Additive Anticonvulsive Effects of Sumatriptan and Morphine on Pentylenetetrazole-Induced Clonic Seizures in Mice

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Background and Purpose: Sumatriptan protects the brain from damage and enhance the anti-seizure effect of morphine. There is evidence that nitric oxide (NO) may mediate these effects of both drugs. In the present study, we investigated the effects of sumatriptan (0.1-20 mg/kg, intraperitoneal [i.p.]) and morphine (0.1-20 mg/kg, i.p.) alone or in combination on seizure thresholds in an *in vivo* model of seizure in mice. Using various NO synthase inhibitors as well as the NO precursor, we assessed possible involvement of NO signaling in these effects.

Methods: Clonic seizures were induced in male Naval Medical Research Institute mice by intravenous administration of pentylenetetrazol (PTZ).

Results: Acute sumatriptan administration exerted anti-convulsive effects at 0.5 (p<0.01) and 1 mg/kg (p<0.05), but pro-convulsive effects at 20 mg/kg (p<0.05). Morphine had anti-convulsive effects at 0.5 (p<0.05) and 1 mg/kg (p<0.001), but exerted pro-convulsive effect at 20 mg/kg (p<0.05). Combination treatment with sub-effective doses of sumatriptan (0.1 mg/kg) and morphine (0.1 mg/kg) significantly (p<0.05) exerted an anti-convulsive effect. Co-administration of the NO precursor L-arginine (60 mg/kg) with sub-effective doses of sumatriptan and morphine significantly (p<0.05) increased seizure threshold compared with sumatriptan alone, but not sumatriptan+morphine group. While concomitant administration of either the non-selective NO synthase (NOS) inhibitor L-N^G-nitroarginine methyl ester (5 mg/kg) or the selective inducible NOS inhibitor aminoguanidine (50 mg/kg) with combined sub-effective doses of morphine and sumatriptan produced significant anticonvulsive effects, concomitant administration with the selective neuronal NOS inhibitor 7-nitroindazole (30 mg/kg) inhibited this effect.

Conclusions: Our data suggest a possible role for the NO signaling in the anticonvulsive effects of combined sumatriptan and morphine on the PTZ-induced clonic seizures in mice. **(2024;14:9-16)**

Key words: Sumatriptan, Morphine, Seizure, Pentylenetetrazole, Nitic oxide, Mice

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Introduction

Sumatriptan is a drug that activates 5-hydroxytryptamine (5-HT)$_{1B/1D}$ receptors, which are found on serotonergic nerve terminals that release serotonin (5-HT).[1] The levels of serotonin in the brain can have an impact on seizure activity.[2,3] As a result, sumatriptan has been found to have both anti-convulsive and pro-convulsive effects, depending on the dosage, when it comes to seizures triggered by pentylenetetrazole (PTZ).[4] Additionally, sumatriptan has other effects on the nervous system. For instance, it can protect against nerve damage induced by vincristine in rats,[5] and it provides relief from pain during migraine attacks.[6]

Opioids have been used by humans for pleasure and for treating various conditions, particularly pain, for almost 6 millennia.[7] One area of investigation that is not well-known is the impact of opioids on seizure susceptibility. Previous studies have demonstrated that opioids can have different effects on convulsive events. There is a growing body of evidence that indicates opioids have both anti-convulsive and pro-convulsive properties in different experimental models of seizures.[8,9] Specifically, low doses of morphine, an opioid-receptor agonist, have an anti-convulsive effect, while higher doses decrease the seizure thresholds induced by agents like picrotoxin, bicuculline, and PTZ.[10]

Nitric oxide (NO) is one of the most important neurotransmitters in both central (CNS) and peripheral nervous system. It is synthesized by three NO synthase (NOS) enzyme isoforms (neuronal NOS [nNOS], endothelial NOS [eNOS], and inducible NOS [iNOS]) from the amino acid L-arginine.[11] Compelling evidence suggest that NO modulates the susceptibility to seizure in a bimodal direction.[12,13] Additionally, studies reported that low doses of sumatriptan exert anti-convulsive effects on the PTZ-induced seizure in mice via mediating the NO pathway.[4] In addition, the involvement of NO in the biphasic effects of morphine on the PTZ-induced seizure susceptibility has been reported.[10,14] In the present study, we aimed to investigate the effects of combining sumatriptan and morphine on the seizure threshold and explore the possible role of the NO pathway.

Methods

Animals

All animal procedures were performed under the guidelines for the care and use of laboratory animals, Tehran University of Medical Sciences as well as the National Institutes of Health (NIH publication NO. 85-23; revised 1985). Male adult Naval Medical Research Institute mice, weighting 23-30 g and aged 6-8-week, were provided from the Department of Pharmacology, Tehran University of Medical Sciences. Animals were housed in standard polycarbonate cages at an ambient temperature of 24°C and a cycle of 12 horus-light/12 horus-dark. They also had free access to food and tap water. All behavioral trials were carried out at the same time of the day between 8:00 and 11:00 AM to minimize diurnal variations. Each mouse was used only once, and each group consisted of four to eight animals.

Chemicals

Sumatriptan, morphine sulfate, PTZ, L-arginine (a NO precursor), L-N^G-nitroarginine methyl ester (L-NAME, a non-specific NOS inhibitor), aminoguanidine (a specific iNOS inhibitor), and 7-nitroindazole (a specific nNOS inhibitor) were purchased from Sigma (St. Louis, MO, USA). All drugs were dissolved in normal saline (0.9%) except 7-nitroindazole, which was suspended in an aqueous solution of Tween® (Sigma) 80 (1%). All drug solutions and suspensions were prepared freshly on the day of the experiment. PTZ was injected intravenously in the tail vein (0.5%). Sumatriptan, morphine sulfate, L-NAME, 7-nitroindazole, and aminoguanidine were administered intraperitoneally (i.p.). The dosage selections, route of drug administration, and injection time of different compounds were based on previously published data, preliminary experiments, and pharmacokinetic considerations.[4,10,15,16]

Experimental groups

In experiment 1, animals received different doses of sumatriptan (0.1, 0.5, 1, 10, and 20 mg/kg, i.p.) or its vehicle (normal saline, as control group) 30 minutes before PTZ injection. In this step, the sub-effective dose of sumatriptan was selected for subsequent experiments.

In experiment 2, animals received different doses of morphine (0.1, 0.5, 1, 10, and, 20 mg/kg, i.p.) or its vehicle (normal saline, as control group) 30 minutes before PTZ injection. In this step, the sub-effective dose of morphine was selected for subsequent experiments.

In experiment 3, a sub-effective dose of sumatriptan (0.1 mg/kg, i.p.) was concurrently administered with a sub-effective dose of morphine (0.1 mg/kg, i.p.). The seizure was induced by PTZ injection 30 minutes after the drugs injection.

In experiments 4 through 7, the role of NO and opioids in the anticonvulsant effects of sumatriptan injection was assess. The non-effective doses of the NO donor L-arginine (60 mg/kg, i.p.), the non-selective NOS inhibitor L-NAME (5 mg/kg, i.p.), the selective iNOS inhibitor aminoguanidine (50 mg/kg, i.p.), and the selective nNOS inhibitor 7-nitroidazole (30 mg/kg, i.p.) were administered 15 minutes before saline, sumatriptan (0.1 mg/kg, i.p.), morphine (0.1 mg/kg, i.p.), and combined sumatriptan (0.1 mg/kg, i.p.)+morphine (0.1 mg/kg, i.p.) injection in separate groups. The seizure was induced by PTZ injection 30 minutes after the drugs injection.

Evaluation of seizure threshold

To assess the clonic seizure thresholds, a 30-gauge butterfly needle was inserted into the tail vein and fixed by a piece of adhesive tape while the animal was placed in a mouse restrainer. The infusion pump was adjusted to deliver PTZ (0.5%) at a constant rate of 1 mL/minutes in all the experiments, and animals were allowed to move freely. All these steps take less than 10 seconds and normally no mortality was seen. The infusion of PTZ was immediately discontinued when forelimb clonus was observed which is normally followed by full clonus of the body.[16] The minimal dose of PTZ (mg/kg of mice weight) needed to induce general clonus was recorded as an index of clonic seizure threshold.[15,16]

Statistical analysis

The data were analyzed using the Graph pad Prism data analysis program (Graph pad Software; San Diego, CA, USA) and presented as the mean±standard error of mean. One-way ANOVA (Graph pad Software) followed by Tukey's multiple comparisons of variances was used to analyze data where appropriate. In all experimental groups, differences were considered statistically significant in case the probability of type I error (p-value) was less than 0.05.

Results

Effect of sumatriptan and morphine on the PTZ- induced seizure threshold

Fig. 1A illustrates the biphasic effects of sumatriptan (0.1, 0.5, 1, 10, and 20 mg/kg, i.p.) on PTZ-induced seizure thresholds ($F_{5,33}$=15.01; p<0.001). Sumatriptan at doses of 0.5 mg/kg (p<0.01) and 1 mg/kg (p<0.05) exerted anti-convulsive effects, whereas sumatriptan at 20 mg/kg decreased the seizure threshold compared with the control (saline-treated) group (p<0.05). Notably, sumatriptan at 0.1 mg/kg did not exert any significant anti-convulsive effects on the seizure threshold compared to the control group and was selected as a sub-effective dose for anti-convulsive activity in our experiments.

Fig. 1B shows the effects of different doses of morphine (0.1, 0.5, 1, 10, and 20 mg/kg, i.p.) on the PTZ-induced seizure thresholds ($F_{5,30}$=12.19; p<0.001). Morphine at 0.5 mg/kg (p<0.05) and 1 mg/kg (p<0.001) increased seizure thresholds compared to the saline-treated control group. However, morphine at the highest dose (20 mg/kg, i.p.) exerted a significant (p<0.05) pro-convulsive effect compared to the control group. Notably, morphine at 0.1 mg/kg did not exert any anti-convulsive effects and was considered as a sub-effective dose.

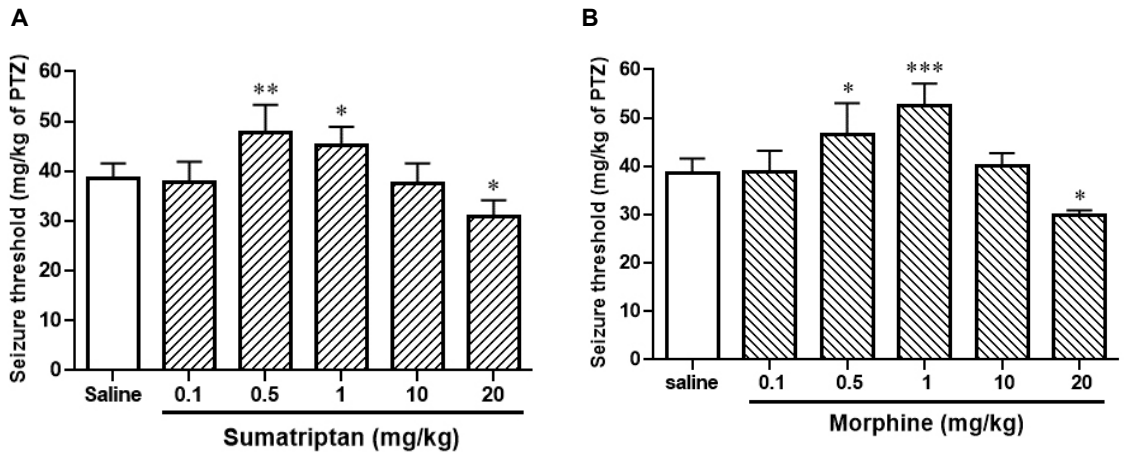

Figure 1. Effects of different doses of (A) sumatriptan (0.1, 0.5, 1, 10, and 20 mg/kg, i.p.) or its solvent (saline, i.p., as control group) and (B) morphine (0.1, 0.5, 1, 10, and 20 mg/kg, i.p.) or its solvent (saline, i.p., as control group) on the pentylenetetrazole (PTZ)-induced clonic seizure threshold in mice. Sumatriptan or morphine were administered 30 minutes before determination of PTZ-induced clonic seizure threshold. Data are expressed as the mean±standard deviation of seizure threshold in each group. Each group consisted of 5 to 8 mice. i.p., intraperitoneal. *p<0.05; **p<0.01; and ***p<0.001 compared with corresponding saline-treated control group.

Effect of acute co-administration of morphine and sumatriptan on the PTZ-induced seizure threshold

Fig. 2 demonstrates the effects of co-administration of the sub-effective doses of morphine (0.1 mg/kg, i.p.) and sumatriptan (0.1 mg/kg, i.p.) on the PTZ-induced seizure threshold ($F_{3,26}$=8.771; p<0.001). This combined administration significantly increased the seizure thresholds in comparison with sumatriptan (p<0.05) or morphine (p<0.05) alone groups.

Effects of L-arginine on the anti-convulsive effects of sumatriptan, morphine, or their combination on the PTZ-induced seizure threshold

As shown in Fig. 3, L-arginine at 60 mg/kg did not have any significant effect on the seizure threshold compared with the saline-treated control group. Moreover, L-arginine (60 mg/kg, i.p.) plus sub-effective dose of sumatriptan (0.1 mg/kg, i.p.) or L-arginine (60 mg/kg, i.p.) plus sub-effective dose of morphine (0.1 mg/kg, i.p.) did not exert any significant effect on the PTZ-induced seizure threshold compared with sumatriptan (0.1 mg/kg, i.p.) or morphine (0.1 mg/kg, i.p.) alone group. Notably, co-administration of L-arginine (60 mg/kg, i.p.), sumatriptan (0.1 mg/kg, i.p.), and morphine (0.1 mg/kg, i.p.) significantly (p<0.05) increased seizure threshold compared with sumatriptan (0.1 mg/kg) alone group. However, this combined three-agent treatment did not significantly alter the seizure threshold (p>0.05) in comparison with sumatriptan (0.1 mg/kg, i.p.) plus morphine (0.1 mg/kg, i.p.) group ($F_{7,43}$=4.766; p<0.001).

Effects of NOS inhibitors on the anti-convulsive effects of sumatriptan, morphine, or their combination on the PTZ-induced seizure threshold

Fig. 4A shows that L-NAME at 5 mg/kg (i.p.) alone did not have any significant effect on the PTZ-induced seizure threshold compared with the saline-treated control group. However, co-treatment of L-NAME (5 mg/kg, i.p.) with the sub-effective dose of sumatriptan (0.1 mg/kg, i.p.) significantly increased the seizure threshold in comparison with sumatriptan (0.1 mg/kg, i.p.) alone (p<0.001). Moreover, co-administration of L-NAME (5 mg/kg, i.p.) with a sub-effective dose of morphine (0.1 mg/kg, i.p.) did not alter the seizure threshold in comparison with the morphine (0.1 mg/kg) alone. Notably, concurrent administration of L-NAME (5 mg/kg, i.p.), sumatriptan (0.1 mg/kg, i.p.), and morphine (0.1 mg/kg, i.p.) significantly increased the PTZ-induced seizure threshold compared with sumatriptan alone (p<0.001), morphine alone (p<0.001), or morphine plus sumatriptan (p<0.05) groups ($F_{7,42}$=13.18; p<0.001).

Fig. 4B shows that aminoguanidine at 50 mg/kg (i.p.) alone did not exert any significant effect on the PTZ-induced seizure threshold

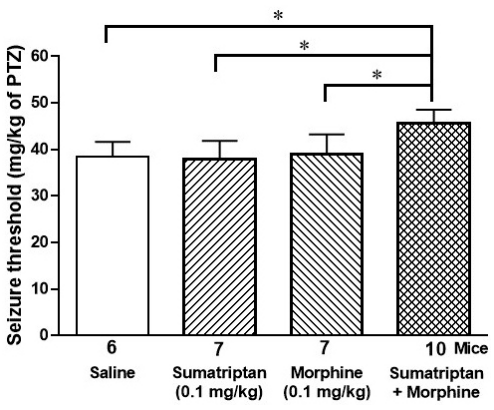

Figure 2. Effect of co-administration of sub-effective doses of morphine (0.1 mg/kg, i.p.) and sumatriptan (0.1 mg/kg, i.p.) on the pentylenetetrazole (PTZ)-induced clonic seizure threshold in mice. Morphine and sumatriptan were administered concurrently 30 minutes before PTZ. Data are expressed as mean±standard deviation. The number of mice in each group is indicated below the corresponding bar. i.p., intraperitoneal. *p<0.05 compared with corresponding group as shown by lines connecting each of the two comparison groups.

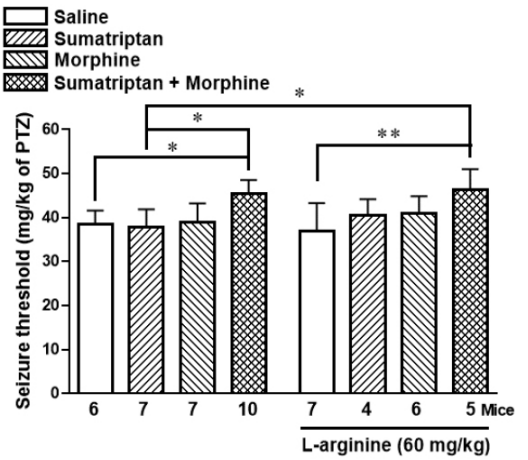

Figure 3. Effect of pretreatment with L-arginine (60 mg/kg, i.p.) on the effects of sumatriptan (0.1 mg/kg, i.p.) and morphine (0.1 mg/kg, i.p.), alone or in combination, on the pentylenetetrazole (PTZ)-induced seizure threshold in mice. L-arginine was administered 15 minutes before sumatriptan or morphine, or their co-administration, and 45 minutes before PTZ. Data are expressed as mean±standard deviation. The number of mice in each group is indicated below the corresponding bar. i.p., intraperitoneal. *p<0.05 and **p<0.01 compared with corresponding group as shown by lines connecting each of the two comparison groups.

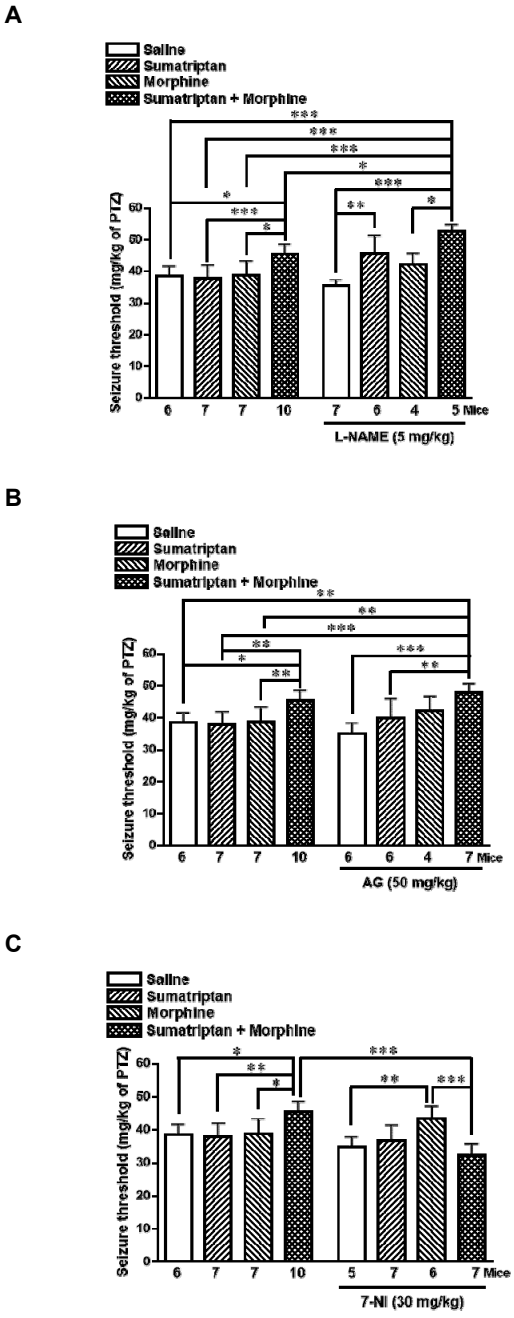

Figure 4. Effect of pretreatment with (A) L-NAME (5 mg/kg, i.p.), (B) aminoguanidine (AG, 50 mg/kg, i.p.), and (C) 7-NI (30 mg/kg, i.p.) on the effects of combined morphine (0.1 mg/kg, i.p.) and sumatriptan (0.1 mg/kg, i.p.) on the PTZ-induced seizure threshold in mice. L-NAME, 7-NI, or AG were administered 15 minutes before sumatriptan or morphine, or their co-administration, and 45 minutes before PTZ. Data are expressed as mean± standard deviation. The number of mice in each group is indicated below the corresponding bar. PTZ, pentylenetetrazole; L-NAME, L-N^G-nitroarginine methyl ester; 7-NI, 7-nitroindazole; i.p., intraperitoneal. *p<0.05; **p<0.01; and ***p<0.001 compared with corresponding group as shown by lines connecting each of the two comparison groups.

compared with the saline-treated control group. Moreover, co-administration of aminoguanidine (50 mg/kg, i.p.) with either the sub-effective dose of sumatriptan (0.1 mg/kg, i.p.) or morphine (0.1 mg/kg, i.p.) did not exert any significant effect on the PTZ-induced seizure thresholds in comparison with either sumatriptan or morphine group, respectively. Notably, the administration of aminoguanidine (50 mg/kg, i.p.) along with sumatriptan (0.1 mg/kg, i.p.) and morphine (0.1 mg/kg, i.p.) significantly increased seizure threshold compared with sumatriptan (p<0.001) and morphine (p<0.01) groups ($F_{7,44}$=8.771; p<0.001). However, this combined three-agent treatment did not alter the seizure threshold compared to sumatriptan plus morphine group (p>0.05).

Fig. 4C shows that 7-nitroindazole at 30 mg/kg (i.p.) did not have any significant effect on the seizure threshold compared with the saline-treated control group. Moreover, co-treatment of 7-nitroindazole (30 mg/kg, i.p.) with the sub-effective dose of either sumatriptan (0.1 mg/kg, i.p.) or morphine (0.1 mg/kg, i.p.) did not exert any significant effect on the PTZ-induced seizure threshold compared with corresponding sumatriptan or morphine group, respectively. Notably, co-administration of 7-nitroindazole (30 mg/kg, i.p.) with combined sumatriptan (0.1 mg/kg, i.p.) and morphine (0.1 mg/kg, i.p.) significantly reduced seizure activity induced by PTZ in comparison with morphine (p<0.001) or sumatriptan plus morphine (p<0.001), and not with sumatriptan alone group ($F_{7,47}$=5.154; p<0.001).

Discussion

Seizure induction by PTZ is a recognized and widely used experimental model for studying clonic seizures in a clinical setting.[17,18] PTZ specifically binds to the picrotoxin site of the γ-aminobutyric acid (GABA) receptor complex, disrupting GABA-mediated inhibition and potentially activating the N-methyl-D-aspartate receptor. This in turn increases activity in key epileptogenic centers of the forebrain such as the amygdala and piriform cortex.[19] These neurochemical processes contribute to the onset and spread of PTZ-induced seizures.[20]

While it was previously believed that sumatriptan only had an effect on the peripheral nervous system, recent findings have shown that it also affects the CNS.[4,21-23] An increase in sumatriptan concentration in the spinal fluid after oral administration and high levels of sumatriptan in the brain after intravenous injection confirm this.[23] Sumatriptan is commonly used to treat migraines and works by targeting 5-$HT_{1B/1D}$ receptors. There is a connection between migraines and epilepsy, known as migralepsy,[24] suggesting that seizures can be

triggered by migraines and followed by headaches and more migraines. This raises questions about how sumatriptan affects seizures. It has been found that lower dose of sumatriptan (1 mg/kg) can increase the threshold for clonic seizures induced by PTZ via involvement of the nNOS/NO pathway in the hippocampus and temporal cortex, whereas higher dose (20 mg/kg) can actually lower the seizure threshold via the activation of iNOS/NO pathway in the brain.[4] A study in mice showed anti-convulsive effects of sumatriptan,[22] but another study in rats reported pro-convulsive effects on the PTZ-induced seizures.[21] Our present study found that low doses of sumatriptan (0.5 and 1 mg/kg) showed anti-convulsive effects, but a higher dose (20 mg/kg) had a pro-convulsive impact on the PTZ-induced seizures in mice.

Opioids, such as morphine, have a biphasic effect on seizure threshold depending on the dosage and models used.[8,10,25-27] Lower doses of morphine typically have an anti-convulsive effect against seizures induced by substances that block GABA transmission, such as PTZ, picrotoxin, bicuculline, and isoniazid, in animal studies.[28] Conversely, higher doses of morphine actually increase the susceptibility of animals to seizures.[25] Moreover, endogenous opioids have the potential to protect against seizures induced by electroconvulsive shocks[29] and can play an anti-convulsive role in acute stress.[30] However, at very high doses, morphine can induce seizures and heighten susceptibility to them.[27] The biphasic impact of morphine on seizures suggests that it activates both excitatory and inhibitory mechanisms.[31] Moreover, opioids can modulate the activity of stimulatory guanine nucleotide-binding (Gs)[32] and inhibitory guanine nucleotide-binding (Gi) proteins,[33] which are involved in signal transduction pathways.[34] Low doses of opioids have been shown to activate Gs proteins,[32] which stimulate adenylate cyclase and increase cyclic adenosine monophosphate (AMP) levels. High doses of opioids, on the other hand, have been shown to activate Gi proteins,[33] which inhibit adenylate cyclase and decrease cyclic AMP levels. Confirming earlier research, we also observed that administering low doses of morphine (0.5 and 1 mg/kg) increased the seizure threshold. On the other hand, higher doses (20 mg/kg) decreased the threshold and intensified susceptibility to seizures induced by PTZ in mice.

The NO pathway has been shown to influence seizure threshold in different ways depending on the study.[35-37] Some researchers have suggested that the alterations in the activity of three enzymes that produce NO in a biological system are responsible for the seizures induced by PTZ in animals.[38,39] Kirkby et al.[38] reported that the effects of NOS inhibitors on seizures, whether pro or anti-convulsive, depend on genetic factors, the models employed in the seizures, and the dosage administered. Moreover, there is evidence that sumatriptan reduces levels of NO.[39] Additional studies have suggested that sumatriptan aids in alleviating migraine headaches by decreasing the production of NO.[40] In our recent study,[41] we observed an increase in NO levels following the induction of status epilepticus (SE) in rats, but treatment with sumatriptan resulted in a significant decrease in NO levels in the rats' brain tissues post-seizure. On the other hand, a more substantial reduction in NO levels could potentially lead to more brain damage. This was evident in our experiment when we combined sumatriptan with NOS inhibitors (L-NAME and 7-nitroindazole), which resulted in more brain injury. Moreover, there is evidence that the NO pathway mediates both the anti- and pro-convulsive effects of morphine in seizures.[10,29,42] For instance, Homayoun et al.[10] demonstrated that the non-selective NOS inhibitor L-NAME was able to completely reverse the anticonvulsive effects of morphine on the PTZ-induced clonic seizures in mice, whereas the pro-convulsive impact was only partially neutralized by L-NAME. Other study also found that pre-treatment with different NOS inhibitors, such as L-NAME (10 mg/kg, i.p.), 7-nitroindazole (30 mg/kg, i.p.), and aminoguanidine (50 mg/kg, i.p.), significantly reversed the inhibitory effects of morphine on the lithium chloride/pilocarpine-induced SE.[43] Another study also demonstrated that constitutional NOS isoforms (i.e., eNOS and nNOS) may play an important role in the modulation of anticonvulsive effects of morphine on electroshock-induced seizure in mice.[29]

In a study conducted in 2013, the researchers investigated the potential role of the NO pathway in the effects of combining morphine with agmatine (a compound derived from the chemical arginine) on seizures induced by PTZ in mice.[42] The results showed that when L-NAME (1 and 5 mg/kg, i.p.) or 7-nitroindazole (15 and 30 mg/kg, i.p.) were co-administered with a combination of morphine (0.1 mg/kg) and agmatine (1 mg/kg), a significant anticonvulsive effect was observed. Additionally, the administration of the NO precursor L-arginine (30 and 60 mg/kg, i.p.) inhibited the anticonvulsive effects of co-administering agmatine (3 mg/kg) and morphine (0.5 mg/kg), suggesting the involvement of the NO pathway.[42] These findings are consistent with our present data that showed that different NOS inhibitors could influence the effects of combined sumatriptan and morphine administration on PTZ-induced seizures in mice. The anticonvulsive effect of morphine is connected to an increase in GABAergic transmission within the CNS.[26,44] Interestingly, several studies have found a correlation between NOS activation and an increase in GABAergic activity in the brain.[45,46] Therefore, it is likely that

NO plays a role in mediating the anticonvulsive effect of morphine by enhancing GABAergic function. However, further investigation is needed to clarify this point.

Conflicts of Interest

The authors report no conflicts of interest related to the present study.

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