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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

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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.

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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