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Original Article

Involvement of GABAergic System in Increased Pentylenetetrazole-Induced Seizure Threshold in Cholestatic Mice

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Abstract

Gamma-aminobutyric acid (GABA) is an important inhibitory transmitter in central nervous system and is involved in pathophysiology of epilepsy. Pentylenetetrazole (PTZ), a convulsant agent, partly acts via anion channel of $GABA_A$ receptor. Ivermectin, an antiparasitic agent and a $GABA_A$ agonist, has anticonvulsant effect in animal seizure models. Cholestasis increases the threshold of PTZ-induced clonic seizure in mice. The object of this study was to clarify the involvement of GABAergic pathways in increasing PTZ-induced seizure threshold (ST) in cholestatic mice. The result of this study showed that cholestasis increases clonic ST three days after surgery while sham-operated control (SOC) and unoperated control (UOC) groups did not show any alteration in clonic ST. Bicuculline, a $GABA_A$ antagonist, reversed but ivermectin increased the clonic ST in UOC, SOC and bile duct-ligated mice, respectively. In conclusion, our results showed that: (1) acute cholestasis is associated with an increase in PTZ-induced clonic ST in mice and this phenomenon may be the result of reinforcement of GABAergic system, and (2) GABA plays a physiological role in regulation of ST.

Keywords: Bicuculline; Cholestasis; GABAergic system; Ivermectin; PTZ-induced seizure threshold.
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1. Introduction

Gamma-aminobutyric acid (GABA) is the major neurotransmitter mediating fast inhibitory neurotransmission in the mammalian central nervous system (CNS). Activation of the $GABA_A$ receptor leads to opening of chloride channel, with increased chloride conductance resulting in postsynaptic hyperpolarization. GABA serves to regulate the excitability of virtually all neurons in the brain and has been implicated in pathophysiological events that underlie brain dysfunction. Several experimental models of epilepsy or increased seizure susceptibility

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have been shown to be associated with variation of $GABA_A$ receptor numbers or function [1]. GABA maintained inhibitory tone that counterbalances neurons excitation. When this balance is disturbed, seizures ensue. The $GABA_A$ receptor has been targeted for the pharmacological control of anxiety, sleep and epilepsy. Various drugs such as barbiturates, benzodiazepines and picrotoxin, interact with $GABA_A$ receptor at distinct sites [2]. $GABA_A$ receptor agonists, as well as drugs which allosterically modulate the $GABA_A$ receptor channel complex, are therapeutically active against convulsive seizures [3]. Drugs which enhance the GABAergic activity, such as muscimol ($GABA_A$ agonist) and diazepam (GABA modulator), are thought to act via GABA receptor and hyperpolarization of GABA-sensitive neurons. Bicuculline, a $GABA_A$ antagonist, blocks the Cl^- permeability and creates convulsion. This convulsant compound has no therapeutic use but is a useful experimental tool for studying GABA-mediated transmission.

Pentylenetetrazole (PTZ) is a convulsant frequently used in the study of seizures. Squires and coworkers [4] have shown that its convulsant effect is at least partly mediated by interactions with the anion channel of the $GABA_A$ receptor.

Ivermectin, a semisynthetic derivative of avermectin family, is used as an antiparasitic drug. Avermectins exert their antiparasitic activity through activation of a glutamate-gated chloride channel present in the invertebrate nervous system [5] and have additional effects on invertebrate GABA-receptors [6]. In vertebrates where no glutamate-gated chloride channel has been reported, avermectins also have effects [7]. A number of studies have shown that ivermectin has anticonvulsant effect in animal seizure models [8-10]. Dawson and coworkers [7] assessed 25 avermectin analogs in mouse seizure model and have concluded that avermectins have anticonvulsant effect in mice treated with PTZ. They suggested that the anticonvulsant effect of avermectins possibly is mediated via the $GABA_A$ receptor, because the efficacy of avermectins at $GABA_A$ receptor is correlated most closely with their anticonvulsant potency. However, it is also likely that the lethal actions of avermectins in mice after doses that have anticonvulsant effect are also mediated through the $GABA_A$ receptor, therefore, the therapeutic potential of avermectins as anticonvulsant agents is limited.

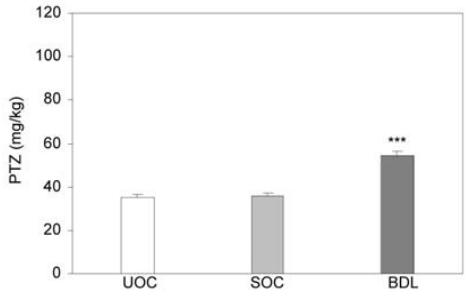

Figure 1: Comparison of clonic seizure threshold in bile duct ligated (BDL), sham operated (SOC) and unoperated (UOC) mice three days after surgery. ***Significantly different from the UOC and SOC groups (p<0.001).

It has been reported that in cholestatic mice, the threshold of PTZ-induced clonic seizures increases in a time-dependent manner, reaches a peak on the third day after bile duct-ligation (BDL) and declines partially after the fourth day, while in sham-operated control (SOC) and unoperated control (UOC) the seizure threshold (ST) did not show any alteration in the clonic ST [11]. The role of opioids in hyporeactivity to PTZ-induced seizure may be related to inhibition of GABA reuptake and release of glutamate [12]. Homayoun and coworkers [11] have shown that opioids and nitric oxide (NO) are both involved in hyporeactivity to PTZ-induced seizures in BDL animals and opioid antagonists and/or NOS-inhibitors were able to restore the ST to the normal level, but did not cause any change in clonic ST in UOC and SOC animals, denoting that endogenous opioids has no physiological role in regulation

of ST. Different neurotransmitters and neuromodulators are known to play significant roles in the system of excitation and various neurotransmitter abnormalities, especially those of GABA and glutamate which have been reported to play a key role in the pathophysiology of epilepsy. With this background, we carried out experiments to elucidate the involvement of GABAergic system in the rise of clonic ST in cholestatic mice and its physiological role in regulation of ST.

2. Material and methods

2.1. Materials

PTZ, bicuculline methochloride and ivermectin were purchased from Sigma Chemical Co. (Poole, UK). Ketamine HCl was obtained from Alfasan (Woerden, the Netherlands) and chlorpromazine HCl from Daroupakhsh (Tehran, Iran). PTZ and bicuculline methochloride were dissolved in normal saline. Ivermectin was suspended in 10% dimethyl sulfoxide. All vehicle and drugs were injected intraperitoneally in a constant volume (10 ml/kg), before intravenous infusion of PTZ.

2.2. Animals and grouping

Male NMRI mice (25-30 g) were purchased from Razi Institute of Iran (Tehran, Iran). The animals were housed under standard laboratory conditions, maintained under a natural light and dark cycle and had free access to food and water. The experiments were performed in accordance with the recommendations and guidelines of the ethics committee for use and care of experimental animals of the Medical School, Tehran University of Medical Science. Each animal was used only once and each group consisted of at least ten animals. In each experiment, animals were randomly divided into three groups: UOC, SOC and BDL.

2.3. Surgery

Mice were laparotomized under general anesthesia (ketamine HCl, 50 mg/kg and chlorpromazine, 10 mg/kg) [13, 14]. In the SOC group, the bile duct was identified, manipulated with forceps and left *in situ*, but in the BDL group, the bile duct was doubly ligated. Then the abdominal wall was closed in two layers. The experiments took place between 9 a.m. and 15 p.m., 3 day after surgery. Ivermectin was given 90 min. and bicuculline 15 min. before assessment of ST, according to the result of our time-dependency studies.

2.4. Determination of threshold of PTZ-induced clonic seizure

The threshold of PTZ-induced clonic seizures was determined by inserting a 30-gauge butterfly needle into the tail vein of mice and infusion of 0.5% PTZ solution at a constant rate of 0.5 ml/min. to unrestrained freely moving animals [15-17]. Minimal dose of PTZ (mg/kg) needed to induce forelimb clonus followed by full clonus of the body was recorded as an index of clonic ST.

2.5. Statistical analysis

Data are expressed as mean±SEM. ANOVA followed by Tukey's post-hoc test was used to compare the means of changes in PTZ-induced seizure threshold. Differences with $p<0.05$ was considered significant between groups.

3. Results

Two days after BDL, the animals revealed obvious signs of cholestasis (jaundice, dark urine and steatorrhea) and these signs persisted thereafter. The mortality rate was less than 20%. Vehicles did not show significant alteration on PTZ-induced seizure threshold.

3.1. Increase in clonic seizure threshold in cholestatic mice

In the BDL group, a significant rise in clonic ST was observed three days after operation ($p<0.001$), while the SOC and UOC groups did not show any alteration in clonic

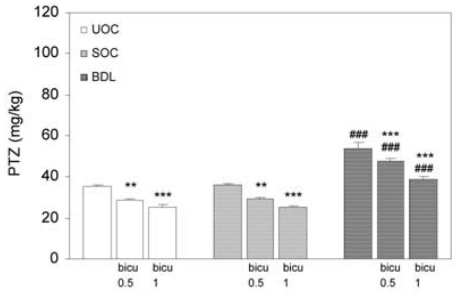

Figure 2: The effect of acute administration of different doses of bicuculline (0.5 and 1 mg/kg) on clonic seizure threshold in bile duct ligated (BDL), sham operated (SOC) and unoperated (UOC) groups 0.01; ***p<0.001, Significantly different from the control group. ###p<0.001, Significantly different from the UOC and SOC groups.

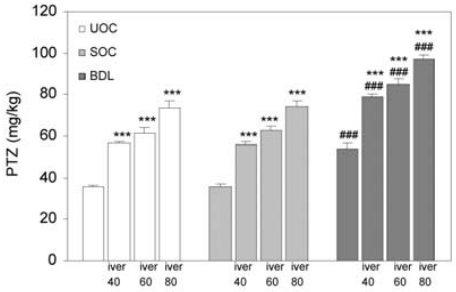

Figure 3: The effect of acute administration of different doses of ivermectin (40, 60 and 80 mg/kg) on clonic seizure threshold in bile duct ligated (BDL), sham operated (SOC) and unoperated (UOC) groups. ***p<0.001, Significantly different from the control group. ###p<0.001, Significantly different from the UOC and SOC groups.

ST (Figure 1).

3.2. Reversal of cholestasis-induced increase in clonic seizure threshold by bicuculline pretreatment

Acute pretreatment of animals with bicuculline (0.5 or 1 mg/kg) decreased the clonic ST dose-dependently in the UOC, SOC and BDL groups on the third day after surgery (p<0.01) (Figure 2).

3.3. Increase in clonic seizure threshold in UOC, SOC and BDL groups by ivermectin

Acute pretreatment of animals with ivermectin (40, 60 or 80 mg/kg) elevated the clonic ST dose-dependently in the UOC, SOC and BDL groups on the third day after surgery (p<0.001) (Figure 3).

3.4. Antagonistic effect of bicuculline on clonic seizure threshold in UOC, SOC and BDL groups pretreated with ivermectin

Bicuculline (1 mg/kg) decreased clonic ST in the UOC, SOC and BDL groups pretreated with different doses of ivermectin (40, 60 or 80 mg/kg) (p<0.001) (Figure 4).

4. Discussion

Squires *et al*. [4] have suggested that the convulsant effect of PTZ is at least partly mediated by interactions with the anion channel of $GABA_A$ receptor. The results of

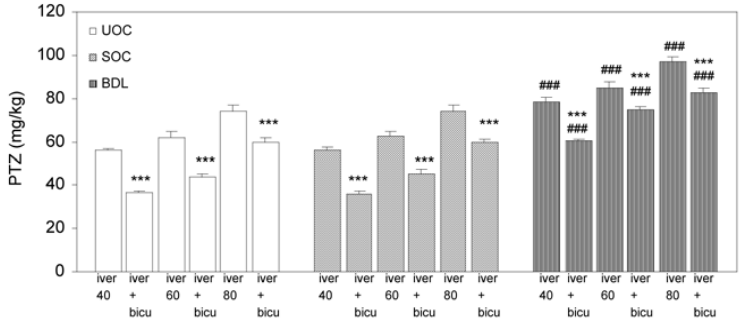

Figure 4: The effect of acute administration of bicuculline (1 mg/kg) on clonic ST in UOC, SOC and BDL animals in pretreated with ivermectin (40, 60 and 80 mg/kg). ***p<0.001 compared with ivermectin only. ###p<0.001 compared with UOC and SOC groups.

this study demonstrated that in cholestatic mice, the threshold of PTZ-induced clonic seizures increases significantly, while in SOC and UOC animals the threshold did not show any alteration in the clonic ST. Hyporeactivity to PTZ-induced seizure in BDL animals may be related to alteration in activity of different neurotransmitters and neuromodulators.

Our results demonstrated that bicuculline, a $GABA_A$ antagonist, significantly decreased the rise in clonic ST in BDL animals, implying that GABAergic tone in cholestasis has serious effects on the CNS, and decreasing the PTZ-induced threshold by bicuculline indicates that GABAergic system is involved in increasing PTZ-induced ST in cholestatic mice.

Dawson *et al*. [7] have suggested that both anticonvulsant activity and toxicity of ivermectin is correlated with its activity at GABA receptors. Our results show that ivermectin significantly increases clonic ST in BDL, SOC and UOC animals and bicuculline decreases the rise in ST induced by ivermectin and ivermectin plus BDL.

Although blockade of opioid receptor with naltrexone failed to reduce clonic ST in UOC and SOC animals [11], bicuculline reduced the PTZ-induced clonic ST in SOC and UOC in addition to BDL. This may denote that the endogenous opioid do not play a physiologically significant role in regulation of ST contrary to GABA.

In conclusion, the present study indicated that: (1) acute cholestasis is associated with a significant increase in the PTZ- induced clonic ST in mice and this phenomenon may be the result of overactivity or reinforcement of GABAergic pathways, and (2) GABA plays a physiological role in regulation of seizure threshold.

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