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The influence of ovariectomy on anti-convulsant effect of pioglitazone in mice

Leila Moezi[a,b,c], Mahsa Hassanipour[d], Meysam Zaeri[a,c], Hassan Ghorbani[a,c], Hamed Shafaroodi[e,*]

[a] Department of Pharmacology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[b] Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[c] Nanomedicine and Nanobiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[d] Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[e] Department of Pharmacology and Toxicology, Pharmaceutical Sciences Branch and Pharmaceutical Sciences Research Center, Islamic Azad University, Tehran, Iran

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ABSTRACT

The anti-convulsant effects of pioglitazone in male animals have been reported in previous studies. Both clinical and animal studies demonstrated that ovarian hormones can influence seizure activity. Pioglitazone has direct effects on ovaries and changes the level of gonadal hormones. In the current study, we examined the influence of ovariectomy on seizure threshold in pioglitazone-treated female mice. Two models of intravenous and intraperitoneal pentylenetetrazole-induced clonic seizures were used to analyze the effect of pioglitazone in sham and ovariectomized female mice. Different doses of pioglitazone were administered orally for 10 days in different groups. We demonstrated that chronic administration of pioglitazone (10 and 20 mg/kg) increased clonic seizure threshold in intravenous pentylenetetrazole seizure model of female mice. We also indicated that chronic treatment with pioglitazone (10 and 20 mg/kg) increased clonic seizure latency in intraperitoneal pentylenetetrazole seizure model in female mice, while the incidence of tonic seizure and death remained unchanged. Ovariectomy abolished anti-seizure effect of pioglitazone in both seizure models of intravenous and intraperitoneal pentylenetetrazole. In conclusion, pioglitazone exerts anti-convulsant effect in both seizure models of intravenous and intraperitoneal pentylenetetrazole possibly through gonadal hormones of ovary in female mice.

1. Introduction

Epilepsy is one of the most common neurologic problems in the world. Epilepsy is identified by recurrent and unprovoked seizures and sometimes is accompanied with some neurobiological, psychological and social consequences. On the contrary, the signs and symptoms of seizures transiently occur and represent abnormal excessive or synchronous neuronal activity in the brain [1].

Both clinical and animal studies suggest that ovarian hormones can influence seizure activity [2,3]. Clinical evidence indicating that seizure frequency changes over the course of the menstrual cycle has been available for more than a century [4]. Estrogens affect neurotransmitter release, neurotransmitter receptors and neuronal excitability and thus may play an important role in seizure disorders [5]. The effects of estrogen on epilepsy and seizures are controversial. The results of some clinical and animal studies suggest that estrogens may have pro-convulsant effects [6,7]. It has been recently shown that estrogens may also produce anti-convulsant effects [8]. These opposite effects of estrogens may be due to the duration of treatment, the latency prior to seizure testing, mode of administration, the applied dose of estrogen and hormonal status, the region of nervous system or neurotransmitter system involved, seizure-inducing models and the sex [5]. On the other hand, a decrease in seizure frequency during the mid-luteal phase has been attributed to the high levels of progesterone at this time [9] and intravenous infusions of luteal phase levels of progesterone in women with epilepsy suppress epileptiform spikes in some patients [10]. However, there are also reports inconsistent

* Corresponding author. Fax: +98 21 22600099.
E-mail address: Shafaroodih@yahoo.com (H. Shafaroodi).

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with this postulate. In humans, one study showed exacerbation of the absence seizure rate by progesterone [11], and a second study determined that only high, anesthetic doses of progesterone have anti-convulsant effects in amygdala kindled rats [12].

Pioglitazone, a thiazolidinedione derivative drug, is a useful glucose-lowering agent for patients with type 2 diabetes. Through peroxisome proliferator-activated receptors (PPAR)-γ-mediated effects, these drugs not only improve insulin sensitivity and glycemia, but also have beneficial effects on lipid metabolism [13]. PPAR-γ is showed to be highly expressed in various brain regions such as striatum, substantianigra, cortex and hippocampus [14,15]. Thiazolidinediones have been shown to be beneficial in several cellular and animal models of central nervous system diseases. Their efficacy in improving insulin sensitivity correlates with the reduction of diabetes-induced acute brain damage, since chronic hyperglycemia is a major risk factor for neuropathy and vasculopathy [16]. Antiepileptic efficacy of pioglitazone was reported for the first time by Okada et al. [17] in genetically epileptic EL male mice. After that, the anti-convulsant effect of acute and chronic pioglitazone treatment has been revealed in several pentylenetetrazole models in male mice [18–20].

Recent studies suggested that, in addition to reducing circulating androgen levels [21,22], thiazolidinediones can normalize (increase) circulating progesterone levels, which are commonly reduced in patients with polycystic ovary syndrome (PCOS) and in a primate model of this syndrome [23]. Seto-Young et al. showed that rosiglitazone or pioglitazone stimulated progesterone production in the human ovary. They also reported that pioglitazone inhibited baseline and follicle stimulating hormone (FSH)-stimulated estradiol production [23]. Aromatase is an enzyme of cytochrome P450 super-family, which converts androgens to estrogens. Published reports of the thiazolidinediones effects on aromatase activity in the ovary are controversial. Gasic et al. reported absence of troglitazone effects on aromatase in porcine granulosa cells [24], while others reported troglitazone-induced suppression of aromatase expression and activity in human granulosa cells and in granulosa carcinoma cell lines [25,26]. It has been suggested that rosiglitazone or pioglitazone directly inhibit estrogen synthesis in human ovarian cell culture containing stromal, thecal, and granulosa cells [23].

In the current study we examined if chronic pioglitazone treatment could exert anti-convulsant effect in female mice using two models of intravenous (IV) and intraperitoneal (IP) pentylenetetrazole. We also investigated the role of ovarian hormones in anti-convulsant effect of pioglitazone in ovariectomized female mice.

2. Materials and methods

2.1. Chemicals

The following drugs were used throughout the study: pioglitazone, ketamine hydrochloride, xylazine hydrochloride pentylenetetrazole (PTZ) and carboxymehyl cellulose. Pioglitazone and PTZ were purchased from Sigma (USA). Ketamine hydrochloride was obtained from Rotexmedica (Trittau, Germany) and xylazine hydrochloride purchased from Alfasan (Holland). Pioglitazone suspension was prepared in 0.5% sodium carboxy methyl cellulose and was administered orally by gavage once in a day for 10 days. Ketamine hydrochloride and xylazine hydrochloride administered IP in a volume of 10 ml/kg of the mice body weight. To assess clonic seizure experiments, PTZ was administered intravenously (0.5%, IV) while to assess generalized tonic–clonic seizures it was administered IP (85 mg/kg). Seizure experiments were done by a researcher who was blind to the administered drugs.

2.2. Animals

Adult female Swiss mice aged 6–8 weeks and weighing 20–25 g were used in the study. Each mouse was used only once, and each treatment group consisted of six to seven animals in IV method and at least 10 animals in IP method. Animals were housed under standard laboratory conditions that included controlled ambient temperature ($22 \pm 1\,^{\circ}C$), a 12-h-dark/12-h-light cycle, and free access to food and water. All experiments were performed between 10:00 and 14:00 h. The study was conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85–23, revised 1985). The animal protocol was reviewed and approved by animal care and use committee of Shiraz University of Medical Sciences.

2.3. Ovariectomy

After induction of general anesthesia with ketamine (50 mg/kg, IP) and xylazineHCl (10 mg/kg, IP), the lumbar dorsum was shaved and surgically prepared by scrubbing with 10% povidone–iodine and then wiping with sterile saline. The skin was opened through a small posterior midline incision, and the ovaries with oviducts were exposed and removed bilaterally. After hemostasis was achieved, the incision was sutured (5–0 silk) and the animal returned to its home cage. The mortality rate was less than 5%. Pioglitazone administration started one day after ovariectomy and lasted 10 days.

2.4. Determination of seizure susceptibility

The primary objective of this study was to examine the role of pioglitazone in modulation of susceptibility to myoclonic seizures induced by PTZ that is a standard experimental model of clinical myoclonic petit mal seizures with both face and construct validity [27,28]. PTZ acts as a GABA receptor antagonist to induce seizure [27]. It has been also shown that PTZ induced single and repeated seizures result in increased oxidative damage and lipid peroxidation, and decreased antioxidant defense mechanisms [29]. Modulation of extracellular adenosine levels is another mechanism of PTZ administration [30].

To assess the seizure susceptibility, we used the more sensitive method of IV administration of PTZ that allows better detection of modulatory effects on convulsive tendency [27]. The threshold of PTZ was determined by inserting a 30-gauge butterfly needle into the tail vein of mice and infusion of PTZ (0.5%) at a constant rate of 0.5 ml/min to unrestrained freely moving animals. Infusion was halted when forelimb clonus followed by full clonus of the body was observed and the dose of PTZ administered (mg/kg of mice weight) was measured as an index of clonic seizure threshold. As such, seizure threshold is dependent on PTZ dose administered and time-related [31].

To further characterize the effects of pioglitazone on seizure latency in another seizure model, we examined the effect of different treatment groups on generalized tonic–clonic seizures induced by IP injection of high dose PTZ. This method is an experimental model for grand-mal seizures [27,32]. The method used was as previously reported [33,34]. In brief, acute IP administration of PTZ (85 mg/kg, CD97 for generalized tonic–clonic seizures in the current experiment) was used to evaluate the incidence and latency of generalized tonic–clonic seizures and the incidence of death. Time of observation following PTZ was limited to 30 min and a latency of 1800 s was recorded for experiments in which no generalized seizure occurred.