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SB 334867, a selective orexin receptor type 1 antagonist, elevates seizure threshold in mice

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ABSTRACT

Aim: Orexins A and B are hypothalamic neuropeptides involved in a number of physiological and behavioral processes. They work via OX_1 and OX_2 receptors. Recent studies revealed that orexins may be implicated in seizure activity. Therefore, the present study was undertaken to evaluate the influence of SB 334867 (a selective OX_1 receptor antagonist) and EMPA (a selective OX_2 receptor antagonist) on the seizure thresholds in mice. We also aimed to determine the changes of orexin A level following different types of seizures.
Main methods: The intravenous pentylenetetrazole (i.v. PTZ) seizure test, the maximal electroshock seizure threshold (MEST) test and the 6 Hz seizure test were used in the present study. Brain orexin A level was determined via enzyme-linked immunoassay (ELISA).
Key findings: SB 334867 did not affect the seizure threshold for myoclonic twitches and tonic seizures in the i.v. PTZ seizure test. This compound, however, significantly raised the threshold for the PTZ-induced clonic seizures, for tonic hindlimb extension in the MEST test as well as for psychomotor seizures induced by 6 Hz stimulation. In comparison, EMPA did not alter the seizure thresholds in the i.v. PTZ test. Both EMPA and SB 334867 did not affect motor coordination and muscular strength. ELISA showed the increase of total brain orexin A level following different types of seizures.
Significance: Our results provide further evidence for the role of orexins in seizure activity and suggest that pharmacological blockade of the OX_1 receptors may represent a novel therapeutic approach in the treatment of seizure disorders.

1. Introduction

Orexin A and B, also known as hypocretin 1 and 2, are neuropeptides produced by relatively small group of orexin-expressing neurons located in the lateral hypothalamus. Orexinergic neurotransmission is mediated via two G-protein coupled receptors termed orexin 1 and orexin 2 receptors (OX_1 and OX_2 receptors, respectively), which have partially overlapping distribution in the brain. The OX_1 receptors have much higher affinity for orexin A than orexin B, while the OX_2 receptors have equal affinity for either orexin A or B [1,2]. Originally, orexin receptors were considered to signal via G_q/phospholipase C/protein kinase A pathway. However, the orexin signaling seems to be more versatile and complicated [3].

Although orexin-producing neurons represent a very restricted group of cells, their ascending projections are extremely widespread and they reach many brain regions including thalamus, hippocampus, amygdala, brainstem, midbrain and cortex [4]. Initially, orexins were thought to be involved in control of food intake. However, a wide distribution of orexinergic neurons throughout the brain indicates that orexins are implicated in control of many different brain and body function such as sleep-wake cycle, arousal, locomotion, reward and addiction, cognition, stress response or hormone release [5,6]. Moreover, the projection of orexin-expressing neurons to the C1–C3 regions of the hippocampus, where the OX_1 and OX_2 receptors are also widely expressed, suggests the possible involvement of orexinergic system in seizure activity [7]. It was reported that intracerebroventricular (i.c.v.) as well as intracortical injection of orexins cause seizures in rats [8,9]. The orexinergic system was also suggested to be involved in the mechanism of seizures induced by sleep deprivation. Inactivation of both OX_1 and OX_2 receptors with selective antagonists (SB 334867 and TCS OX2 29, respectively) prolonged the latency and reduced the duration of pentylenetetrazole (PTZ)-induced seizures in sleep-deprived rats [7]. Moreover, the anticonvulsant activity of SB 334867 and TCS OX2 29, injected directly into the hippocampus, against seizures induced by PTZ in naïve rats was observed [10]. Noteworthy, almorexant (a dual

Abbreviations: CB_1 receptor, cannabinoid receptor type 1; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunoassay; i.c.v., intracerebroventricularly; i.p., intraperitoneally; i.v., intravenously; MES, maximal electroshock seizure; MEST, maximal electroshock seizure threshold; OX_1 receptor, orexin receptor type 1; OX_2 receptor, orexin receptor type 2; PTZ, pentylenetetrazole; SEM, standard error of the mean.

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orexin receptors antagonist) was devoid of anticonvulsant activity in the pilocarpine-induced limbic seizures model in mice [11]. The same compound, however, produced antiepileptic activity and improved sleep in Kcna1-null mouse model of temporal lobe epilepsy [12].

According to the above-mentioned reports, it seems that orexins, especially in the hippocampus, have excitatory effect and may cause seizures. However, there are also some opposite findings. Doreulee et al. [13] found that orexin A inhibits spontaneous epileptiform after discharges induced by bicuculline in hippocampal slices. In another study, orexin A ameliorated the impairment of spatial learning and memory in PTZ-kindled rats by promoting neurogenesis in the dentate gyrus. This protective effect of orexin A was abolished by treatment with SB 334867 [14]. Data obtained in clinical studies are also contradictory [15,16].

In order to further elucidate the role of the OX_1 receptors in seizure activity, we investigated the influence of SB 334867, a selective OX_1 receptors antagonist, on the seizure thresholds in three acute seizure threshold tests mice. Since OX_1 has much higher affinity for orexin A than B, the changes in total brain orexin A level following different types of seizures were determined. In order to compare the role of OX_1 and OX_2 receptors in control of seizure threshold, we also evaluate the effect of EMPA, a selective OX_2 receptors antagonist, on seizure thresholds in the i.v. PTZ test.

2. Materials and methods

2.1. Animals

Experimental subjects were male Albino Swiss mice weighing 25–30 g. The animals were purchased from a licensed breeder (Laboratory Animals Breeding, Słaboszów, Poland) and housed under strictly controlled laboratory conditions (temperature maintained at 22–23 °C, relative humidity about 45–55%) with an artificial 12-h light/dark cycle (light on at 6:00 a.m.). A nutritionally-balanced rodent chow diet (Agropol S.J., Motycz, Poland) and tap water were continuously available. Before being used in the experiments, mice were allowed an adaptation period of at least 7 days. All experiments were performed between 8:00 a.m. and 14:00 p.m. to minimize circadian influences, after minimum 30-min acclimatization to the experimental room. The animals were randomly assigned to the experimental groups. Each animal was used only once.

The study was carried out under experimental protocols approved by the Ethical Committee of the Medical University in Lublin. Housing and experimental procedures were conducted in accordance with the European Union Directive of 22 September 2010 (2010/63/EU) and Polish legislation concerning animal experimentation. All efforts were made to minimize animal suffering as well as the number of animals used in the study.

2.2. Treatment

N-ethyl-2-[(6-methoxy-3-pyridinyl)[(2-methylphenyl)sulfonyl]amino]-*N*-(3-pyridinylmethyl)-acetamide (EMPA, Sigma-Aldrich, St. Louis, MO, USA), *N*-(2-methyl-6-benzoxazolyl)-*N′*-1,5-naphthyridin-4-yl urea (SB 334867, Tocris Bioscience, Bristol UK) and sodium valproate (Sigma-Aldrich, Poznań, Poland) were used in the present study. EMPA and SB 334867 were dissolved in a small volume of dimethyl sulfoxide (DMSO, ICN Biomedicals, Inc., Aurora, OH, USA) and diluted to the appropriate concentration with 1% solution of Tween 80 (POCH, Gliwice, Poland) in normal saline. The final concentration of DMSO did not exceed 1%. EMPA and SB 334867 were administered by intraperitoneal (i.p.) route in a volume of 10 ml/kg, 30 min before behavioral tests. The dosage, route of administration and pretreatment time for both of the studied compounds were based on literature search [17–21]. In the first experiment (i.e. the i.v. PTZ test), the initial doses were 3 mg/kg for SB 334867 and 10 mg/kg for EMPA. The doses were then increased. In the MEST test and the 6 Hz seizure test, the initial dose for SB 334867 was 10 mg/kg. The dose was then increased or decreased depending on the observed effect.

Sodium valproate (a positive control) was dissolved in normal saline and injected i.p. 15 min before the tests. Control animals received i.p. injections of vehicles.

2.3. Intravenous pentylenetetrazole (PTZ) seizure threshold test

Mice were placed in the cylindrical plastic restrainer (12-cm long, 3-cm inner diameter). The lateral tail vein was catheterized with a 2-cm long 27-gauge needle attached by polyethylene tubing PE20RW (Plastics One Inc., Roanoke, VA, USA) to a 5-ml plastic syringe containing 1% aqueous solution of PTZ (Sigma-Aldrich, St. Louis, MO, USA). The syringe was mounted on a syringe pump (model Physio 22, Hugo Sachs Elektronik–Harvard Apparatus GmbH, March-Hugstetten, Germany). The accuracy of needle placement in the vein was confirmed by appearance of blood in the tubing. The needle was secured to the tail by an adhesive tape. After correct needle placement into the tail vein, mice were placed in a Plexiglas arena for behavioral observation. The PTZ solution was infused at a constant rate of 0.2 ml/min. The time latencies from start of infusion to the appearance of (1) the first myoclonic twitch, (2) generalized clonus with loss of righting reflex and (3) tonic forelimb extension were recorded. The seizure thresholds were calculated separately for each endpoint using the following formula: threshold dose of PTZ (mg/kg) = (infusion duration (s) × infusion rate (ml/s) × PTZ concentration (mg/ml) × 1000) / body weight (kg). Seizure thresholds were expressed as the amount of PTZ (in mg per kg) ± SEM (standard error of the mean) needed to produce the first apparent sign of each endpoint in each experimental group (9–15 mice/group). Tonic convulsions were lethal for mice.

For measurement of brain orexin A level, mice were infused with the PTZ solution in the same manner as described above and decapitated immediately after tonic forelimb extension.

2.4. Maximal electroshock seizure test

A constant-current stimulator (Rodent Shocker, Type 221; Hugo Sachs Elektronik, Freiburg, Germany) was used to evoke electroconvulsions in mice. Mice were challenged with saline-soaked transcorneal copper electrodes. Before stimulation, the corneal electrodes were wetted with saline to provide good electrical contact. Stimulus duration was 200 ms at a frequency of 50 Hz (sine-wave pulses). During stimulation mice were restrained manually and immediately following stimulation were placed in a transparent box for behavioral observation for the presence or absence of seizure activity. Tonic hindlimb extension (i.e., hindlimbs of animals outstretched at 180° to the plane of body axis) was taken as an endpoint.

Two experimental approaches to induce electroconvulsions were used in the present study: (1) the maximal electroshock seizure threshold (MEST) test that employed stimulation at varied current intensities (6.6–15.1 mA) and (2) the maximal electroshock seizure (MES) test which employed stimulation at a fixed current intensity (25 mA). In the MEST test, the current intensity was established according to an 'up-and-down' method described by Kimball et al. [22]. Current intensity was lowered or raised by 0.06-log intervals depending on whether the previously stimulated animal did or did not exert tonic hindlimb extension, respectively. The data obtained in groups of 20 animals were used to determine the threshold current causing endpoint in 50% of mice (CS_{50} with confidence limits for 95% probability). The MES test was performed in order to determine the brain orexin A level after tonic hindlimb extension. Mice were divided into three groups and stimulated at a fixed current intensity of 25 mA, at which all animals exhibited tonic hindlimb extension. The animals were decapitated 0, 60 and 240 min after the endpoint.