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Differential modulatory actions of $GABA_A$ agonists on susceptibility to $GABA_A$ antagonists-induced seizures in morphine dependent rats: Possible mechanisms in seizure propensity

Siyavash Joukar [a,b,c,*], Nafiseh Atapour [a], Tajpari Kalantaripour [d], Hamideh Bashiri [e], Alireza Shahidi [b]

[a] Neuroscience Research Center, Kerman University of Medical Sciences, Kerman, Iran
[b] Physiology Research Center, Kerman University of Medical Sciences, Kerman, Iran
[c] Department of Physiology and Pharmacology, Afzalipour Medical Faculty, Kerman University of Medical Sciences, Kerman, Iran
[d] Midwifery and Nursing Faculty, Islamic Azad University, Kerman Branch, Kerman, Iran
[e] Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

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ABSTRACT

In order to clarify the mechanisms involved in the susceptibility to $GABA_A$ antagonists-induced seizures in morphine dependent rats, we investigated how $GABA_A$ agonists modulate this vulnerability. Seizures were induced to animals by infusion of $GABA_A$ antagonists: pentylenetetrazole (PTZ), picrotoxin (PIC) and bicuculline (BIC). $GABA_A$ agonists, muscimol (MUS) and 4,5,6,7-tetrahydroisoxazolo [5,4-c]pyridin-3-ol (THIP), were administered intravenous (i.v.) before antagonists. Morphine-dependence significantly decreased the PTZ threshold dose (19.16 ± 1.89 versus 25.74 ± 1.25 mg/kg) while, it had no effect on PIC induced seizures. BIC doses for both threshold and tonic–clonic seizures induction were significantly lower in morphine dependent rats (0.10 ± 0.01 and 0.12 ± 0.02 versus 0.25 ± 0.02 and 0.39 ± 0.07 mg/kg respectively). In morphine-dependence, although pre-treatment with MUS significantly increased the required dose of PTZ for seizures threshold, THIP significantly decreased the required dose of PTZ for tonic–clonic convulsion. Moreover, MUS pretreatment completely recovered the effect of morphine dependency on BIC seizure activity.

The results suggest that the capability of $GABA_A$ agonists on modulation of propensity to seizures induced by different antagonists in morphine-dependence is dissimilar. Therefore, it seems that long-term morphine alters some properties of GABA system so that the responsive rate of $GABA_A$ receptors not only to its antagonists, but also to its agonists will change differently.

1. Introduction

Morphine can exert biphasic effects on seizure threshold with anticonvulsant effect at lower doses and pro-convulsant effect at higher doses (Homayoun et al., 2002; Honar et al., 2004; Shafaroodi et al., 2004). Previous studies reported that activation of multiple receptor systems including opioid (Saboory et al., 2007), glutamatergic (Schroeder et al., 1998) and adrenergic (Homayoun et al., 2002) receptors or inhibition of GABAergic neurotransmission (Werz and Macdonald, 1982) is involved in pro-convulsive effects of opioids.

In a study, morphine-dependence increased the severity of seizures induced by pentylenetetrazole (PTZ) primary injections in kindling models, decreased the threshold dose of PTZ-induced seizures, and increased the dose of N-methyl-D-aspartate for tonic–clonic seizures, but had no effect on caffeine or picrotoxin (PIC)-induced convulsion (Atapour et al., 2000).

GABAergic as the most important inhibitory system in the central nervous system (CNS) can be undermined due to the alteration in circuits containing GABAergic interneurons (Levitt, 2005), the loss of GABAergic interneurons and inadequate release of GABA (André et al., 2001; Löscher et al., 2006) and eventually the changes in GABA receptor expression (Sperk et al., 2004). γ-Aminobutyric acid (GABA), formed within GABAergic axon terminals, is released and acts via $GABA_A$ and $GABA_B$ receptors. Synaptic and extrasynaptic $GABA_A$ receptors (Belelli et al., 2009) are ligand-gated ion channel receptors, which show phasic and tonic inhibitory effects respectively by increasing inward chloride conductance. This receptor is a pentameric heterooligomer that contains binding sites for some ligands including GABA as an endogenous agonist, and neurosteroids and some

Abbreviations: PTZ, pentylenetetrazole; CON, control; DEP, morphine dependent; THIP, 4,5,6,7-tetrahydroisoxazolo [5,4-c]pyridin-3-ol; MUS, muscimol; BIC, bicuculline.

* Corresponding author at: Neuroscience Research Center, Physiology Research Center and Department of Physiology and Pharmacology, Afzalipour Medical Faculty, Kerman University of Medical Sciences, P.O. Box 7616914115, Kerman, Iran. Tel./fax: +98 341 3220081.
E-mail addresses: sjokar@gmail.com, jokar@kmu.ac.ir (S. Joukar).

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exogenous steroids, benzodiazepines and barbiturates as allosteric agonists. The convulsant agents, t-butylbicyclophosphorothionate (TBPS) and PIC as non-competitive blockers of $GABA_A$ receptor bind to sites located within or close to the chloride channel (Korpi et al., 2002). PTZ is the other $GABA_A$ antagonist that binds to the Cl-channel as PIC (Olsen, 1981).On the other hand, muscimol (MUS) as a selective agonist, THIP (Gaboxadol) as a partial agonist and bicuculline as a competitive antagonist of this receptor have been introduced (D'Hulst et al., 2009). Because of its diversity in subunits and assembly, hundreds of GABAA receptor subtypes can be found in the CNS (Sieghart and Sperk, 2002). The assembly and subunit compositions affect the receptor kinetics, affinity for ligands and its pharmacological profiles (Olsen and Sieghart, 2008; Winsky-Sommerer, 2009). Moreover, existence of different sites on $GABA_A$ receptor for different ligands is the other property of this receptor that affects the pharmacological responses profile of receptors. In addition, some alterations in subunit expression occur in response to the administration of different drugs, e.g. alcohol (Grobin et al., 1998), barbiturates (Ito et al., 1996), neurosteroids (Grobin and Morrow, 2000) and benzodiazepines (Liu and Glowa, 1999).

Although there is evidence that the pro-convulsive effect of morphine-dependence is partly linked to GABAergic system, the property of this association is not clear. Considering the previous study indicating morphine dependent animals are more susceptible to PTZ but no PIC-induced seizures as two $GABA_A$ antagonists (Atapour et al., 2000), we hypothesized that morphine-dependence may change some special properties of GABA system that lead to alteration in the pattern of receptor response to both GABA agonists and antagonists. To test this hypothesis, in the present study, we used three different $GABA_A$ antagonists for comparison of its ability to seizure-induction in morphine dependent rats. Moreover, we tested the effects of two $GABA_A$ agonists, MUS and THIP in order to elucidate whether morphine will also change the responsive rate of $GABA_A$ receptor to its agonists and its ability in seizure suppression.

2. Materials and methods

This study was conducted in accordance with the national guidelines for the care and use of laboratory animals (ethic committee permission No 86/123KA—Kerman University of Medical Sciences).

2.1. Animals

One hundred fourteen adult male Wistar rats weighting 200–250 g were used and divided into two groups randomly (control group = 57 and morphine dependent group = 57). Animals were housed at constant temperature (21 ± 2 °C) with 12/12 h light–dark cycle and free access to food and water except during the experiments.

2.2. Drugs

Sucrose from Merck and PTZ, BIC, PIC, THIP, MUS, morphine sulfate (M.S.) and naloxone hydrochloride were purchased from Sigma. All drugs were dissolved in saline except sucrose and M.S., which were dissolved in tap water. BIC was dissolved in HCl 10 normal and then immediately dissolved in saline and under titration with NaOH, pH of solution was stable in 5.6–5.8.

2.3. Chronic morphine administration

Morphine dependence was induced as described in the previous study (Atapour et al., 2000). Briefly, animals were made dependent with free access to morphine solution at concentrations of 0.1, 0.2 and 0.3 mg/ml each for 48 h and 0.4 mg/ml during the following 19 days in drinking water as its sole source of fluid. The mean amount of morphine consumption was about 42 mg/kg/day when animals received the solution with 0.4 mg/ml concentration of morphine. Solution bitter taste was masked by sucrose (3% w/v). Naloxone HCl (2 mg/kg, i.p.) was randomly administered to some of the rats (n = 12) that were treated chronically with morphine for 25 days and to the animals used as matched controls (n = 8). Then withdrawal syndrome behaviors as indicators of morphine dependence progress were observed. These behaviors include: teeth chattering, chewing, paw tremor, ptosis, writhing, wet-dog shakes, head shakes, diarrhea, ejaculation, erection, weight loss and irritability to touch and handling.

2.4. Convulsion tests

After restraining and keeping the animal in a clear Plexiglas cylinder, a 22 angiocut was inserted into the lateral vein of the tail and the insertion was verified by appearance of blood in the angiocut tube. The angiocut was firmly fixed and connected to syringe of drug by an appropriate tube. Then, the animal was released from restrainer to a Plexiglas cage to allow free movement. Every drug was infused at constant rate and rat was observed during the infusion. The onset of the first myoclonic jerk was recorded as seizure threshold (Guillet, 1995) and the onset of generalized tonic–clonic convulsions or clonic movements which exceeded 5 s was the end of the infusion (Lauretli et al., 1994). The durations of the convulsant infusion to see a seizure threshold and tonic–clonic convulsion were measured. The amount of convulsant agent required for induction of threshold or tonic–clonic convulsion was calculated by the following parameters: the concentration of convulsant in the injected liquid, duration of convulsant infusion, infusion rate, and the animal weight. Convulsants were infused at a rate of 1 ml/min and at the following concentrations: BIC, 0.02 mg/ml; PIC, 1 mg/ml; and PTZ, 2 mg/ml (Guillet, 1995). $GABA_A$ agonists-MUS, 5 mg/kg and THIP 50 mg/kg were individually injected i.v. ten minutes before convulsant infusion on protocol necessary (Waszczak et al., 1980).

2.5. Statistics

Data analysis was performed using unpaired *t*-test for picrotoxin, One-Way ANOVA followed by LSD (least significant difference) multiple comparison post hoc test for other data and P-value ≤ 0.05 was considered as statistically significant.

3. Results

3.1. Requirement doses of convulsant agents for threshold and tonic–clonic induction with or without morphine dependency

In morphine dependent group mean doses of PIC for threshold and tonic–clonic seizures were not significantly different compared to the control group (4.6 ± 0.3 versus 4.7 ± 0.25 mg/kg for threshold and 6.9 ± 0.2 versus 6.7 ± 0.3 mg/kg for tonic–clonic seizure). While, in PTZ convulsion model, morphine dependency significantly decreased the threshold dose ($P<0.05$) but had no significant effect on the tonic–clonic dose of PTZ in comparison with the control group (Fig. 1). In addition, mean doses of BIC for both threshold and tonic–clonic convulsions were significantly lower in dependent subjects compared to the control group ($P<0.05$), (Fig. 2).

3.2. Effects of $GABA_A$ agonist pre-treatment on convulsant doses for threshold and tonic–clonic seizures

Interestingly, the control group that was pre-treated with THIP required a lower dose of PTZ for induction of seizure threshold when compared to CON group alone ($P<0.01$) (Fig. 1). Morphine-dependence alone caused significant decline of threshold dose of PTZ. Threshold dose further reduced when dependent animals were pretreated with THIP but was not statistically significant. However, in DEP + THIP

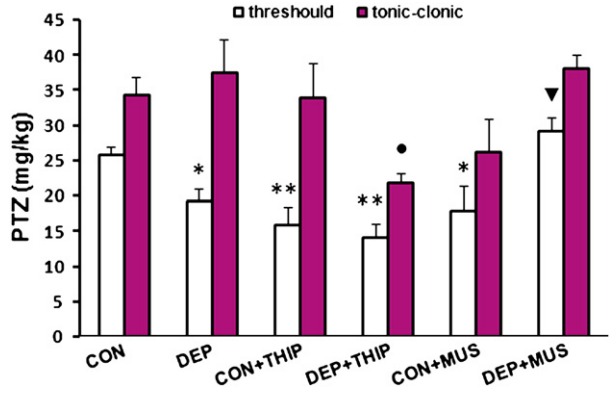

Fig. 1. Requirement doses of PTZ (pentylenetetrazole) for induction of threshold and tonic–clonic seizures in the presence and absence of $GABA_A$ agonists (THIP and MUS) on control and morphine dependent rats. Data are presented as mean ± SEM, n = 7–9. *P<0.05 and **P<0.01 compared with CON group. ▼p<0.01 compared with DEP, DEP + THIP. ● p<0.01 compared with CON + THIP, DEP and DEP + MUS groups. CON: control, DEP: morphine dependent, THIP: tetrahydroxyisoxazole pyridine, MUS: muscimol, n: number of animals.

group, the PTZ dose for tonic–colonic seizure induction significantly decreased as compared with DEP group. On the other hand, muscimol pre-treatment completely reversed the threshold dose to the control level without significant effect on the tonic–colonic dose of PTZ when compared to DEP group (Fig. 1).

Regarding the BIC, pre-treatment with THIP or muscimol, had no significant effects on threshold dose but only THIP caused significant increase in tonic–clonic dose when compared to CON group. On the other hand, THIP injection failed to change the BIC doses for threshold and tonic–clonic seizure induction in morphine dependent group. However, muscimol administration caused a significant increase in BIC doses of threshold and tonic–clonic of DEP + MUS group compared to DEP group alone ($p<0.01$) to a level that there was no significant difference with results of CON group (Fig. 2).

3.3. The latency times to occurrence of the threshold and tonic–clonic seizure

3.3.1. PTZ experiment

As shown in Table 1, morphine-dependence, THIP and MUS each alone significantly decreased the threshold latency time in PTZ

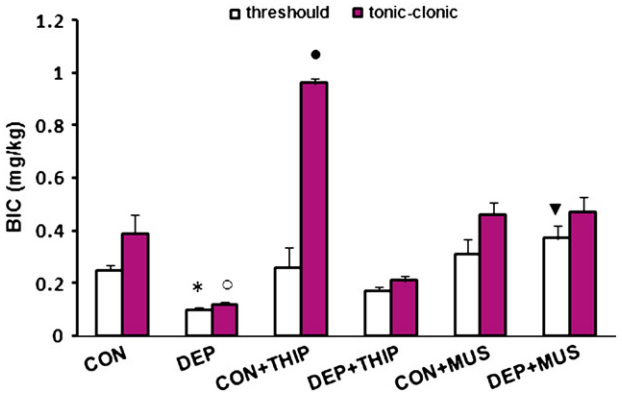

Fig. 2. Requirement doses of BIC (bicuculline) for induction of threshold and tonic–clonic seizures in with or without of $GABA_A$ agonists (THIP and MUS) on control and morphine dependent rats. Data are presented as mean ± SEM, n = 7–9. *Significant compared to all groups except DEP + THIP ($p<0.01$ compared with MUS groups and $P<0.05$ compared with other groups), ▼$p<0.01$ compared to DEP + THIP group, ○compared with CON group ($P<0.05$) and DEP + MUS ($p<0.01$), ●($p<0.01$) compared to all groups. CON: control, DEP: morphine dependent, THIP: tetrahydroxyisoxazole pyridine, MUS: muscimol, n: number of animals.

Table 1
Threshold and tonic–clonic latency time in second (Mean ± SEM).

	CON		DEP	
	Threshold	Tonic–clonic	Threshold	Tonic–clonic
PTZ (9)	183 ± 14	241 ± 18	126 ± 17*	236 ± 26
PTZ + THIP (9)	121 ± 20**	259 ± 39	101 ± 16	159 ± 9●♣
PTZ + MUS (7)	88 ± 17**	130 ± 22▼▼♣	159 ± 12 ♦	203 ± 11
BIC (9)	168 ± 17	227 ± 54	76 ± 7♬	97 ± 16□
BIC + THIP (9)	149 ± 46	557 ± 120□□	111 ± 15	141 ± 15■■
BIC + MUS (7)	162 ± 32	229 ± 27■■	218 ± 28○○◊	286 ± 40 ♪

*P<0.05 and **P<0.01 compared with threshold latency time of PTZ group. ▼▼P<0.01 compared with tonic–clonic latency time of PTZ group. ♦P<0.05 compared with threshold latency time of PTZ + THIP + DEP group. ●P<0.05 compared with tonic–clonic latency time of PTZ + DEP group. ♣P<0.01 compared with tonic–clonic latency time of PTZ + THIP group. ♬P<0.01 compared with threshold latency time of BIC group. ○○P<0.01 compared with threshold latency time of BIC + DEP group. ◊P<0.01 compared with threshold latency time of BIC + THIP + DEP group. □P<0.05 and □□P<0.01 compared with tonic–clonic latency time of BIC group. ♪P<0.05 compared with tonic–clonic latency time of BIC + DEP group.■■ P<0.01 compared with tonic–clonic latency time of BIC + THIP group. CON: control, DEP: morphine dependent, THIP: tetrahydroxyisoxazole pyridine, MUS: muscimol. Numbers in parentheses show number of animals in each group.

experiment. In dependent animals, pre-treatment with MUS increased threshold latency time to the extent that had no significant difference with CTL group. Although, MUS pre-treatment reduced the tonic–clonic latency time of CON group but did not show significant effect on this index in DEP group. Conversely, THIP pre-treatment decreased significantly tonic–clonic latency time in dependent animals.

3.3.2. BIC experiment

In BIC experiment, morphine-dependence significantly decreased the time needed to reach seizure threshold and tonic–clonic convulsion compared with CON group. However, pre-treatment with MUS completely recovered this effect. This latency time reduction of seizure occurrence by morphine-dependence also attenuated in some extent by THIP but not as much as of MUS effect (Table 1).

4. Discussion

In the present study, we investigated that how chronic consumption of morphine can affect the pattern of the $GABA_A$ receptors response to its agonists and antagonists. According to the obtained results, $GABA_A$ agonists modulated the susceptibility to $GABA_A$ receptor antagonists-induced seizures in morphine dependent rats differently.

In relation to PTZ-induced convulsion, morphine dependent rats showed significant reduction in seizure threshold latency and seizure threshold, while there was no such effect on tonic–clonic doses. In agreement with our results, others reported that morphine-dependence significantly decreases the threshold dose of PTZ (Atapour et al., 2000) and facilitates convulsive movements (Homayoun et al., 2002; Honar et al., 2004; Lauretli et al., 1994; Shafaroodi et al., 2004). Pre-treatment with muscimol reversed the effect of morphine and lead to significant increase in both latency time and threshold dose of PTZ up to the amounts of control values. However, pre-treatment with THIP not only had no beneficial effects but also further reduced the tonic–clonic dose of PTZ. In our study, there was no significant difference between PIC-induced seizures of control and morphine-dependent groups, which is consistent with the previous study (Atapour et al., 2000).

In BIC experiment, threshold, tonic–clonic doses and latency times in morphine dependent rats were significantly lower than control group. In line with our study, other researchers showed that morphine administration facilitates BIC-induced seizures (Aleman and Demuns, 1983; Lauretli et al., 1994; Foote and Gale, 1984; Foote and Gale, 1983). On the other hand, in BIC experiment, MUS pre-treatment completely

abolished the morphine-dependence effects on susceptibility to seizure but the effect of THIP was negligible.

The most important finding of this study is that muscimol is able to correct the facilitating effects of morphine on convulsion, while THIP does not have such ability. Moreover, morphine-dependent animals showed more susceptibility to BIC than PTZ or PIC-induced seizures.

Muscimol is a power selective $GABA_A$ receptor agonist that its effect is mediated through interaction with GABA site of receptor. In addition, BIC is a competitive antagonist that directly binds to GABA site (Barolet et al., 1985; Olsen, 1981). The competitive effect of these two substances to occupy the $GABA_A$ site can be the reason of BIC-induced seizures suppression by muscimol therapy. On the other hand, PIC and PTZ are non-competitive antagonists of $GABA_A$ receptor and bind to Cl-channel mostly via the t-butylbicyclophosphorothionate (TBPS) site of the $GABA_A$ receptor (Olsen, 1981 and Korpi et al., 2002). However, another study indicated that these agents interact on distinct domains of $GABA_A$ receptor (Huang et al., 2001) that along with other PTZ actions including change of potassium and calcium channels conductance (Madeja et al., 1994; Sugaya et al., 1989) could justify different responses of dependent animals to these agents from the point of seizure induction and seizure suppression.

THIP as a mixed agonist/antagonist of GABA receptor (Braestrup et al., 1979) did not show a significant attenuating effect on morphine pro-convulsive action, and even in some extent increased the severity of PTZ-induced tonic–clonic seizures in DEP + THIP group. It may be due to the overcoming of antagonist and the convulsive effect against agonist and the anticonvulsive effect of this substance that is reported in some studies (Fisher, 1989). Mentioned evidence, raises two possibilities that morphine-dependence involved mechanisms leading to firstly, reduction in the release of GABA hence increase of susceptibility to seizures, and secondly, reduction of $GABA_A$ receptor efficacy and response to neurotransmitter. Previous studies reporting morphine inhibitory effect on the release of GABA through suppression of $GABA_A$ergic synaptic transmission (Vaughan et al., 1997) support the first possibility. Morphine-dependence ineffectiveness on PIC-induced seizures, the discrepancy in response to other $GABA_A$ antagonists and different modulatory effects of $GABA_A$ agonists are the reasons in favor of the second possibility. Genomic studies documented that animals with long-time self-administer morphine show significant change of 29 transcripts abundance in amygdala. Additionally, acute as well as chronic intraperitoneal morphine administration has changed the abundance of PKC γ, γ1 subunit of $GABA_A$ and hsp70 (heat shock protein 70) genes (Rodriguez Parkitna et al., 2004). Moreover, previous studies also reported GABA receptor contribution in other morphine effects such as the development of morphine tolerance (Rahman et al., 1995).

Therefore, it is predictable that morphine consumption similar to other agents e.g. alcohol (Grobin et al., 1998), barbiturates (Ito et al., 1996), neurosteroids (Grobin and Morrow, 2000) and benzodiazepines (Liu and Glowa, 1999), may change the expression of $GABA_A$ receptor subunits, affects the receptor performance and increases susceptibility to seizures.

Overall, the results of this study showed that: a) morphine-dependence changes both the seizures propensity to $GABA_A$ antagonists and the ability of $GABA_A$ agonists on modulation of this susceptibility.

b) Morphine dependency has a more dominant positive effect on BIC than PTZ-induced seizures activity and this effect is suppressed by MUS not THIP. In addition, long-term morphine administration has no effect on propensity to PIC-induced convulsion. Since BIC is a competitive antagonist of $GABA_A$ receptor and MUS is a powerful agonist of $GABA_A$ receptor, it is suggested that pro-convulsive effects of chronic morphine exposure are partly mediated through changes in receptor efficacy and hence the reduction in response to endogenous ligand, which ultimately leads to increased susceptibility to convulsion. Moreover, the contribution of non-dependent GABA receptor pathways or non-GABAergic system is possible. However, further studies are needed to elucidate the pathways in which morphine-dependence attenuates the GABA inhibitory action and to promote epileptiform activity. Our findings may provide a new attitude in relation to the application of anticonvulsive drugs to deal with pro-convulsive effects of opioids.

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