1 mg/kg, Dia2:Diazepam 2 mg/kg, NaHS5: Hydrogen sulfide donor 5 mg/kg, NaHS10: Hydrogen sulfide donor 10 mg/kg, CORM5: CO donor 5 mg/kg, CORM10: CO donor 10 mg/kg, LARG30: L-arginine 30 mg/kg, LARG100: L-arginine 100 mg/kg, LNAME30; L-nitroarginine methy ester 30 mg/kg. (**:p <0.01 vs. PTZ)

DISCUSSION

In the present study, it was shown that diazepam and H_2S donor NaHS significantly increased seizure latency compared to PTZ alone group whereas the remaining agents showed no effect. In addition, NaHS, similar to diazepam, lowered seizure duration of the rats. On the other hand, the other substances did not yield a significant effect when compared with that of PTZ alone group. Finally, all the agents but diazepam and NAHS failed to affect seizure score. Diazepam is a benzodiazepine class agent known for its anxiolytic, muscle relaxant and anticonvulsant effects, and is frequently used in emergency departments, especially in convulsions (20). In this study it was used as a positive control and was shown to be protective against PTZ seizures at a dose of 2 mg/kg. H_2S, CO and NO are gas molecules that are naturally produced and metabolized in the body acting as mediators in physiological situations in the emergence of diseases (21). These mediators provide protection against reactive oxygen and nitrogen species which are among the main causes of neuroinflammation. Therefore, it has been suggested that they play a protective role in various neurodegenerative diseases (22). Although it has been suggested that gas neurotransmitters may also have effects on epilepsy studies regarding its beneficial effects are limited (23).

H_2S is a gas transmitter that has demonstrated vital biological effects in various physiological events including insulin release, blood pressure, cytoprotection, muscle relaxation and in the pathophysiology of various diseases such as inflammation, hypertension, atherosclerosis, diabetes, myocardial infarction, Alzheimer's and Parkinson's diseases (24,25). It has been shown that H_2S can provide inhibitory/excitatory balance in neurotransmission and regulate the activities of targets in seizures such as ion channels and NMDA receptors. However, information based on the mechanism of action of H_2S in seizures is limited (26, 27). In our study, it was observed that NaHS reduced seizure latency at both doses (5 and 10 mg/kg). However, it was found that NaHS (10 mg/kg), significantly reduced the seizure duration. In addition, the average of seizure score was remarkably lower in NaHS (10 mg/kg) group than in PTZ group According to this result, NaHS (10 mg/kg) was able to protect against PTZ-induced seizures.

Although CO is well known regarding its toxicity, it has beneficial biological effects in controlled doses and plays a role as a molecular messenger in various physiological processes of the nervous system (28). Various studies indicated that carbon monoxide has anti-inflammatory (29), antiproliferative (30), antiatherogenic (31), antihyperalgesic (32) and antiapoptotic effects (33). CORM-2 molecules are capable of carrying CO to tissues and cells (34). Studies have shown that heme-oxygenase enzyme, which catalyzes biosynthesis of carbon monoxide in the central nervous system, plays a protective role in various pathologies such as cerebrovascular diseases, pain and Alzheimer's and Parkinson's diseases (35). CORM-A1 molecule, another carbon monoxide-releasing agent, has been found to be protective in neonatal vascular

damage due to epileptic seizure (36). According to our literature review, there has been no report about the effects of CORM-2 molecules and CO molecule on seizures. It is thought that it may have effects on seizures due to its protective properties in the nervous system. However, in our study, it was seen that CORM-2 did not change the seizure latency at a dose of 10 mg/kg and increased the duration of the seizure similar to the PTZ group. It was observed that the averages of seizure score of CORM-2 at both 5 and 10 mg/kg dose were close to each other. Therefore, we did not observe any effect of CORM-2 in PTZ-induced seizures.

It has been suggested that NO may play a role in epilepsy and epileptogenesis due to the second messenger, neuromodulator and neurotransmitter roles in the central nervous system and NO has anti- or pro-convulsive properties, depending on the seizure model, the dosage of pharmacological agents in the experiments and the route of drug administration (37). L-NAME has been shown to be proconvulsant in PTZ-induced seizures at doses of 10 and 40 mg/kg (38). LARG and L-NAME did not change the seizure latency relative to the PTZ group in this study. Although it has been seen that L-NAME (30 mg/kg) decreased the seizure duration compared to PTZ group, but the difference was not statistically significant. The mean seizure score was also found to be high in both LARG and L-NAME groups.

In conclusion, our study showed that NaHS may have protective effects on PTZ-induced convulsions. However, these data are not yet sufficient to prove the antiepileptic effects of NaHS as a drug. More studies are needed that focus on the anticonvulsant effects of this agent in other animal species, as well as to obtain more information about its anticonvulsant effects

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