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Involvement of ATP-sensitive potassium channels and the opioid system in the anticonvulsive effect of zolpidem in mice

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ABSTRACT

Zolpidem is a hypnotic medication that mainly exerts its function through activating γ-aminobutyric acid $(GABA)_A$ receptors. There is some evidence that zolpidem may have anticonvulsive effects. However, the mechanisms underlying this effect have not been elucidated yet. In the present study, we used the pentylentetrazole (PTZ)-induced generalized seizure model in mice to investigate whether zolpidem can affect seizure threshold. We also further evaluated the roles of ATP-sensitive potassium (K_{ATP}) channels as well as μ-opioid receptors in the effects of zolpidem on seizure threshold. Our data showed that zolpidem in a dose-dependent manner increased the PTZ-induced seizure threshold. The noneffective (i.e., did not significantly alter the PTZ-induced seizure threshold by itself) doses of K_{ATP} channel blocker (glibenclamide) and nonselective opioid receptor antagonist (naloxone) were able to inhibit the anticonvulsive effect of zolpidem. Additionally, noneffective doses of either K_{ATP} channel opener (cromakalim) or nonselective μ-opioid receptor agonist (morphine) in combination with a noneffective dose of zolpidem exerted a significant anticonvulsive effect on PTZ-induced seizures in mice. A combination of noneffective doses of naloxone and glibenclamide, which separately did not affect zolpidem effect on seizure threshold, inhibited the anticonvulsive effects of zolpidem. These results suggest a role for K_{ATP} channels and the opioid system, alone or in combination, in the anticonvulsive effects of zolpidem.

1. Introduction

Zolpidem is an effective nonbenzodiazepine sedative hypnotic medication that activates benzodiazepine binding sites on γ-aminobutyric acid $(GABA)_A$ receptors [1,2]. The selective affinity of zolpidem for α_1-containing $GABA_A$ receptors causes different effects including sedation, muscle relaxation, and anxiolytic effects, which represents different pharmacological profiles from the classic benzodiazepines. Additionally, there is some evidence that zolpidem can act as a potent anticonvulsant in animal studies [3]. Zolpidem has approximately 10-fold lower affinity for the α_2 and α_3 subunit-containing $GABA_A$ receptors than benzodiazepines and with no appreciable affinity for α_5 subunit-containing receptors [4,5]. There is a consensus among researchers that, except for anxiolytic effects of zolpidem, other effects of zolpidem such as sedation, amnesia, and potential anticonvulsant properties are mainly due to its effect on α_1-containing $GABA_A$ receptors [1,2,6–10]; however, the exact underlying mechanism of action of zolpidem in increasing seizure threshold has not been completely understood.

It is well established that central opioidergic neurotransmission plays a crucial role in modulating seizure threshold [11–15]. Opioid receptor agonists depending on the doses used exert both anticonvulsive and proconvulsive effects in different models of experimental seizures [13–17]. Low doses of the nonselective μ-opioid receptor agonist morphine have an anticonvulsive effect, while higher doses increase the seizure susceptibility induced by GABA-transmission blockers (i.e., picrotoxin, bicuculline, pentylentetrazole [PTZ]) and in isoniazid models of seizures [16,18]. It has been shown that μ-opioid receptors are responsible for both anticonvulsive and proconvulsive effects of morphine on chemical and electrical models of seizures and, accordingly, naloxone, as a nonselective opioid receptor antagonist, reverses these effects [16]. Although several investigations have shown that GABAergic neurotransmission could participate in seizure modulation in association with the central opioidergic system [19,20], whether the possible anticonvulsive effects of zolpidem, as a $GABA_A$ receptor agonist, could be modulated by the opioidergic system has not yet been assessed in the recent studies.

The ATP-sensitive potassium (K_{ATP}) channels are a group of potassium channels that are sensitive to alterations in the intracellular concentration of the ATP and the ATP/ADP ratio, linking the electrical activity of

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the cell to its metabolic state [21,22]. They are expressed in many excitable cells such as cardiac myocytes, pancreatic β cells, vascular smooth muscles, skeletal muscles, and neurons [23–27]. These channels are also expressed pre- and postsynaptically in some brain regions such as hippocampus [28,29]. Increasing lines of evidence have demonstrated that K_{ATP} channels play an important role in control of seizure threshold in in vivo and in vitro models [15,29–35]. Activation of K_{ATP} channels prolongs seizure onset through inhibition of excitatory neurotransmitters (e.g., glutamate) [36]. It has been shown that the lack and overexpression of K_{ATP} channels are respectively responsible for reducing the threshold of generalized seizure and increasing the threshold for kainite-induced seizures [32,36]. Additionally, several lines of evidence have suggested the involvement of K_{ATP} channels in the central and/or peripheral actions of nonselective μ-opioid receptor agonists (e.g., morphine). In the central nervous system (CNS), these actions include the following: antinociceptive effect, tolerance, withdrawal, hyperthermia, noradrenaline turnover-enhancing effect, morphine state-dependent memory of passive avoidance, and bicuculline (a competitive $GABA_A$ receptor antagonist)-induced convulsions [34,37–42]. Thus, K_{ATP} channel modulators play a major role in the effects of morphine on neurotransmitter release in the CNS [43] and may be involved in their effects on modulation of the central GABAergic transmission. To the best of our knowledge, there is no published evidence regarding the possible involvement of K_{ATP} channels, directly or indirectly through involving other systems such as opioid systems, in the central effects of zolpidem, as a $GABA_A$ receptor agonist, such as its possible anticonvulsive effects.

Therefore, in the present study, we first evaluated the effects of zolpidem on the seizure threshold in a mouse model of clonic seizures induced by PTZ, and then we evaluated whether the μ-opioidergic receptors as well as K_{ATP} channels could potentially be involved in the anticonvulsive effects of zolpidem on the PTZ-induced seizures in mice. Of note, PTZ is a noncompetitive $GABA_A$ receptor antagonist [44]. The onset and intensity of PTZ-induced seizure can be modified by drugs having anticonvulsive and/or proconvulsive effects [44,45]. There is an intravenous (i.v.) PTZ seizure threshold model which is used as a laboratory evaluation for anticonvulsive drugs [46], and we used this model in our present study.

2. Materials and methods

2.1. Chemicals

Drugs used were as follows: cromakalim, glibenclamide, pentylenetetrazole (Sigma, Bristol, UK), morphine (Sigma, Bristol, UK), and naloxone (Sigma, Bristol, UK). Glibenclamide was dissolved in 1% of DMSO. Cromakalim and morphine were dissolved in saline. All injections were administered at a volume of 5 ml/kg. Appropriate vehicle controls were performed for each experiment. Morphine, naloxone, cromakalim, and glibenclamide were administered intraperitoneally (i.p.). To assess clonic seizure experiments, PTZ was administered intravenously at a constant rate of 1 ml/min to unrestrained animals. The doses were chosen based on previously published studies [1,15,31,47–49] and pilot experiments.

2.2. Experimental animals

Male NMRI mice weighing 20–25 g (Pasteur Institute) were used throughout this study. Animals were housed in groups of 4–5 and were allowed free access to food and water except for the short time that animals were removed from their cages for testing. All behavioral experiments were conducted during the period between 10:00 a.m. and 13:00 p.m. with normal room light (12-h regular light/dark cycle) and temperature (22 ± 1 °C). All procedures were carried out in accordance with the institutional guidelines for animal care and use. Each group consisted of 8–10 animals.

2.3. Determination of clonic seizure threshold

Pentylentetrazole-induced clonic seizure threshold was determined by inserting a 30-gauge butterfly needle into the tail vein of mice which was fixed by adhesive tape and the infusion of PTZ (0.5%) at a constant rate of 1 ml/min to animals using a 40-cm flexible tube as a connector between infusion pump syringe and butterfly needle which provides an unrestrained freely moving condition. Infusion was halted when forelimb clonus followed by full clonus of the body was observed. A minimal dose of PTZ (mg/kg of mice weight) needed to induce clonic seizure was considered as an index of seizure threshold. As such, seizure threshold is dependent on PTZ dose administered and time-related [1,15,31,47–49].

2.4. Experimental protocol

We investigated the effect of three different doses of zolpidem (1, 3, and 10 mg/kg) [50,51] on PTZ-induced seizures compared with the seizure threshold of the control group, which had been given normal saline. Also in separate groups of animals, zolpidem at a dose of 10 mg/kg was injected 5, 15, and 30 min before the PTZ infusion to acquire the best time of action for our subsequent experiments.

Next, for the determination of probable contribution of K_{ATP} channels in the anticonvulsive activity of zolpidem, we administered different doses of the K_{ATP} channel blocker glibenclamide (0.3, 0.1, and 1 mg/kg) [15,49] 30 min prior injection of zolpidem (10 mg/kg) and 60 min prior to PTZ-induced seizure threshold measurement.

In an additional set of experiments, we further assessed the interaction between zolpidem and K_{ATP} channels in seizure threshold alteration in mice. We administered the noneffective dose of the K_{ATP} channel opener cromakalim (10 μg/kg) [52] 15 min before the administration of the noneffective dose of zolpidem (1 mg/kg, i.p.). The seizure threshold was then assessed 30 min after zolpidem injection.

In order to assess the interaction between the opioid system and zolpidem in provoking the anticonvulsive effects, noneffective doses of the opioid receptor antagonist naloxone (0.03, 0.1, and 1 mg/kg, i.p.) [53] were injected 15 min prior to zolpidem (10 mg/kg) and 45 min before PTZ infusion.

Finally, we evaluated the potential interaction between K_{ATP} channels and opioid system in modulating to anticonvulsive effect of zolpidem. Thus, glibenclamide (0.03 mg/kg, i.p.) or naloxone (0.03 mg/kg, i.p.), separately or combined together, was injected prior to the injection of zolpidem (10 mg/kg, i.p.).

2.5. Statistical analysis

Data are expressed as mean ± S.E.M. of clonic seizure threshold in each experimental group. The one-way or two-way analysis of variance (ANOVA) followed by Newman–Keuls post hoc test was used to analyze the data. In all experiments, a P value less than 0.05 was considered statistically significant.

3. Results

3.1. Anticonvulsive effect of zolpidem

Fig. 1A shows the effect of zolpidem (1, 3, and 10 mg/kg, i.p.) on PTZ-induced seizure threshold 30 min after zolpidem injection. Doses of 3 mg/kg and 10 mg/kg of zolpidem significantly increased the threshold ($P < 0.001$ and $P < 0.0001$, respectively; $F_{3,16} = 102.1$), whereas zolpidem at 1 mg/kg did not show significant anticonvulsive effects. As depicted in Fig. 1, the maximum dose of zolpidem for an anticonvulsive effect was observed at 10 mg/kg. Fig. 1B also shows the effects of zolpidem (10 mg/kg, i.p.) on seizure threshold when administered 5, 15, and 30 min before the PTZ injection. The maximum response time