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Aggravating Effects of Spermine and Spermidine but not Agmatine on Pentylenetetrazole (PTZ)-Induced Seizures in Rats

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Abstract

Objective: Epilepsy, a chronic and complex disorder of the brain, is an important neurological problem worldwide. Novel drug objectives in the central nervous system (CNS) may present more effective choices in treatment of epilepsy. The present study is designed to examine the effects of certain polyamines on seizures induced by pentylenetetrazole (PTZ) in rats.

Materials and Methods: Female adult (250-300 g) Wistar albino rats were used in our study. They were randomly allocated into several groups (n=8 for each group). Spermine (1-4 mg/kg), spermidine (20-80 mg/kg), agmatine (160 mg/kg) or saline were injected to animals through intraperitoneal (IP) route 30 minutes prior to PTZ (40 mg/kg, IP) treatment. The onset time and severity of the seizures were assessed immediately after the treatment with PTZ.

Results: Spermidine treatments significantly shortened onset time of seizures, at all doses used in the study (p_s=0.0001). It significantly increased the severity of seizures at doses of 20 and 80 mg/kg (p=0.007 and p=0.03, respectively). Treatment with spermine significantly shortened onset time of seizures at dose of 4 mg/kg (p=0.002). While spermine (4 mg/kg) increased severity of seizures significantly (p=0.01; Dunnet's test), it did not cause any noteworthy alteration on the severity of seizures at other doses. Agmatine (100 mg/kg) did not have any statistically significant effect on seizures.

Conclusion: Our results suggest that spermine and spermidine but not agmatine cause some aggravating effects on the seizures induced by PTZ. The data indicate that polyamines in the CNS may be an important target for epilepsy.

Keywords: Seizure, spermine, spermidine, agmatine, pentylenetetrazole, rat(s)

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INTRODUCTION

Epilepsy, a chronic and complex disorder of the brain, is an important neurological problem worldwide. It is characterized by unpredictable seizures caused by abnormal (i.e., excessive) electrical discharges within the nerve cells in the brain, and is one of the most common disorders, influencing around 1% of the population (1, 2). There is no absolute drug-based treatment for epilepsy. Currently available drug treatments aim to prevent the occurrence of seizures with sustained and sometimes lifelong drug use. They do so by increasing the activity of inhibitory neurochemicals such as gamma-aminobutyric acid (GABA) or suppressing the activity of excitatory neurochemicals such as glutamate thereby compensating for abnormal activity in voltage-regulated ion channels (i.e. Na^+, K^+, and Ca^{2+}), which can lead to abnormal discharges in the neuronal membrane (3-5). Many studies are carried out to develop new drugs which are more effective and better tolerated (6). Further studies on the mechanisms involved in the disorder are required for a better understanding of epilepsy, and the development of targeted therapies. New drug targets in the central nervous system (CNS) may lead more effective choices for epilepsy treatment.

Polyamines are aliphatic molecules containing two or more amine groups such as putrescine, spermidine, spermine and agmatine. Along with their biosynthetic enzymes, polyamines are found in all parts of the body, including the CNS. They are involved in several cellular functions such as DNA stabilization, regulation of gene expression, ion channel function and cell proliferation (7). Recently, a polyamine, agmatine –the decarboxylated derivative of L-arginine–, has been noted as a prominent neuromodulator in mammalian brain (8, 9). Agmatine binds to α2-adrenergic and imidazoline receptors (8, 10). It inhibits the enzyme nitric oxide synthase (NOS) that contributes to nitric oxide (NO) formation and is found to antagonize glutamatergic N-methyl-D-aspartate (NMDA) receptors in rat hippocampus (11, 12). It has been shown that NOS inhibitory agents (13) and NMDA antagonists (14) prevent seizures in experimental animal models.

Conflicting results have been reported regarding the effects of polyamines on epileptic seizures. Some experimental studies indicated anticonvulsant effects of polyamines such as agmatine (15, 16) and spermine (17). However, other studies have also reported certain unfavorable effects of polyamines on epileptic seizures. For example, it has been demonstrated that spermidine induces proepileptic effects by shortening seizure latency in rats, due to increase NO production (18). Hayashi et al. also suggested that increases in the concentrations of some polyamines such as putrescine, spermidine and spermine are involved in neuronal excitability in brain during seizures (19). A study by Luszczki et al. showed that agmatine significantly reduced the anticonvulsant effects of vigabatrin against clonic seizures induced by pentylenetetrazole at a level higher than its anticonvulsant doses (20). On the other hand, stress-induced increases in polyamine levels and/or metabolism are referred to as the polyamine stress response (PSR) (21). In this context, increased polyamine levels and PSR are related to DNA fragmentation and programmed cell death (22). PSR has also been associated with some mental disorders such as suicidal behavior and schizophrenia (23). However, studies on the effects of polyamines or PSR and epileptic seizures are limited and inconclusive in terms of their findings.

Given this background, the main objective of the present study is to investigate the effects of three polyamines –spermine, spermidine and agmatine–, on the onset time and severity of seizures induced by pentylenetetrazole (PTZ), which serves as a readily available and valid experimental model in rodents (24). To this end, we recorded the onset time and severity of the seizures in rats injected with spermidine, spermine, or agmatine, 30 minutes before the administration of a subeffective dose (40 mg/kg) of PTZ in rats.

MATERIALS AND METHODS

Animals and Laboratory

Female adult (250-300 g) Wistar albino rats (Üsküdar University Experimental Research Unit – USKUDAB, Türkiye) were subjects in our study. Four animals were housed per Plexiglass cage. The rats were placed in a quiet and temperature- and humidity-controlled room (22 ± 2°C and 60 ± 5%, respectively) in which a 12/12 h light–dark cycle was maintained (light from 07:00 to 19:00). Food and water were available ad *libitum*. All processes in this study were accomplished in agreement with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (Publication No. 85-23, revised 1985). The Local Ethics Committee for Animal Experiments (HADYEK) of Üsküdar University approved the study on January 22, 2021, with the decision number 2020-14.

Drugs

PTZ, agmatine, spermine, and spermidine were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Drugs were dissolved in 0.9% saline and injected intraperitoneally (IP) to the rats in the same volume of 0.5 ml/250 g. Solutions for injection were prepared freshly before the tests.

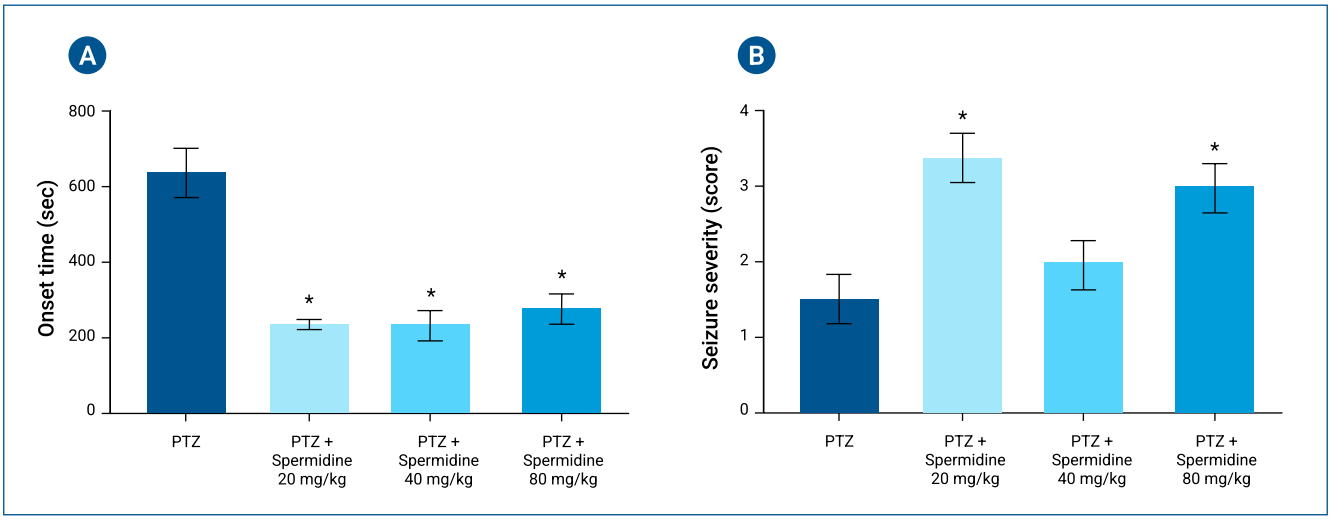

FIGURE 1. Effects of spermidine on onset time (A) and severity (B) of seizures induced by PTZ. (*p<0.05 significantly different from control; n=8 for each group).

Procedure

As the present study was based on female rats, vaginal smear screening was performed on the days on which the experiments were to be carried out, and rats in the metestrus stage were taken for experiments. Rats were then randomly assigned to specific groups (n=8 for each group).

Spermine (2, 4 and 8 mg/kg), spermidine (20, 40 and 80 mg/kg), agmatine (160 mg/kg) or saline were injected to animals by IP route 30 minutes prior to PTZ (40 mg/kg, IP) treatment. Immediately after PTZ injections, rats were placed in a plexiglas cage and recorded for onset times of "first myoclonic jerk (FMJ)", "generalized clonic seizures (GCS)" and "tonic generalized extensions (TGE)" as previously described (13). The onset times were recorded as seconds. The severity of seizures was assessed using a modified eight-point semi-quantitative scale defined previously (25). The scale can be summarized as follows: 0: no convulsion; 1: ear and facial twitching; 2: convulsive waves through the body; 3: myoclonic jerks, rearing; 4: generalized clonic seizures, turn over into side position; 5: turn over into back position, generalized clonic-tonic seizures. The observation period for seizures was restricted to 60 min. Duration of 600 sec was established as the cut-off time for computing the onset time of seizures induced by PTZ. All experimental procedures were conducted during the light period (10:30 - 12:30 am).

In our study, we used agmatine only at a high dose of 160 mg/kg. This is because agmatine has neuroprotective and anticonvulsant effects in rodents at doses ranging from 1 to 100 mg/kg. Agmatine has the potential to cause PSR at doses such as 160 mg/kg (9). We did not use higher doses because they could be toxic.

Statistical Analysis

The data were presented as the means ± standard error of the mean (SEM) and were evaluated using the Statistical Package (SPSS Version 20.0) software. The effects of spermine and spermidine on the onset time of seizures were evaluated by one-way analysis of variance (ANOVA) test followed by Dunnett test for *post-hoc* comparisons. In addition, the effects of spermine and spermidine on seizure severity were also evaluated by the Kruskal-Wallis (KW) test followed by Dunnett's T3 test for *post-hoc* analyses. Student's t and Mann-Whitney U tests were applied to evaluate the effects of agmatine on onset time and severity of seizures, respectively. The statistical significance was accepted at the level of p<0.05.

RESULTS

The effects of spermidine on onset time and severity of PTZ-induced seizures in rats

The effects of spermidine on onset time and severity of the seizures have been shown in Figure 1 A and B, respectively. One-way ANOVA and KW tests revealed some significant changes in onset time and severity of seizures induced by PTZ when the rats were subjected to spermidine treatments [F(3,28)=19.409, p=0.000 and KW=13.756, p=0.003, respectively].

Administration of spermidine shortened significantly onset time of the seizures at all doses applied in our study (p_s<0.0001; Dunnett's T3 test, Figure 1A).

Spermidine treatments also increased significantly the severity of seizures at doses of 20 and 80 mg/kg (p=0.007 and p=0.03, respectively; Dunnett's T3 test). The increase

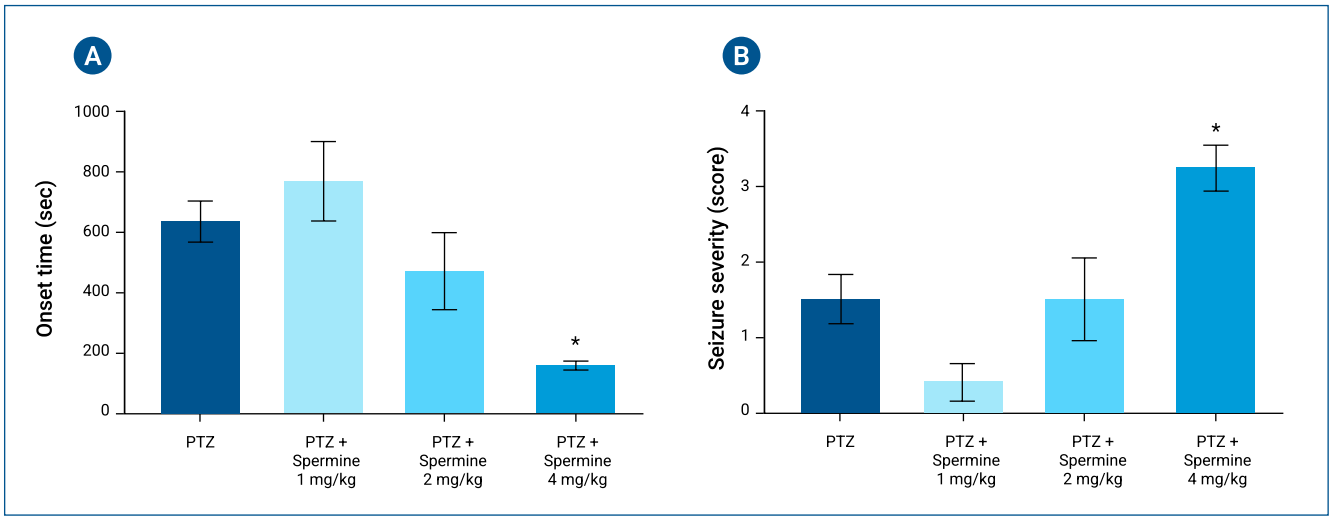

FIGURE 2. Effects of spermine on onset time (A) and severity (B) of seizures induced by PTZ. (*p<0.05 significantly different from control; n=8 for each group).

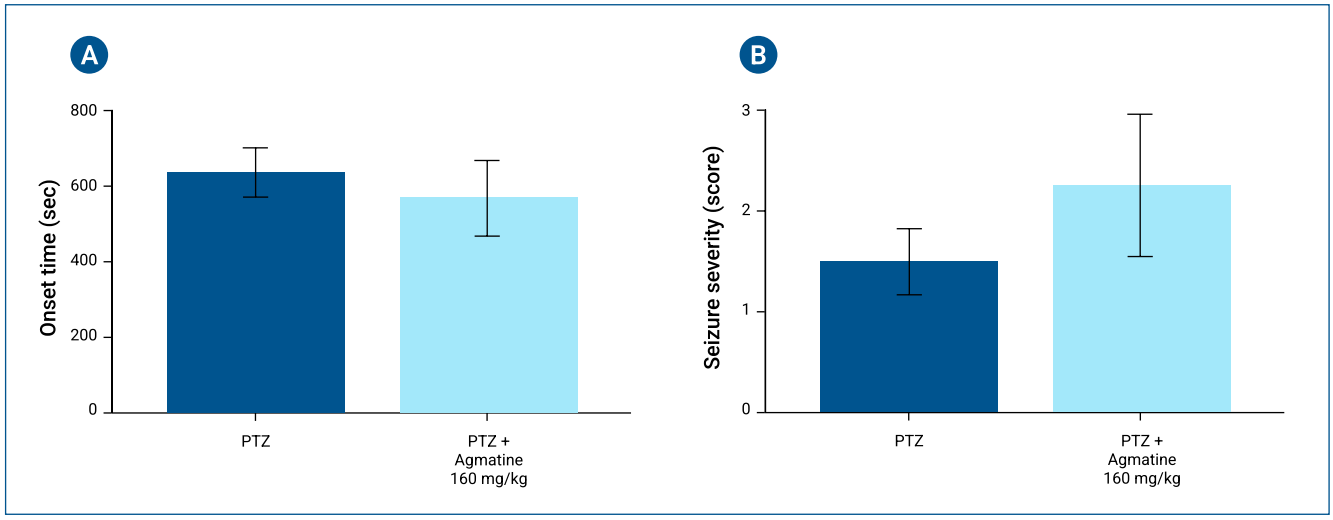

FIGURE 3. Effects of agmatine on onset time (A) and severity (B) of seizures induced by PTZ. (*p<0.05 significantly different from control; n=8 for each group).

observed in the severity of seizures at dose of 40 mg/kg was not statistically significant (p=0.851; Dunnett's T3 test; Figure 1B).

The effects of spermine on onset time and severity of PTZ-induced seizures in rats

The effects of spermine on onset time and severity of the seizures have been shown in Figure 2A and B, respectively. One-way ANOVA and KW tests revealed some remarkable changes in onset time and severity of seizures when the rats were subjected to spermidine treatments [F(3,28)=7.811, p=0.001 and KW=13.278, p=0.004, respectively].

Spermine shortened significantly onset time of the seizures at 4 mg/kg (p=0.002; Dunnett's T3 test). It also shortened onset time of the seizures at doses of 1 and 2 mg/kg but these effects did not reach a statistically significant level (p=0.694 and p=0.427, respectively; Dunnett's T3 test, Figure 2A). In addition, while spermine treatment (4 mg/kg) increased the severity of seizures significantly (p=0.01; Dunnett's T3 test), we did not observe any statistically significant difference on the severity of seizures at doses of 1 and 2 mg/kg (p=0.107 and p=1.0, respectively; Dunnett's T3 test; Figure 2B).

Spermine shortened significantly onset time of the seizures at 4 mg/kg (p=0.002; Dunnett's T3 test). It also shortened onset time of the seizures at doses of 1 and 2 mg/kg but these effects did not reach a statistically significant level (p=0.694 and p=0.427, respectively; Dunnett's T3 test, Figure 2A). In addition, while spermine treatment

(4 mg/kg) increased the severity of seizures significantly (p=0.01; Dunnett's T3 test), we did not observe any statistically significant difference on the severity of seizures at doses of 1 and 2 mg/kg (p=0.107 and p=1.0, respectively; Dunnett's T3 test; Figure 2B).

The effects of agmatine on onset time and severity of PTZ-induced seizures in rats

The effects of agmatine (160 mg/kg) on onset time and severity of the seizures have been shown in Figure 3A and B, respectively. Agmatine produced no significant change in onset time (p=0.593, Student's t test) or severity (p=0.369, Mann-Whitney U test) of the seizures.

DISCUSSION

We investigated the effect of three polyamines –spermine, spermidine, and agmatine– on epileptic seizure threshold and severity in an experimental model. Because we predicted that the PSR would cause a lower seizure threshold and exacerbate the severity of seizures, we used a relatively low dose (40 mg/kg) of PTZ for seizure induction. PTZ is generally used at higher doses (i.e. 60 and 80 mg/kg) to induce severe seizures in rats (26-28). Our results clearly demonstrated that while spermine and spermidine potentiated PTZ-induced seizures, agmatine did not cause any statistically significant difference on onset time and the severity of seizures. Our findings regarding spermine and spermidine are in line with those of previous reports indicating neuronal excitability in the epileptic brain with increased polyamine concentrations (19) and spermidine-induced proepileptic effects in rats (18).

Kumar and Kumar reported that at doses of 5 and 10 mg/kg, spermine helped prevent PTZ-induced seizures in mice (17). Kaasinen et al. also suggested that the over expression of spermidine/spermine N1-acetyltransferase, an enzyme in the catabolic pathway of polyamine metabolism, elevated the threshold for PTZ-induced seizure activity in transgenic mice (29). However, in our study, while lower doses of spermine (1 and 2 mg/kg) were ineffective on the onset time and the severity of seizures, at 4 mg/kg, it shortened the onset time and caused a significant increase in seizure severity. Such a conflict between the results of different studies may be related to the use of different animal species. Mice and rats may have different sensitivity levels to the effects of spermine. Nevertheless, while spermine was found to be ineffective at low doses, the shortened latency and increased severity of epileptic seizures at higher doses may be related to PSR. In addition, aggravating effects of spermine and spermidine on seizures support the hypotheses suggesting a relationship between PSR and neuropsychiatric disorders.

It is not known where in the CNS the effects of spermine and spermidine may be important in aggravating PTZ-induced seizures. At the molecular level, there is some evidence that these polyamines interact with glutamatergic receptors such as NMDA, AMPA and kainite (30-32). In addition, Masuko et al. reported that spermine enhances NMDA receptor activation at depolarized membrane potential and increases NMDA receptor currents in the presence of glutamate and glycine (33). Moreover, it has been shown that spermine induces convulsions by CNS excitation via NMDA receptors in mice (34, 35). The role of ionotropic glutamate receptors, especially in NMDA type receptor activation, in pathogenesis of epileptic seizures is well known (36). Thus, glutamatergic activation via NMDA receptor activation may be responsible for the worsening effects of spermine and spermidine on PTZ-induced seizures. However, this hypothesis needs to be confirmed by further studies.

An interaction with GABA, the major inhibitory neurotransmitter in CNS, could also explain the aggravating effects on seizures. Several reports suggesting a relationship between GABA inhibition and PTZ-induced epileptic seizures have been published (37-39). Interestingly, PTZ also stimulates polyamine catabolism in rat brain (40). Polyamines' effects on epileptic seizures via GABA also merit further studies.

In contrast to prospects, in the present study, 160 mg/kg agmatine (a higher dosage level than its anticonvulsant doses) was found to be ineffective on seizures. Although it reduced the onset time and increased the severity of PTZ-induced seizures, the effects were not statistically significant, nonetheless. Several studies have previously shown that intraperitoneal administration of agmatine has anticonvulsant activity in rodents at doses ranging from 5 mg/kg to 100 mg/kg (15, 41, 42). Thus, we used a much higher dose of agmatine (160 mg/kg) expecting it to produce PSR, which could induce epileptic activity. We also previously observed that this dose of agmatine induced a schizophrenia-like model in rats by disrupting prepulse inhibition of the acoustic startle reflex (43). In addition, in a population-based retrospective cohort study, Chang et al. found a strong bidirectional relationship between schizophrenia and epilepsy (44). The sex-associated variations in the effects of agmatine may be related to this ineffectiveness. Agmatine may cause some sex-related effects in mice. For example, it has been shown that agmatine antagonized the caffeine-induced open-field locomotor hyperactivity in male but not in female mice (45). As the present study was carried out with female rats, the effect or lack thereof, may be related to the gender of the animals. We did not prefer higher doses of agmatine since in preliminary studies some toxic effects such as excessive sedation was observed and the use of another administration route such as subcutane-

ous administration, produces toxic effects such as ulcerative necrotic cutaneous lesions in rats (46).

The interaction between polyamines, PSR and neuropsychiatric disorders has been increasingly debated in scientific community (9, 23, 27). Although some remarkable results were obtained in the present study, detailed laboratory or clinical studies are required to confirm the findings, given the presence of conflicting results in the literature. The effects of polyamines merit in-depth investigation as some of them such as agmatine are used as food supplements.

In conclusion, our results suggest that significant changes in epileptic seizures could be produced by spermidine and spermine but not agmatine. Agmatine seems to be potentially less dangerous than spermine and spermidine, in terms of seizures. All these observations and scientific data point out that the polyamine pathway in the CNS is a novel, important, and worthwhile area for the pathogenesis, diagnosis, and treatment of epilepsy. New data to be obtained from follow-up studies will contribute to clarifying the role of polyamines in this context, and to offering new and more effective treatment options.

Ethics Committee Approval: The Local Ethics Committee for Animal Experiments (HADYEK) of Üsküdar University approved the study on January 22, 2021, with the decision number 2020-14.

Informed Consent: N.A

Peer-review: Externally peer-reviewed.

Author Contributions Concept – D.İ.K., T.U.; Design – T.U., T.G., A.Ö.Ş., B.Ç., D.İ.K.; Supervision – T.U.E., D.İ.K., T.U., B.Ç.; Data Collection and/or Processing – D.İ.K., B.Ç., A.Ö.Ş., T.U.E.; Analysis and/or Interpretation – D.İ.K., A.Ö.Ş., T.U.; Literature Review – D.İ.K. T.U.; Writer – T.U., D.İ.K.; Critical Reviews – T.U.

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