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Modulatory effect of opioid ligands on status epilepticus and the role of nitric oxide pathway

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ABSTRACT

Epilepsy is a chronic disorder that causes unprovoked, recurrent seizures. Status epilepticus (SE) is a medical emergency associated with significant morbidity and mortality. Morphine has been the cornerstone of pain controlling medicines for a long time. In addition to the analgesic and opioid responses, morphine has also revealed anticonvulsant effects in different epilepsy models including pentylenetetrazole (PTZ)-induced seizures threshold. Some authors suggest that nitric oxide (NO) pathway interactions of morphine explain the reason for its pro or anticonvulsant activities. To induce SE, injection of a single dose of lithium chloride (127 mg/kg, intraperitoneal (i.p.)) 20 h before pilocarpine (60 mg/kg, i.p.) was used. Administration of morphine (15 mg/kg, i.p.) inhibited the SE and decreased the mortality in rats when injected 30 min before pilocarpine. On the other hand, injection of L-N^G-nitro arginine methyl ester (L-NAME, a nonselective NO synthase (NOS) blocker; 10 mg/kg, i.p.), 7-nitroindazole (7-NI, a neuronal NOS (nNOS) blocker; 30 mg/kg, i.p.), and aminoguanidine (AG, an inducible NOS (iNOS) blocker; 50 mg/kg, i.p.) 15 min before morphine, significantly reversed inhibitory effect of morphine on SE. Subsequently, measurement of nitrite metabolite levels in the hippocampus of SE-induced rats displayed high levels of nitrite metabolite for the control group. However, after injection of morphine in SE-induced rats, nitrite metabolite levels reduced. In conclusion, these findings demonstrated that NO pathway (both nNOS and iNOS) interactions are involved in the anticonvulsant effects of morphine on the SE signs and mortality rate induced by lithium–pilocarpine in rats.

1. Introduction

Epilepsy is a symptom of a variety of diseases that manifests itself in various clinical forms. Epilepsy is a chronic disorder in brain function caused by an abnormal electrical discharge of brain neurons. An epileptic attack involves a period of uncontrolled, abnormal motor–sensory behaviors (neurologists name epilepsy as a brain electrical disorientation) [1]. A study in 2015 on school-aged children (5–15 years old) showed that combined healthcare, social care, and education costs over 18 months for a child with active epilepsy are about two times higher compared with those without impairment [2]. There is some evidence showing that multiple mechanisms are involved in manifestations of this central nervous system (CNS) disorder such as CNS inflammations [3], gamma-aminobutyric acid (GABA) receptors [4], N-methyl-D-aspartate (NMDA) receptors [5], and opioid receptors [6,7]. In addition to all, nitric oxide (NO) is implicated in the pathogenesis of seizures and epilepsy [8–13].

In this study, the model used to induce seizure is lithium–pilocarpine intraperitoneal (i.p.) injection. Pilocarpine is a muscarinic and cholinergic agonist and can cause limbic motor seizures. These seizures spread over 1–2 h and slowly lead to persistent epilepsy, which is named status epilepticus (SE) [14].

Opioid receptors are found in the CNS and periphery. The ligands that interact with these receptors (opioids) cause a number of different physiological functions such as analgesia, respiration, and hormonal regulation [15]. There are plenty of literature that indicated the impacts of opioid receptors' agonists and antagonists on different models of epilepsy in both human and animals. Mazarati et al. in 1999 reported that opioid receptors in the hippocampus of rat intervened the initiation and maintenance of SE [7]. Adler M W et al. showed that morphine increases the pentylenetetrazole (PTZ)- and flurothyl-induced seizures threshold in adult male Sprague–Dawley rats [16]. There are number of studies that demonstrate anticonvulsant actions of morphine. In 1983, Hanan Frenk observed that morphine presents anticonvulsant properties that are mediated by opioid receptors [17]. Further, in 1986, Frey H. reported

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that morphine suppresses the generalized seizures [18]. Also, Riazi et al. showed the anticonvulsant effects of morphine, which was potentiated by agmatine [19].

Nitric oxide has been known as a neuronal messenger in CNS and a mediator of several brain functions [20]. Three enzymes are synthesizing NO: inducible NO synthase (iNOS), endothelial NOS (eNOS), and neuronal NOS (nNOS). Highly selective inhibitor of iNOS has been identified, which can play a role in the treatment of inflammatory conditions [21]. Nitric oxide has been studied in many different experimental models and shown both pro and anticonvulsant effects on seizure paradigms. In a study on NMDA- and kainate-induced seizure models, NOS inhibitor significantly protected against seizure, suggesting NO presents proconvulsant activities [22]; while Edmund Przegalinski et al. showed that NO acts as an endogenous anticonvulsant in kainate-induced seizures in mice [23]. In addition, the interaction of NO pathway and opioid receptors was reported in previous studies. For instance, it was shown that agmatine as an endogenous polyamine and a metabolite of L-arginine (L-Arg) precursor of NOS- enhanced the anticonvulsant effects of morphine in PTZ-induced seizures threshold in mice [24].

Therefore, the current study aimed to investigate whether morphine alters signs or mortality in lithium–pilocarpine-induced SE in rats and to investigate the possible role of NO pathway.

2. Materials and methods

2.1. Materials

The drugs used in this study are the following: L-Arg, a NO precursor; L-N^G-nitro arginine methyl ester (L-NAME), a nonselective NOS inhibitor; aminoguanidine (AG), a specific iNOS inhibitor; 7-nitroindazole (7-NI), a specific nNOS inhibitor; hyoscine-N-bromide (HNBr); pilocarpine and lithium chloride (LiCl, Sigma, St Louis, MO, USA); morphine sulfate (Temad Pharmaceuticals, Alborz, Iran). All these drugs were purchased from Sigma Aldrich, St Louis, MO, USA, except morphine sulfate obtained from Temad Pharmaceuticals, Alborz, Iran.

For preparation, all drugs were dissolved in sterile isotonic saline solution, except 7-NI, which was dissolved in minimum amount of Dimethyl sulfoxide (DMSO) and then reached to desired concentration by adding saline (0.4% DMSO-Saline, v/v). During the study, all drugs injected intraperitoneally.

In this study, male adult Wistar rats from the Pharmacy Faculty of Tehran University of Medical Science (TUMS) were used, which weighted 180 to 250 g. All rats were kept in 23 °C (±2 °C) temperature. At the breeding grounds for these rats, the day/night cycle was 12 h light and 12 h dark. Water and food were accessible to the rats. Rats were divided into groups of 6, and each group was tested only once. The study was conducted in accordance with the Declaration of Helsinki, and approved by the independent ethics committee of TUMS (IR.TUMS.PSRC.REC.1396.2356).

2.2. Methods

Wistar rats were treated by lithium (127 mg/kg, i.p.), after 20 h pilocarpine (60 mg/kg, i.p.) was administered to induce SE [25–29]. Hyoscine-N-bromide (2 mg/kg, i.p.) was injected 30 min before pilocarpine to prevent the peripheral cholinergic side effects of it.

The rats were observed for 2 h, the seizure onset times were recorded, and stages were scored from 1 to 5 based on a modified version of Racine scale (according to Table 1) [30]. In the next step, the rats were transferred to the storage room and followed for the next 24 h to investigate the survival of animals.

2.3. Experiments

Two groups of naïve rats were chosen to serve as the control and sham group. Saline was injected to the sham group, and lithium–pilocarpine (127 mg/kg and 60 mg/kg, respectively, i.p.) and saline were administered to the control group.

Next, to understand the impact of morphine on seizures induced by lithium–pilocarpine, six groups of rats were chosen, and morphine (0.5, 1, 3, 10, 15, and 30 mg/kg, i.p.) was injected 30 min before SE induction to the groups.

Finally, in eight groups of rats SE induced by lithium–pilocarpine, L-NAME (10 mg/kg), L-Arg (60 mg/kg), 7-NI (30 mg/kg), and AG (50 mg/kg) were injected 15 min before either morphine or saline administration.

Table 1
A modified version of the Racine scale using stages 1 to 5.

Stage	Behavior
1	Facial automatism and tail stiffening
2	Head nodding and wet-dog shakes
3	Low-intensity tonic–clonic seizure marked bay unilateral forelimb myoclonus
4	Bilateral forelimb myoclonus and rearing
5	Bilateral fore and hind limb myoclonus and transient loss of postural control

2.4. Nitric oxide measurement

Griess reaction was used to investigate NO levels in the hippocampus of treated rats [31]. Rats were euthanized via chloroform according to the ethical principles, and the hippocampi were excised and stored in 2 ml microtubes at −80 °C in order to measure NO concentration.

The samples were blended with the same amount of Griess reagent to form a purple azo dye in a reaction path. The Griess reagent, 1.25% HCl containing 5 g/l sulfanilamide with 0.25 g/l N-naphthyl ethylenediamine, was added with a rate of 0.1 ml/min. The absorbance of the dye product was measured at 540 nm in accordance with a standard nitrite curve produced by $NaNO_2$ using ELISA method. The results were displayed as μMol/ml.

2.5. Statistical analysis method

Mann–Whitney test was run to compare seizure behaviors between groups, and P-value ≤0.05 was considered as the significant difference between groups. Normality of each group's scores was investigated and ruled out. In addition, chi-square test was used to compare mortalities. In terms of studying nitrite metabolite levels, one-way Analysis of variance (ANOVA) and t-test were used.

3. Results

Lithium–pilocarpine-treated (control group) rats presented remarkable signs of SE in comparison with saline-treated rats (sham group).

The onset of seizure has been the same for all groups in all tests of this study.

3.1. Effect of acute morphine injection on seizure stages and mortality rates after 24 h induced by lithium–pilocarpine

Morphine was injected to six groups of rats (0.5, 1, 3, 10, 15, and 30 mg/kg). All rats had been put in the setting of lithium–pilocarpine-induced SE. The acute injection of 15 and 30 mg/kg (i.p.) morphine 30 min before pilocarpine made a significant ($P < 0.001$) decrease in SE signs in comparison with the control group, while administration of 0.5, 1, 3, and 10 mg/kg (i.p.) did not change the scores of SE stages compared with the control group (Fig. 1). In addition, chi-square test demonstrated (Table 2) a significant decrease ($P < 0.05$) in mortality rates after 24 h in the group injected 15 mg/kg (i.p.) morphine (0%) compared with the control; however, it did not show

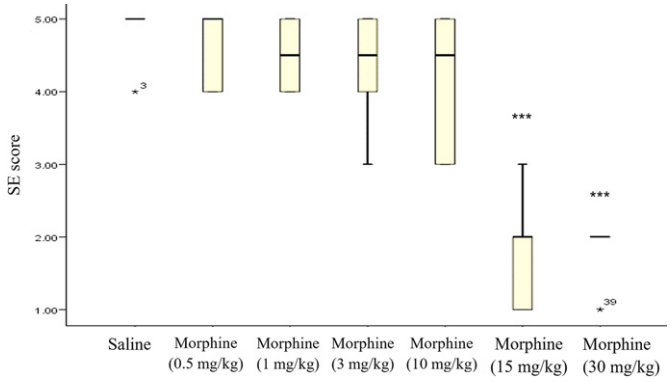

Fig. 1. Effect of acute morphine injection on lithium–pilocarpine-induced SE. Group 1 served as control and groups 2 to 7 injected morphine (0.5, 1, 3, 10, 15, and 30 mg/kg, respectively, i.p.). Each group consisted of six rats, and there were 42 rats in this test. ^{3} determined rat number 3, and ^{39} determined rat number 39. *** ($P < 0.001$) compared with the control group.

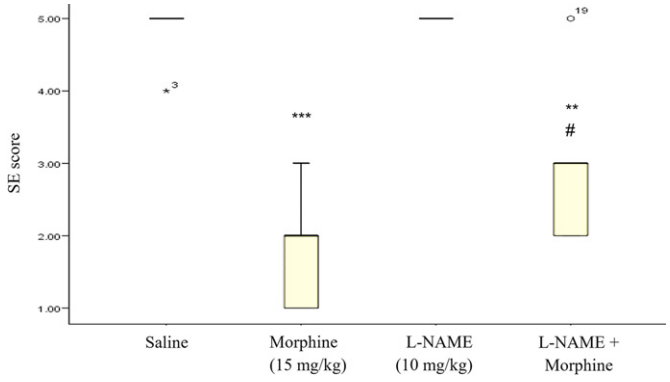

Fig. 2. Effect of L-NAME (10 mg/kg, i.p.) injection on morphine inhibitory effect on SE. Group 1 was the control group, group 2 was morphine (15 mg/kg, i.p.), group 3 was L-NAME (10 mg/kg, i.p.), and group 4 was L-NAME + morphine. Each group consisted of six rats, and there were 24 rats in this test. ^{3} determined rat number 3 and ^{19} determined rat number 19. *** ($P < 0.001$) compared with the control group. ** ($P < 0.01$) compared with the control group. # ($P < 0.05$) compared with morphine (15 mg/kg, i.p.) group.

any difference between the groups treated by morphine (0.5, 1, 3, 10, and 30 mg/kg, i.p.) and the control (50%).

3.2. Effect of L-N^{G}-nitro arginine methyl ester (L-NAME) injection on morphine pretreated and lithium–pilocarpine-induced status epilepticus

L-N^{G}-nitro arginine methyl ester was injected 15 min before either morphine or saline in lithium–pilocarpine-treated rats. The acute injection of L-NAME (10 mg/kg, i.p.) did not change lithium–pilocarpine-induced seizure signs but significantly ($P < 0.05$) diminished the inhibitory effect of morphine (15 mg/kg, i.p.) on lithium–pilocarpine-induced SE (Fig. 2). Moreover, chi-square test showed (Table 2) a significant increase ($P < 0.001$) in mortality rates after 24 h in the L-NAME + morphine group (100%) compared with the morphine (15 mg/kg, i.p.) group (0%).

Table 2
Effect of morphine (0.5, 1, 3, 10, 15, and 30 mg/kg), L-NAME (10 mg/kg, i.p.), L-Arg (60 mg/kg, i.p.), 7-NI (30 mg/kg, i.p.), and aminoguanidine (50 mg/kg, i.p.) injection on mortality rates after 24 h.

Groups	Mortality rate	Significance
Saline	50%	
Morphine (0.5 mg/kg, i.p.)	33%	
Morphine (1 mg/kg, i.p.)	17%	
Morphine (3 mg/kg, i.p.)	50%	
Morphine (10 mg/kg, i.p.)	33%	
Morphine (15 mg/kg, i.p.)	0%	* ($P < 0.05$)
Morphine (30 mg/kg, i.p.)	33%	
L-NAME (10 mg/kg, i.p.)	100%	* ($P < 0.05$)
L-Arg (60 mg/kg, i.p.)	100%	* ($P < 0.05$)
7-NI (30 mg/kg, i.p.)	66%	
Aminoguanidine (50 mg/kg, i.p.)	100%	* ($P < 0.05$)
Morphine (15 mg/kg, i.p.)	0%	* ($P < 0.05$)
L-NAME + morphine	100%	* ($P < 0.05$) ### ($P < 0.001$)
L-Arg + morphine	83%	* ($P < 0.05$) ## ($P < 0.01$)
7-NI + morphine	100%	* ($P < 0.05$) ### ($P < 0.001$)
Aminoguanidine + morphine	83%	* ($P < 0.05$) ## ($P < 0.01$)

##, and ### compared with morphine (15 mg/kg, i.p.).
* Compared with the control group.

3.3. Effect of L-arginine on morphine pretreated and lithium–pilocarpine-induced status epilepticus

L-Arginine was injected 15 min before either morphine or saline in lithium–pilocarpine-treated rats. The acute injection of L-Arg (60 mg/kg, i.p.) did not significantly ($P < 0.05$) change lithium–pilocarpine-induced SE signs nor did prevent morphine inhibition (Fig. 3). However, chi-square test revealed (Table 2) a significant increase ($P < 0.01$) in mortality rates after 24 h in the L-Arg + morphine group (83%) in comparison with the morphine (15 mg/kg, i.p.) group (0%).

3.4. Effect of 7-nitroindazole on morphine pretreated and lithium–pilocarpine-induced status epilepticus

7-Nitroindazole was injected 15 min before either morphine or saline in lithium–pilocarpine-treated rats. The acute injection of 7-NI (30 mg/kg, i.p.) did not notably ($P < 0.05$) alter lithium–pilocarpine-induced seizure signs; however, it significantly ($P < 0.01$) prevented the inhibitory effect of morphine (15 mg/kg, i.p.) on lithium–pilocarpine-induced SE. Moreover, chi-square test illustrated (Table 2) a significant increase ($P < 0.001$) in mortality rates after 24 h between the 7-NI + morphine (100%) and morphine (0%) groups (Fig. 4).

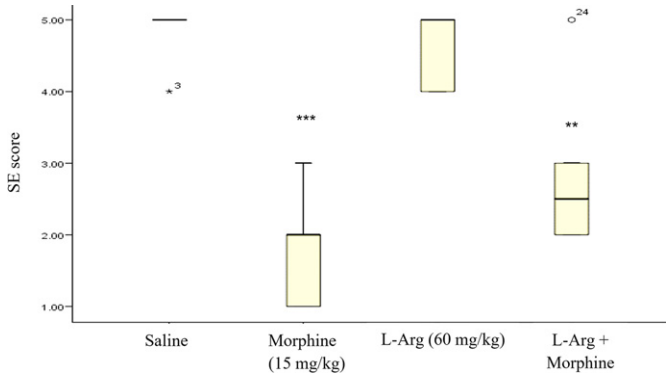

Fig. 3. Effect of L-Arg (60 mg/kg, i.p.) injection on morphine inhibitory effect on SE. Group 1 was the control group, group 2 was morphine (15 mg/kg, i.p.), group 3 was L-Arg (60 mg/kg, i.p.), and group 4 was L-Arg + morphine. Each group consisted of six rats, and there were 24 rats in this test. ^{3} determined rat number 3 and ^{24} determined rat number 24. *** ($P < 0.001$) compared with the control group. ** ($P < 0.01$) compared with the control group.

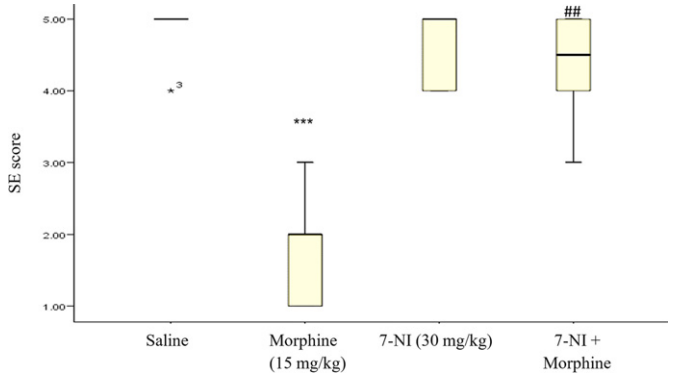

Fig. 4. Effect of 7-NI (30 mg/kg, i.p.) injection on morphine inhibitory effect on SE. Group 1 was the control group, group 2 was morphine (15 mg/kg, i.p.), group 3 was 7-NI (30 mg/kg, i.p.), and group 4 was 7-NI + morphine. Each group consisted of six Wistar rats, and there were 24 rats in this test. *3 determined rat number 3. *** ($P < 0.001$) compared with the control group. ## ($P < 0.01$) compared with the morphine (15 mg/kg, i.p.) group.

3.5. Effect of aminoguanidine on morphine pretreated and lithium–pilocarpine-induced status epilepticus

Aminoguanidine was injected 15 min before either morphine or saline in lithium–pilocarpine-treated Wistar rats. The acute injection of AG (50 mg/kg, i.p.) did not notably change lithium–pilocarpine-induced seizure signs, while it meaningfully ($P < 0.05$) diminished the inhibitory effect of morphine (15 mg/kg, i.p.) on lithium–pilocarpine-induced SE (Fig. 5). Further, chi-square test demonstrated (Table 2) a remarkable increase ($P < 0.01$) in mortality rates after 24 h in the AG + morphine group (83%) compared with the morphine (15 mg/kg, i.p.) group (0%).

3.6. Detection of nitric oxide level in hippocampus

t-Test demonstrated a notable increase in NO metabolite level for the control group in comparison with the sham group ($P < 0.001$). However, one-way ANOVA illustrated that morphine significantly inhibits the increase of NO metabolite level in comparison with the control group (Fig. 6).

Plasma NO metabolite levels were significantly decreased in morphine (15 and 30 mg/kg, i.p.)-treated groups in comparison with the control group (5.15 ± 1.03 and 7.91 ± 3.08, respectively versus 50.40 ± 4.02 for the control group; $P < 0.0001$). However, NO levels were not significantly different in the rats administered L-NAME (10 mg/kg, i.p.), 7-NI (30 mg/kg, i.p.), and AG (50 mg/kg, i.p.) 15 min before morphine (15 mg/kg, i.p.) in comparison with morphine (15 and 30 mg/kg, i.p.).

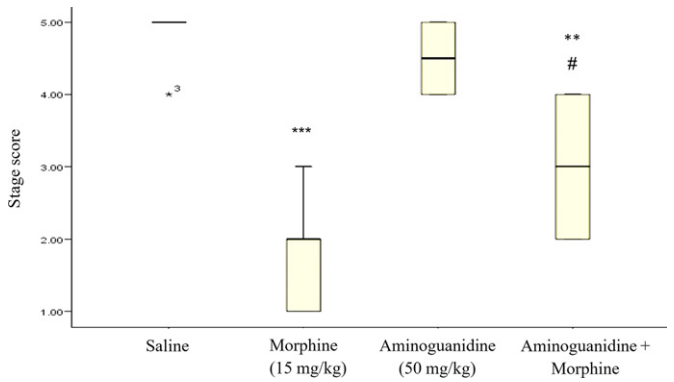

Fig. 5. Effect of aminoguanidine (50 mg/kg, i.p.) injection on morphine inhibitory effect on SE. Group 1 was saline (control group), group 2 was morphine (15 mg/kg, i.p.), group 3 was aminoguanidine (50 mg/kg, i.p.), and group 4 was aminoguanidine + morphine. Each group consisted of six rats, and there were 24 rats in this test. *3 determined rat number 3. *** ($P < 0.001$) compared with the control group. ** ($P < 0.01$) compared with the control group. # ($P < 0.05$) compared with the morphine (15 mg/kg, i.p.) group.

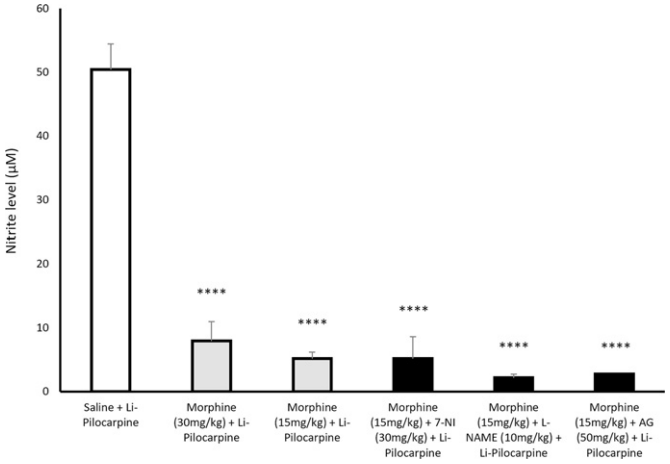

Fig. 6. NO metabolite levels in all groups (15 mg/kg morphine, 30 mg/kg morphine, morphine + aminoguanidine, morphine + L-NAME, and morphine + 7-NI) were notably lower in comparison with saline + lithium–pilocarpine (control) group. **** ($P < 0.0001$) compared with the control group.

4. Discussion

In the current study, we showed that morphine (15 mg/kg, i.p.) presents an anticonvulsant effect on SE induced by lithium–pilocarpine in rats. We also observed that morphine reduces the mortality rate in SE rats. Acute pretreatment of rats with L-NAME (a nonselective NOS inhibitor), 7-NI (a selective nNOS inhibitor), and AG (a selective iNOS inhibitor) not only prevented the anticonvulsant action of morphine but also increased mortality rates in comparison with the morphine (15 mg/kg, i.p.) group (100%, 100%, and 83%, respectively versus 0%). In addition, morphine administration 15 min prior to lithium–pilocarpine decreased the elevated level of NO in epileptic rats. In addition, administration of L-NAME (10 mg/kg), 7-NI (30 mg/kg), or AG (50 mg/kg) 15 min prior to morphine (15 mg/kg) decreased NO metabolite levels in the hippocampus of animals. Therefore, we propose that the NO pathway plays a vital role in the inhibitory effect of morphine on lithium–pilocarpine-induced SE in rats.

Opioids exert their main actions such as analgesia, respiration, and hormone modulation through opioid receptors all over the body, especially in CNS. However, these ligands have been shown to present other effects through different pathways including H1 receptor activation-linked pathway, adenosine triphosphate-sensitive (ATP-sensitive) K^+ channels, and selective stimulation of μ and κ opiate receptors [32–34]. It is also known that NO mediates tramadol's anticonvulsant effect on PTZ-induced seizure in mice [35]. In the current study, we investigated the NO pathway's role in the morphine modulations of seizure in lithium–pilocarpine model.

There are different models (laboratory and clinical) used to investigate pro or anticonvulsant effects of opioids. Morphine and opioid peptides have complex, both pro and anticonvulsant, effects [36]. Based on the clinical and experimental data, morphine presents either proconvulsant [37–40] or anticonvulsant [16,41–43] effects, depending on conditions of administration, species of animals, doses of chemicals, and type of seizures paradigms. In 1977, G. Le Gal La Salle et al. showed that morphine injections enhance seizures induced by amygdaloid stimulation [37]. Further, Turski et al. reported that morphine may present sustained limbic seizure by antagonizing inhibitory amino acid neurotransmission [38]. There is also a study in 2014 that documented similar proconvulsant effect of morphine and tramadol on PTZ

model of seizure, which may be mediated by GABAergic pathways [39]. Gholami et al. observed increasing seizure vulnerability in postnatal day 25 but not in postnatal day 32 rats [40]. In addition to all these data, Gideon Urca et al. had some evidence that morphine exerts both pro and anticonvulsant activities [36].

On the other hand, in a previous study, opioid ligands including morphine, meperidine, methadone, ethylketocyclazocine (EK), d-ala2-met-enkephalinamide, d-ala2-leu5-enkephalin, and beta-endorphin were investigated in the maximal electroshock (MES) model for understanding the modulatory actions of opioids. All these ligands caused a decrease in the length of the tonic component in rats [41]. A meta-analysis including nine randomized controlled trials with a total of 604 participants reported the effects of opioid pretreatment for prevention of etomidate-induced myoclonus in 2018. The meta-analysis illustrated that opioid administration resulted in a fewer number of patients who experienced myoclonic movement following etomidate injection [42]. Adler M W et al. demonstrated that morphine has anticonvulsant effect when administered before flurothyl or PTZ in rats [16]. Also, Honar H. et al. reported that morphine shows an anticonvulsant effect on PTZ-induced seizures threshold in mice, which is potentiated with ultralow doses of naltrexone [43]. Along these studies, our experiment showed that morphine significantly reduces the signs of SE including facial automatism and tail stiffening, head nodding and wet-dog shakes, low-intensity tonic–clonic seizure marked bay unilateral forelimb myoclonus, bilateral forelimb myoclonus and rearing, and bilateral fore and hind limb myoclonus, and transient loss of postural control in rats.

Studies showed that the populations with epilepsy have a mortality rate two to three times higher than the general population. Deaths in epilepsy relate to different reasons; some relate directly to the epilepsy consequences, and some to the epilepsy-caused disorders. Accidents, SE, and sudden unexpected death (SUD) are the causes of deaths that relate to seizures. Risk of SUD is 40 times higher in patients who continue to have seizures [44]. Our study, the treatment with morphine in SE-induced rats by lithium–pilocarpine, showed significant reduction of the mortality rate in treated groups compared with untreated subjects.

Nitric oxide is a critical messenger in CNS and participates in some functions of the brain [20]. So far, in many studies, the contradictory effects of the NOS pathway in seizures have been reported. A study demonstrated that the paradoxical effects of NOS inhibitors on a seizure depend on the models used in the seizure and genetic factors [45]. Several documents revealed that seizures induced by PTZ in animals were associated with different alterations in activity levels of three enzymes involved in NO production in a biological system [46,47]. Del-Bel EA et al. showed that changes in PTZ quantities inverted the NOS inhibitor action [9].

Further studies showed that NO pathway also plays a vital role in anticonvulsant actions of morphine against electroshock seizures [48]. There is evidence that both anti and proconvulsant effects of morphine, which is affected by melatonin, are modulated via the NO pathway [49]. In addition, Payandemehr B et al. showed that coadministration of agmatine – which is naturally created from the chemical arginine – enhances the anticonvulsant effects of morphine, which is mediated by the NO pathway [24]. Another study proved that a nonselective NOS inhibitor (L-NAME) inhibits the anticonvulsant actions of morphine on seizures induced by PTZ in mice [50]. Following these articles, in the current study, NOS inhibitors including L-NAME, 7-NI, and AG were used and prevented the protective effect of morphine on both SE signs and mortality rate.

Bashkatova V et al. reported that NO content in rat cerebral cortex had a fivefold elevation at the peak time of PTZ-induced seizures [51]. Another study displayed an elevation in protein nitrotyrosine by several times in rats exposed to 5 atm 100% O_2 for 2 to 13 min or until the occurrence of electroencephalographic (EEG) seizures [52]. Nitric oxide levels significantly increased in the control group, but the acute injection of morphine (15 and 30 mg/kg, i.p.) remarkably inhibited NO level increase.

5. Conclusion

To conclude, morphine treatment in lithium–pilocarpine-induced SE in rats not only decreased the convulsive properties and NO metabolite levels in the hippocampus but also increased the survival of animals. These effects may be modulated through inducible and neuronal NO pathway.

Declaration of competing interests

There is no conflict of interests between authors of this paper.

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