Neuroscience Letters 556 (2013) 99–103

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Pharmacological characteristics of zolpidem-induced catalepsy in the rat

Pawel Mierzejewski[a], Marcin Kolaczkowski[c,d], Natalia Nowak[a], Agnieszka Korkosz[a], Anna Scinska[a,b], Halina Sienkiewicz-Jarosz[b], Jerzy Samochowiec[e], Wojciech Kostowski[a], Przemyslaw Bienkowski[a,*]

[a] Department of Pharmacology, Institute of Psychiatry and Neurology, Warsaw, Poland
[b] Department of Neurology I, Institute of Psychiatry and Neurology, Warsaw, Poland
[c] Department of Medicinal Chemistry, Collegium Medicum, Jagiellonian University, Krakow, Poland
[d] Adamed Ltd., Pienkow, Poland
[e] Department of Psychiatry, Pomeranian Medical University, Szczecin, Poland

HIGHLIGHTS

- Zolpidem is a hypnotic drug acting preferentially at α1-containing $GABA_A$ receptors.
- Zolpidem, but not diazepam and midazolam, produced cataleptic responses in rats.
- Zolpidem-induced catalepsy was abolished by flumazenil, quinpirole, and MK-801.
- Zolpidem can produce neuroleptic-like catalepsy in rats.

ARTICLE INFO

Article history:
Received 22 August 2013
Received in revised form
26 September 2013
Accepted 4 October 2013

Keywords:
Zolpidem
Flumazenil
The $GABA_A$ receptor
Catalepsy
Rat

ABSTRACT

Zolpidem is a non-benzodiazepine hypnotic drug acting preferentially at α1-containing $GABA_A$ receptors expressed in various parts of the brain, including the basal ganglia. The aim of the present study was to provide preliminary characteristics of zolpidem-induced catalepsy in Wistar rats. Zolpidem (2.5–10.0 mg/kg), but not diazepam and midazolam, produced dose-dependent cataleptic responses in the bar test, which were similar to those produced by a reference antipsychotic drug, haloperidol. Zolpidem-induced catalepsy was abolished by a benzodiazepine site antagonist, flumazenil (5.0 mg/kg), $D_{2/3}$ receptor agonist, quinpirole (1.0 mg/kg), and a non-competitive NMDA receptor antagonist, MK-801 (0.1 mg/kg), but not by a non-selective opioid receptor antagonist, naltrexone (3.0 mg/kg). The present results indicate that systemic injections of zolpidem may produce short-lasting, neuroleptic-like catalepsy in the rat.

1. Introduction

The mammalian brain contains a plethora of structurally diverse γ-aminobutyric $acid_A$ ($GABA_A$) receptors which are targets for a variety of clinically effective medications [3,6,15,18]. Zolpidem is a non-benzodiazepine hypnotic drug acting at the benzodiazepine site of the $GABA_A$ receptor complex [11,12,15]. In contrast to classical benzodiazepines (e.g. diazepam, midazolam), zolpidem binds with some selectivity to the α1-containing $GABA_A$ receptors and produces relatively weak myorelaxant, anxiolytic, and anticonvulsant effects [4,8,15].

Catalepsy defined as the delayed correction of an abnormal posture is considered an animal model of extrapyramidal symptoms observed in Parkinson's disease and in antipsychotic-induced parkinsonism [9,14]. It has been shown repeatedly that dopamine receptor antagonists, opioid receptor agonists, and cannabinoid receptor agonists can evoke strong cataleptic responses in rodents [9,10,16].

GABAergic neurones localized in various parts of the extrapyramidal system play a complex role in the regulation of motor functions [2–4,17]. Although myorelaxation makes scoring of cataleptic responses after peripheral administration of benzodiazepines difficult, $GABA_A$ receptor agonists can induce catalepsy when injected directly into the brain [21,28]. In fact, both cataleptic and anti-cataleptic effects of $GABA_A$ receptor agonists were

* Corresponding author at: Department of Pharmacology, Institute of Psychiatry and Neurology, 9 Sobieskiego Street, 02-957 Warsaw, Poland.
Tel.: +48 22 842 76 44; fax: +48 22 842 76 44.
E-mail address: bienkow@ipin.edu.pl (P. Bienkowski).

0304-3940/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2013.10.011

observed, depending on the site of microinjection. For example, a $GABA_A$ receptor agonist, muscimol produced dose-dependent, neuroleptic-like cataleptic responses when injected into the globus pallidus (GP) or lateral hypothalamus [16,21,28]. In contrast, bilateral microinjections of muscimol into the substantia nigra (SN) produced stereotypies and antagonized haloperidol-induced catalepsy [17,20]. Systemic injections of classical benzodiazepines and indirect GABAergic drugs enhanced antipsychotic-induced catalepsy in rodents [7,29].

Zolpidem-induced catalepsy was serendipitously found in our pilot studies on effects of GABAergic drugs in animal models predictive of antipsychotic activity. The first aim of the present study was to evaluate dose- and time-dependence of zolpidem-induced catalepsy in Wistar rats. Zolpidem-induced immobility responses were compared with those induced by a reference antipsychotic, haloperidol and benzodiazepines, diazepam and midazolam. The second aim of the study was to provide preliminary pharmacological characteristics of zolpidem-induced catalepsy. To confirm the involvement of $GABA_A$ receptors in zolpidem-induced immobility responses, the drug was administered in combination with a benzodiazepine site antagonist, flumazenil [3,8]. Catalepsy induced by antipsychotics and opiates is differentially modulated by dopamine, opioid, and NMDA receptor ligands [10,24,27]. Morphine-induced catalepsy is blocked by opioid receptor antagonists (e.g. naltrexone) [26] and enhanced by NMDA receptor antagonists (e.g. MK-801) [24]. Antipsychotic-induced catalepsy is blocked by NMDA receptor antagonists and dopamine $D_{2/3}$ receptor agonists (e.g. quinpirole) [16,27]. In order to further characterize receptor mechanisms involved in zolpidem-induced catalepsy, the drug was injected in combination with naltrexone, quinpirole, and MK-801.

Our study may shed more light on the involvement of α1-containing $GABA_A$ receptors in the motor control processes [3,4] and provide some clues for identifying mechanisms of side effects observed in zolpidem-treated patients [12].

2. Materials and methods

2.1. Subjects

Male Wistar rats (Charles River, Sulzfeld, Germany) weighting 300–350 g were used. Rats were housed two per standard plastic cage and kept in a room with constant environmental conditions (temperature: 22 ± 1 °C, relative humidity: 60%, a 12:12 light–dark cycle with lights on at 07:00 am). Animals were supplied by the breeder two weeks before the start of experiments. During this time, the subjects were weighed and handled several times. Tap water and standard lab chow (Labofeed H, WPIK, Kcynia, Poland) were available ad libitum. Treatment of rats in the present study was in full accordance with the ethical standards laid down in respective Polish and European (directive No. 86/609/EEC) regulations. All procedures were reviewed and approved by an ethics committee on animal studies.

2.2. Catalepsy assessment

All experiments were carried out between 09:00 am and 1:00 pm in a sound-attenuated experimental room with the constant environmental conditions. Cataleptic responses were assessed in the bar test [23]. Each rat was placed on a clean, smooth table with the wooden bar (2 cm × 3 cm × 25 cm, $H \times W \times L$) suspended 10 cm above the working surface. The animal's hindlimbs were freely placed on the table, the tail laid out to the back, and the forelimbs gently placed over the bar. The length of time the animal touched the bar with both front paws was measured up to a pre-set cut-off time of 300 s. Rats that did not move their front paws but showed head movements and exploratory behavior were not considered cataleptic. On the day before the test, all rats were habituated to the experimental room (for 30 min) and to the working surface and wooden bar (for 1 min). On the test day, subjects were allowed to habituate to the experimental room for 30 min.

Separate groups of drug-naive rats were administered with various doses of zolpidem (2.5–10.0 mg/kg, i.p.), haloperidol (0.1–1.0 mg/kg, s.c.), midazolam (5.0–10.0 mg/kg, i.p.), and diazepam (2.5–10.0 mg/kg, i.p.) ($n = 8$ animals per dose). In zolpidem-, diazepam-, and midazolam-treated rats, catalepsy was assessed 5 min, 15 min, 30 min, and 60 min after the drug injection. In haloperidol-treated rats, catalepsy was assessed 30 min, 60 min, and 120 min after the drug injection.

In a separate experiment, zolpidem (5.0 mg/kg, i.p.) was administered in combination with the ligands of different receptors (antagonism tests). Catalepsy was scored 15 min after zolpidem administration. Flumazenil (5.0 mg/kg), naltrexone (3.0 mg/kg), and quinpirole (1.0 mg/kg) were injected i.p. 30 min before zolpidem. MK-801 (0.1 mg/kg) was injected i.p. 15 min before zolpidem. The doses and pre-treatment times were selected on the basis of previous reports [1,16,18,26,27] and our pilot studies.

2.3. Drugs

Zolpidem (Synthelabo, Bagneux, France), (+)MK-801 (Sigma–Aldrich, Poznan, Poland) naltrexone (Sigma–Aldrich), quinpirole (Sigma–Aldrich), midazolam (Hoffman la Roche, Basel, Switzerland), and flumazenil (Hoffman la Roche) were dissolved in sterile physiological saline and injected in a volume of 1.0 ml/kg. Haloperidol (ampoules 5 mg/ml; WZF Polfa S.A., Warsaw, Poland) was diluted with physiological saline and administered in a volume of 1.0 ml/kg. Diazepam (ampoules 5 mg/ml; WZF Polfa S.A.) was diluted with sterile distilled water and administered in a volume of 2.0 ml/kg.

2.4. Statistics

A two-way analysis of variance (ANOVA; Dose × Time) with repeated measures on Time was used to analyze zolpidem-, haloperidol-, diazepam-, and midazolam-induced catalepsy. A one-way ANOVA was used to assess effects of flumazenil, quinpirole, MK-801, and naltrexone on zolpidem-induced catalepsy. The Newman–Keuls test was used for individual post hoc comparisons. Probability (p) levels less than 0.05 were considered significant. The Statistica 5.0 software package (StatSoft, Tulsa, OK, USA) was used to analyze all data.

3. Results

Zolpidem produced dose-dependent and relatively short-lasting catalepsy (Fig. 1). The ANOVA revealed a significant effect of Dose [$F(3,28) = 76.0$, $p < 0.01$] and Time [$F(3,84) = 102.1$, $p < 0.01$], and a significant Dose × Time interaction [$F(9,84) = 52.2$, $p < 0.01$]. No catalepsy was noted 60 min after zolpidem administration (for post hoc analyses, see Figs. 1–5). A further inspection indicated that the subjects treated with the two higher doses of zolpidem (5.0–10.0 mg/kg) were able to maintain different uncomfortable postures. In agreement with the definition of catalepsy, zolpidem did not eliminate the righting reflex and motor responses to tail pinch [14]. In contrast, diazepam and midazolam failed to induce any catalepsy (F values < 0.1, P's > 0.9; Figs. 2 and 3).

Haloperidol produced dose-dependent and relatively long-lasting catalepsy (Fig. 4). The ANOVA revealed a significant effect of Dose [$F(3,28) = 35.0$, $p < 0.01$] and Time [$F(2,56) = 84.3$, $p < 0.01$], and a significant Dose × Time interaction [$F(6,56) = 8.3$, $p < 0.01$].